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Chemistry of the [beta]-phenylserines

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NOTE TO USERS

CHEMISTRY OF THE β-PHENYLSERINES

by

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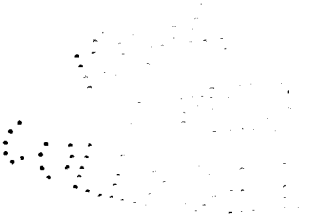

Signature was redacted for privacy.
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TABLE OF CONTENTS

I. INTRODUCTION

The α-amino-β-hydroxy acid, β-phenylserine, is closely related in a structural sense to the naturally occurring amino acids, serine, phenylalanine and threonine. Phenylserine has not been demonstrated as a constituent of proteins. However, its chemical reactivity, in conjunction with the conditions customarily employed for proteolysis and for isolation of the resultant amino acids, would render such a task difficult.

Phenylserine has recently attracted attention in the microbiological field by virtue of its properties as an anti-metabolite. The simultaneous finding in several laboratories that the compound is an admirable synthetic precursor for the antibiotic, chloramphenicol, is not unrelated. In another direction, there are increasing indications that phenylserine, or, more closely, its 3,4-dihydroxy derivative may be involved actively in the biosynthesis of adrenaline. These varied features make phenylserine, its derivatives and its analogues of worthwhile concern from the chemical, pharmaceutical and biological standpoints.

Due to the presence of two asymmetric centres in its molecule, phenylserine can theoretically exist as two diastereomeric pairs of enantiomorphs. Although one diastereomer has been well known for the past six decades, the existence of

the other has been the subject of much contention. Some of the synthetic methods which have been employed may lead not only to phenylserine, but also to the α-hydroxy-β-amino acid, phenylisoserine, thus further complicating the stereoisomeric picture. The confusion in the phenylserine literature is increased by the frequently inadequate characterization of starting compounds and products in respect to both purity and steric form. Similar uncertainty surrounds the materials which have been used in past biochemical studies.

A rapidly growing interest in this field is reflected in the appearance of as many publications during the past three years as in the preceding half century. In this light, the need is evident for a comprehensive review dealing with all phases of phenylserine chemistry which have been studied in the past. As part of this thesis, an attempt to meet this demand has been made. Particular attention has been given to reported findings of a controversial or dubious nature, and, where apparent, points meriting reinvestigation or potential areas for new research have been indicated.

The initial purpose of the investigation described in the present thesis was to effect the synthesis of racemic chloramphenicol from phenylserine, and then to attempt the preparation of analogues of possible chemotherapeutic value. The first objective had been successfully attained when the interest of several industrial groups in the same problem became clearly

manifested. To avoid undesirable duplication of effort, attention was turned to the disputed phenylserine diastereomer, a derivative of which was fortuitously encountered during early work on synthesis of the antibiotic. After the identity and steric configuration of this compound had been conclusively established, an analytical method was sought by which it could be readily distinguished from the well-known phenylserine. With this in hand, it was possible to investigate conditions in synthesis which governed the ratio of the two phenylserine diastereomers produced, and thus to shed some light on the mechanism of reaction. A method for separating the diastereomers in isomerically pure form followed. Finally, the preparation of derivatives suitable for enzymic resolution was undertaken, since the four individual enantiomorphs thus to be obtained are of considerable interest for future biochemical studies.

II. REVIEW OF LITERATURE

A. Methods of Synthesis

1. Condensation of aromatic aldehydes and glycine derivatives

The feasibility of reacting benzaldehyde with glycine became of concern during a period when the Perkin type condensation was receiving considerable attention. Curtius and Lederer (1) noted formation of benzylamine and carbon dioxide upon heating the two substances at 130°. Plöchl (2) observed reaction in presence of acetic anhydride, but was unable to obtain a discrete product. Although these studies provided clear evidence of reaction capability, the need was apparent for less drastic conditions to permit isolation of intermediate products.

Erlenmeyer (3,4) found that benzaldehyde readily condensed with glycine under influence of sodium hydroxide in aqueous ethanol. Rapid clarification of the initially formed emulsion, attended by slight warming, was succeeded by clouding and formation within thirty minutes of a crystalline

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(1) T. Curtius and G. Lederer, Ber., 19, 2462 (1886).
(2) J. Plöchl, Ber., 16, 2815 (1883).
(3) E. Erlenmeyer, Jr., Ber., 25, 3445 (1892).
(4) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).

paste, which set completely solid after several hours. When this material was filter pressed and washed repeatedly with warm ethanol, the alcohol-insoluble sodium salt of N-benzalphenylserine was obtained.

From the ethanol washings was isolated N-benzalisodiphenylhydroxyethylamine, a substance which constituted the main reaction product when three instead of two moles of benzaldehyde were reacted with one mole of glycine (1); in this case, small amounts of the more soluble isomeric N-benzaldiphenylhydroxyethylamine also were recovered from the alcoholic mother liquors. The free racemic bases, isodiphenylhydroxyethylamine, m.p. 129°, and diphenylhydroxyethylamine, m.p. 163°, were readily obtained on acid treatment of the benzal adducts, and were well characterized by detailed investigation (2). As a result of interest in their morphine-like properties, these compounds have been further investigated recently (3).

The sodium salt of N-benzalphenylserine was recrystallized from hot water with only slight attendant

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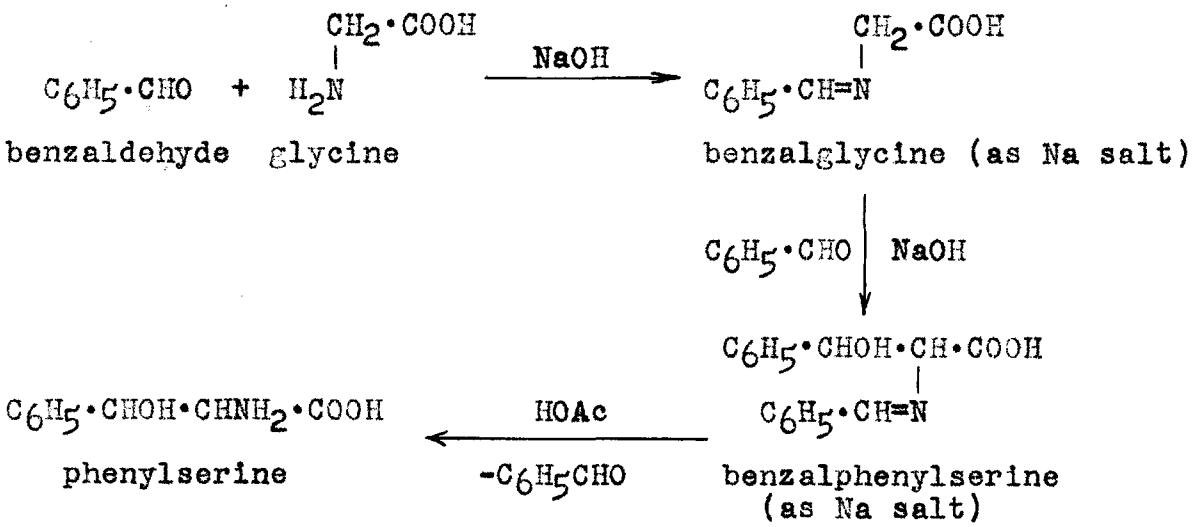
(1) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).
(2) E. Erlenmeyer, Jr., Ber., 28, 1866 (1895); 29, 295 (1896); 30, 1525, 1527, 2896 (1897); 32, 2377 (1899); Ann., 307, 113 (1899).
E. Erlenmeyer, Jr., and A. Arnold, Ann., 337, 307, 329, 342 (1904).
(3) J. Weijlard, K. Pfister, E. F. Swanezy, C. A. Robinson and M. Tishler, J. Am. Chem. Soc., 73, 1216 (1951).

decomposition (1). Treatment with acetic anhydride in the cold gave α-acetamidocinnamic acid, together with O-acetyl-N-benzalphenylserine, m.p. 169-170° (dec.), the latter being further characterized as its crystalline sodium salt. Transformation of N-benzalphenylserine sodium to benzalisodiphenylhydroxyethylamine occurred upon treatment with more benzaldehyde and sodium hydroxide. Free N-benzalphenylserine was not prepared, since cold acids split out benzaldehyde thereby liberating phenylserine.

The following sequence was offered to explain formation of phenylserine (2):



The first intermediate, the sodium salt of benzalglycine, was presumed too soluble to permit its isolation prior to further

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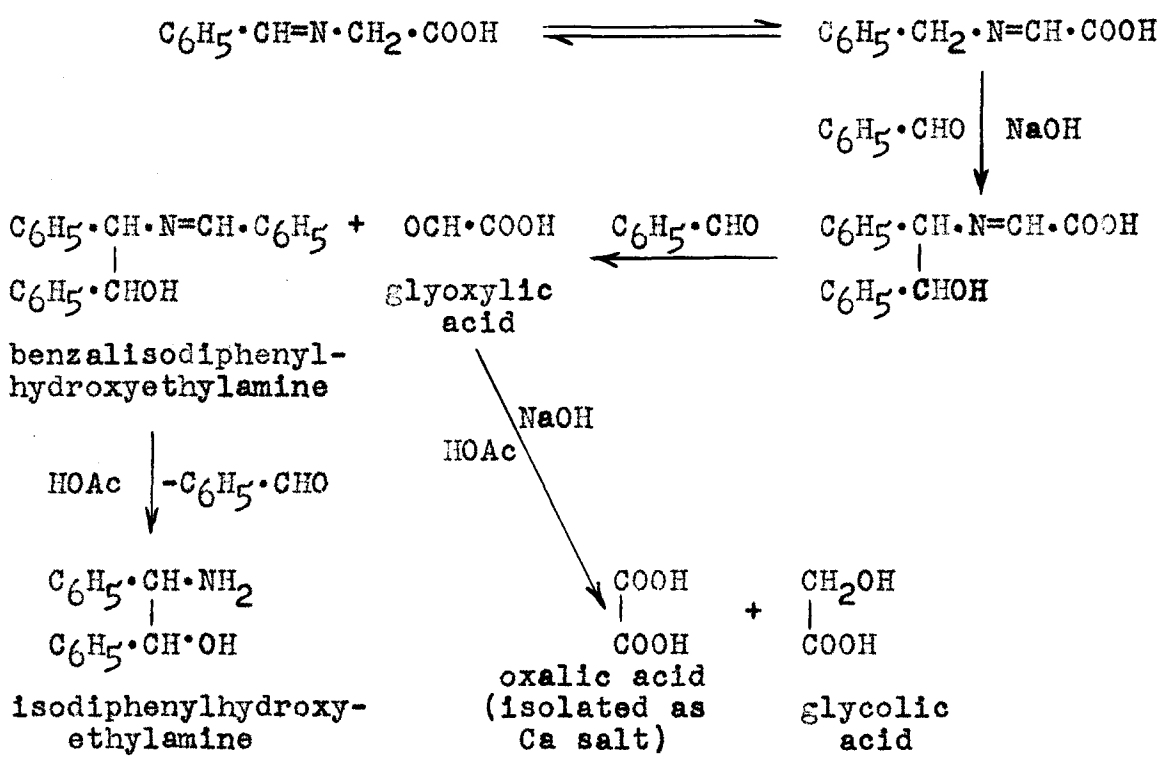
(1) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).
(2) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).

reaction (1). With long reaction time, or with excess benzaldehyde present, an alternative reaction path was followed, due to tautomerism of this adduct (2):



Evidence for participation of the tautomer was provided by isolation, from a partially reacted condensation mixture, of benzylamine (2), which was also formed on heating isodiphenylhydroxyethylamine at 130° or higher (2,3). Thus two alternative sources were provided for benzylamine noted in studies of Curtius and Lederer (4). However, efforts to prepare the

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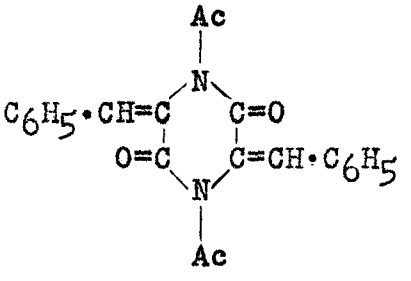
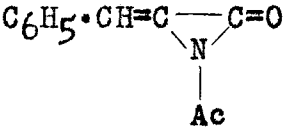
(1) E. Erlenmeyer, Jr., Ann., 337, 205 (1904).
(2) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).
(3) E. Erlenmeyer, Jr., Ber., 28, 1866 (1895).
(4) T. Curtius and G. Lederer, Ber., 19, 2462 (1886).

tautomer from benzylamine and glyoxylic or dichloroacetic acid were unsuccessful (1).

Phenylserine, thus prepared, crystallized from ethanol-water as shimmering colorless plates of the monohydrate, m.p. 192-193° (dec.) (2), 193-194° (dec.) (3). Drying at 100° gave the anhydrous amino acid, m.p. 195-196° (dec.) (1,2), 190° (dec.) (3), poorly soluble in water and insoluble in alcohol. It formed a hydrochloride (not characterized) and a poorly soluble violet copper salt.

Treatment of phenylserine with acetic or benzoic anhydrides yielded the corresponding "lactimide" (azlactone), initially formulated as a diketopiperazine (2) or as an α-lactam (3):





This was converted by action of alkalies to the acylamido-cinnamic acid, which on prolonged basic hydrolysis decomposed to ammonia and phenylpyruvic acid (3):

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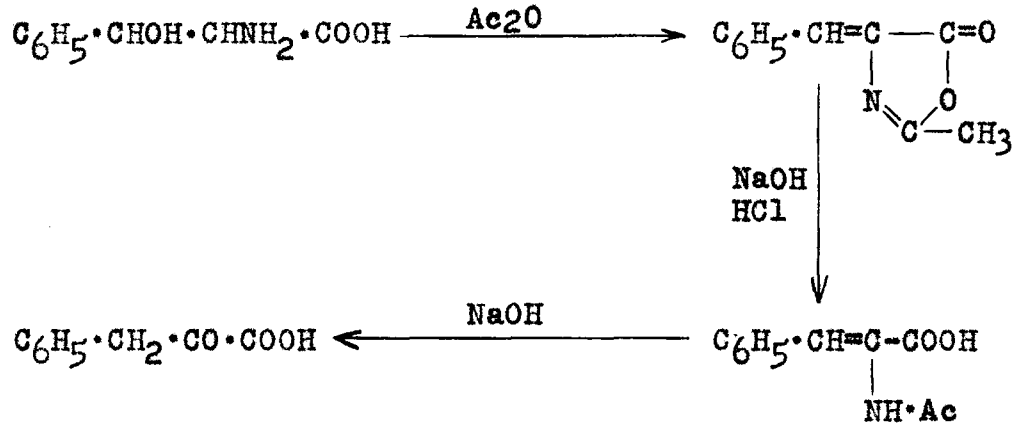
(1) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).
(2) E. Erlenmeyer, Jr., Ber., 25, 3445 (1892).
(3) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).



Repetition of Plöchl's work (1) also gave an azlactone (2). With nitrous acid, phenylserine gave a solution which appeared to contain phenylglyceric acid (2), whereas, on heating with dilute sulfuric acid, the molecule was decarboxylated and rearranged to phenylacetaldehyde (3). These reactions substantiated the structure postulated for phenylserine.

Early attempts to isolate the second expected diastereomer* of phenylserine were unsuccessful (2). On a later occasion (3), the mother liquor from acetic acid cleavage of a large batch of benzalphenylserine sodium was slowly evaporated during some weeks at room temperature to a crystal mass. Fractional solution of sodium acetate present, and

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* This compound is denoted henceforth in this manuscript as allophenylserine, not only to avoid confusion in nomenclature, but also for reasons set forth on p. 178.

(1) J. Plöchl, Ber., 16, 2815 (1883).
(2) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).
(3) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).

crystallization of the residue from ethanol-water led to shining colorless needles of a monohydrated hydroxyamino acid. Drying at 100° caused slight decomposition, with some yellowing. Erlenmeyer mentioned that melting occurred at 188° (dec.), without specifying whether hydrated or anhydrous material was used, and cited no elementary analyses for either. Warming with acetic anhydride gave a "lactimide", but in insufficient quantity for characterization. The copper salt differed considerably from that of the earlier described phenylserine in forming bright blue small plates, approximately eight times more soluble in water. Concentration of the above mother liquor on a water bath caused extensive decomposition, to which fact Erlenmeyer attributed his earlier failure to isolate the new amino acid.

On the basis of comparative solubilities and ease of crystallization, phenylserine and isodiphenylhydroxyethylamine were considered to possess the same steric configuration as racemic tartaric acid, i.e. by modern terminology, threo, whereas allophenylserine and diphenylhydroxyethylamine were related to mesotartaric acid, i.e. erythro configuration (1). At that time, these contentions lacked adequate experimental foundation.

The factors operating in glycine-benzaldehyde

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(1) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).

condensation, which led to formation of the phenylserine or diphenylhydroxyethylamine diastereomers in quite unequal amounts, were of interest to Erlenmeyer. Size, nature, distribution and orienting influence of other substituent groups in the reacting molecules were postulated to be of vital import. The formation of a particular isomer was reasoned to depend not on the structure of the product, but rather on the nature of the starting materials, and the conditions under which they reacted. It followed that failure to secure a specific steric form by one route did not necessarily preclude its existence, but instead suggested the value of search for an alternative path to the desired product. These views are of interest because of their accord with modern organic chemical theory and practice.

Erlenmeyer was unable to generalize the benzaldehyde-glycine condensation as a method of preparation for α-amino-β-hydroxy acids (1). Other amino acids, such as phenylalanine, tyrosine, leucine and aspartic acid, gave with benzaldehyde only the corresponding α-keto acid and isodiphenylhydroxy-ethylamine (2), with no isolable trace of phenylserine analogue.

$$R\cdot CHNH_2\cdot COOH + 2C_6H_5CHO \xrightarrow{NaOH} R\cdot CO\cdot COOH + C_6H_5\cdot CHOH\cdot CHNH_2\cdot C_6H_5$$

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(1) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).
(2) E. Erlenmeyer, Jr., Ann., 307, 70 (1899); 337, 205 (1904); Ber., 30, 2896 (1897).

Aliphatic aldehydes condensed with themselves rather than with glycine. Other aromatic aldehydes were equally disappointing in behavior. Salicylaldehyde and vanillin did not react, presumably due to interference by the anionic phenoxide hydroxyl group. Anisaldehyde, cuminol and piperonal yielded very small amounts of hydroxyamine base, but no α-amino-β-hydroxy acid.

Only o-methoxybenzaldehyde showed reactivity towards glycine comparable to that of benzaldehyde (1). Condensation proceeded more slowly, probably due to steric hindrance, and separation of the two benzal adducts required more care. The intermediate N-benzal-o-methoxyphenylserine sodium salt was derivatized as the monohydrated O-acetyl compound, m.p. 216° (dec.). o-Methoxyphenylserine monohydrate, iridescent plates, m.p. 179° (dec.), and di-(o-methoxyphenyl)-hydroxyethylamine, m.p. 136°, each were isolated in only one diastereomeric form, the proportion varying with condensation time. The former was characterized as the hydrochloride, a grey-blue copper salt and as the acetamido azlactone, m.p. 156°.

A slightly different approach in phenylserine chemistry commenced with the studies of Rosenmund and Dornsaft (2), who sought a general method of synthesis for α-amino-β-hydroxy

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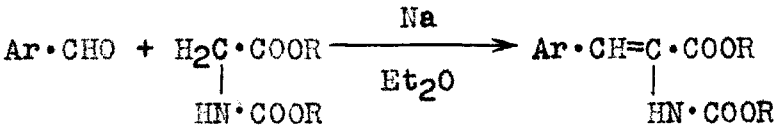
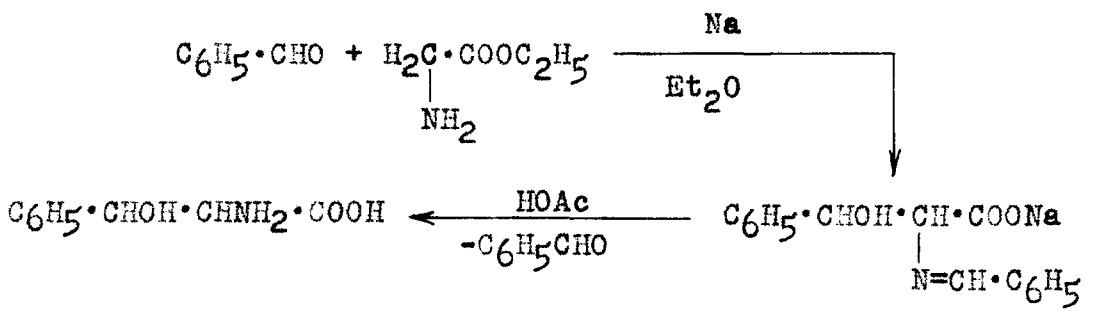
(1) E. Erlenmeyer, Jr., and F. Bade, Ann., 337, 222 (1904).
(2) K. W. Rosenmund and H. Dornsaft, Ber., 52, 1734 (1919): Arb. Pharm. Inst. Univ. Berlin, 12, 73 (1921).

acids. Although condensation of aromatic aldehydes with N-carbethoxyglycine ester gave only α-N-carbethoxyaminocinnamic esters, another modification of the Erlenmeyer method proved



more fruitful. Aromatic aldehydes, wherein any phenolic hydroxyl groups were first carbethoxylated to prevent salt formation, condensed with glycine ethyl ester in ether under influence of sodium during 24-48 hours. The resultant N-arylidene adducts were readily converted to the desired phenylserines.

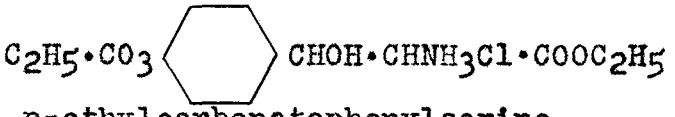
In the case of benzaldehyde, the usual intermediate insoluble sodium salt was cleaved with acetic acid to give a "phenylserine", which was reported to crystallize from water



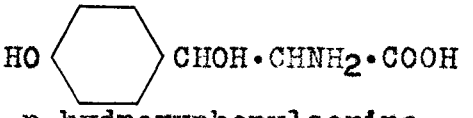
as colorless needles, m.p. 192° (dec.). Degree of hydration was unspecified. Preparation of azlactones already described by Erlenmeyer, by reaction with acetic or benzoic anhydride, was the only mode of characterization. Since this treatment

destroyed molecular asymmetry, structural configuration of this "phenylserine" can not be related to those of Erlenmeyer. p-Methoxyphenylserine, which decomposed on treatment with strong acids and similarly synthesized from anisaldehyde, was described as colorless hydrated needles, m.p. 185-186° (dec.) when dried.

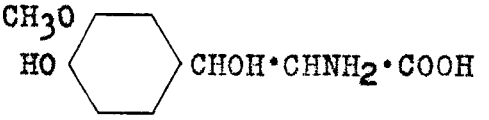
With carbethoxylated hydroxyaldehydes, very little sodium salt was formed, the intermediate consisting predominantly of the corresponding unsaponified ethyl ester, which remained dissolved in the ether. After arylidene cleavage by alcoholic hydrogen chloride, the ring-carbethoxylated hydroxyphenylserine ethyl ester hydrochloride could be isolated, or saponification with cold 1 N sodium hydroxide could be effected to give the desired phenylserine analogue upon final neutralization. Compounds thus prepared included the following:



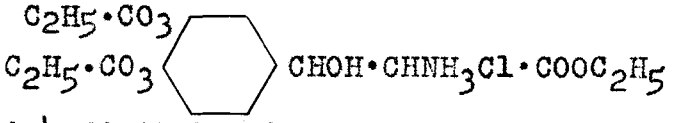
p-ethylcarbonatophenylserine
ethyl ester hydrochloride
37% yield: m.p. 181°
(free ester, m.p. 124°)



p-hydroxyphenylserine
needles or plates
dec. p. 217°



vanillylserine needles
m.p. 195° (dec.)



3,4-diethylcarbonatophenylserine
ethyl ester hydrochloride
m.p. 151-152° (dec.)

3,4-dihydroxyphenylserine
granules
m.p. 208-210° (dec.)

In preparing 3,4-dihydroxyphenylserine, otherwise denoted as DOPS and of interest as a potential biological precursor of adrenaline, final saponification was effected under hydrogen to avoid otherwise rapid atmospheric oxidation under the alkaline conditions employed.

Diphenylhydroxyethylamine analogues were not reported by Rosenmund and Dornsaft in their papers on condensation studies. No attention was given to the possible formation of diastereomers of the various phenylserine analogues prepared. Clarification of these two aspects has yet to be effected by further investigation.

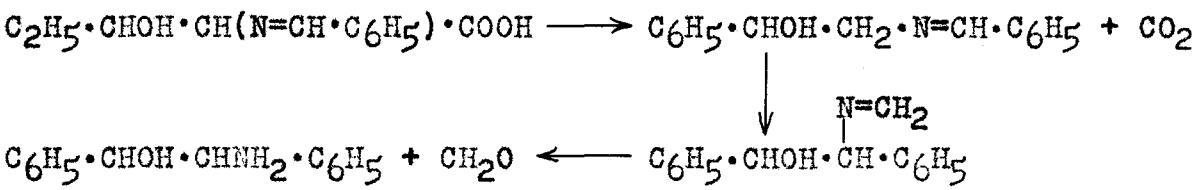
Attempts to convert an aliphatic aldehyde to an α-amino-β-hydroxy acid have remained unsuccessful. Propionaldehyde supposedly gave a product formulated as $C_2H_5 \cdot CH(NH \cdot CH_2COOH)_2$, although no structure proof was offered, nor were processing conditions such that isolation of such a moiety would be likely (1). It was reported some years later (2), that phenylacetaldehyde failed entirely to condense with glycine.

The inefficiency of Erlenmeyer's method for synthesizing phenylserine was apparent from a reported yield in one case of only 13% (3). It was remarked by Forster and Rao that the

---

(1) K. W. Rosenmund and H. Dornsaft, Ber., 52, 1734 (1919): Arb. Pharm. Inst. Univ. Berlin, 12, 73 (1921).
(2) F. Knoop, F. Ditt, W. Hecksteden, J. Maier, W. Merz and R. Härle, Z. physiol. Chem., 239, 30 (1936).
(3) E. Abderhalden and S. Buadze, Fermentforschung, 8, 487 (1926).

prescribed procedure gave isodiphenylhydroxyethylamine consistently as the sole product (1). The finding that 60-65% yield was readily attainable when cold ethanol was used to wash the intermediate benzal sodium salt suggested that earlier poor results were due to rearranging action of sodium hydroxide in the hot ethanol originally specified for washing. A mechanism somewhat different from that propounded by Erlenmeyer was offered to explain the side reaction, without any supporting experimental data, or regard for Erlenmeyer's



extensive study of the problem. The hydroxyamino acid, with a melting point of 200-202° reported for anhydrous material, was redesignated as "trans-phenylserine", in view of the simultaneously claimed discovery of the diastereomeric "cis-phenylserine" (see p. 27). For derivatization, preparation of Erlenmeyer's α-acetamido- and α-benzamidocinnamic acid "lactimides" was repeated. While Erlenmeyer's observation of allophenylserine as a by-product of glycine-benzaldehyde condensation was noted, it was dismissed with the comment, "but the substance was not analyzed, and further reference to it has not been made by any other investigator" (loc. cit., p. 1944).

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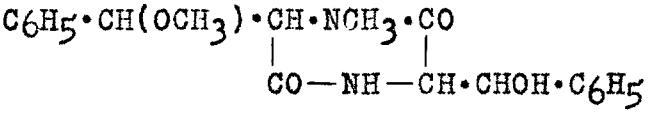
(1) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).

Efforts to prepare a diketopiperazine of phenylserine were unsuccessful. Interest in synthesizing this derivative stemmed from earlier work on picrorocellin (1), to which the structure of a phenylserine diketopiperazine had been assigned. This compound, isolated long years before (2) from an African



lichen, Rocella fuciformis, is the only simple phenylserine derivative which has been reported to occur naturally.

The nomenclature employed by Forster and Rao (3) was poorly chosen. Use of the term "trans" implied rigidity of bonding between α- and β- carbon atoms of phenylserine where rotation is actually free. That such was the actual concept of these workers was clear from their utterance concerning synthesis of α-benzamidocinnamic acid "lactimide" (loc. cit., p. 1945):

> We find that the same substance is produced from Erlenmeyer's acid by a much milder method, namely, action of benzoyl chloride suspended in sodium carbonate solution, thus emphasizing the surprising facility with which removal of water takes place when favored by the cis-relationship of the hydrogen atom and the hydroxyl group in trans-phenylserine.

This unfortunate misnomer has continued to appear in subsequent

---

(1) M. O. Forster and W. B. Saville, J. Chem. Soc., 121, 816 (1922).
(2) J. Stenhouse and C. E. Groves, Ann., 185, 14 (1877).
(3) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).

literature (1,2,3,4). Forster and Rao were also evidently unfamiliar with the fact that Erlenmeyer himself, more than two decades earlier, had considered the "lactimide" formulation erroneous and had adopted the five-membered cyclic azlactone structure as being in better accord with experimental observations.

Further improvement in Erlenmeyer's synthesis of phenylserine was described in a German patent (5), whereby condensation was effected in an entirely aqueous medium, in presence of a minimal quantity of sodium hydroxide. The intermediate N-benzalphenylserine sodium salt was hydrolyzed directly without isolation, strong mineral acids being used in place of acetic acid. While this simplified procedure was claimed to increase yield to 70%, no mention was made of the stereochemical problem.

The Rosenmund-Dornsaft variation of Erlenmeyer's phenylserine synthesis was reinvestigated and extended by Dalgliesh and Mann. The condensation of dicarbethoxyprotocatechualdehyde with glycine ethyl ester was advantageously modified by use of

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(1) H. D. Dakin, J. Biol. Chem., 140, 847 (1941).
(2) D. G. Doherty, J. E. Tietzman and M. Bergmann, J. Biol. Chem., 147, 617 (1943).
(3) J. S. Fruton, Advances in Protein Chemistry, 5, 17 (1949).
(4) E. E. Howe, "Properties of Amino Acids", in Greenberg, ed., "Amino Acids and Proteins", Charles C. Thomas, Springfield, Ill., c1951, p. 43.
(5) Ges. für Kohlentechnik m.b.H. German Patent 632,424. July 8, 1936.

excess sodium to provide maximum surface for reaction (1). Final yield of 3,4-diethylcarbonatophenylserine ethyl ester was found to depend markedly on condensation time, decreasing rapidly after 24 hours. The carbomethoxylated aldehyde proved much less satisfactory than its ethyl homologue. Derivatives prepared of the above free ester included the monohydrate, m.p. 138-139°; hydrochloride, m.p. 152-153°; picrate, m.p. 152°; normal oxalate monohydrate, m.p. 140-141° (2). Alkaline hydrolysis of the ester hydrochloride gave 4% overall yield of free 3,4-dihydroxyphenylserine, m.p. 219-221° (Rosenmund and Dornsaft (3) had listed m.p. 208-210°), characterized further as an orange picrate, m.p. 90-92°. Hydrolysis of the oxalate by refluxing with dilute acetic acid caused decomposition, with paper chromatography revealing no DOPS in the hydrolysate (1).

N-Methyl-3,4-dihydroxyphenylserine (adrenalinecarboxylic acid) was of pharmacological interest in view of its intermediate relation to adrenaline and 3,4-dihydroxyphenylalanine (DOPA) (2,4). Treatment of 3,4-diethylcarbonatophenylserine ethyl ester with diazomethane gave only unchanged reactants; action on the former of benzenesulfonyl chloride failed to

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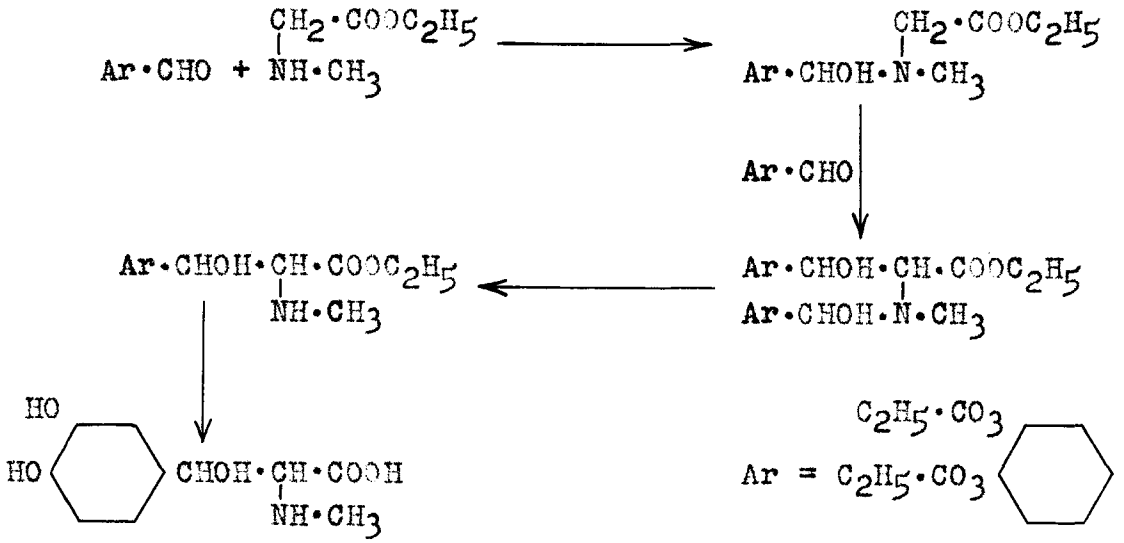
(1) C. E. Dalgliesh, J. Chem. Soc., 90 (1949).
(2) C. E. Dalgliesh and F. G. Mann, ibid., 658 (1947).
(3) K. W. Rosenmund and H. Dornsaft, Ber., 52, 1734 (1919): Arb. Pharm. Inst. Univ. Berlin, 12, 73 (1921).
(4) F. G. Mann and C. E. Dalgliesh, Nature, 158, 375 (1946).

give a crystalline derivative, which, it had been hoped, could be N-methylated. Finally, sarcosine ethyl ester was successfully condensed with dicarbethoxyprotocatechualdehyde under Rosenmund-Dornsaft conditions. Although neither the resultant N-methyl-3,4-diethylcarbonatophenylserine ethyl ester hydrochloride nor the free ester were obtainable in crystalline form, purification was effected via the corresponding picrate, m.p. 144°; acid oxalate monohydrate, m.p. 157° (dec.) or normal oxalate monohydrate, m.p. 147° (dec.). Attempted hydrolysis of the acid oxalate with sodium or barium hydroxide under hydrogen was unsatisfactory. Although refluxing the crude hydrochloride with dilute acetic acid under nitrogen was also unsuccessful, similar treatment of the acid oxalate achieved effective saponification to give adrenalinecarboxylic acid, m.p. 233° (dec.), in 4% yield from initial aldehyde. The compound was moderately water soluble, gave no crystalline picrate and reacted positively, but more slowly than DOPS, in the ninhydrin test.

The mechanism of the reaction leading to adrenalinecarboxylic acid was obscure. Schiff base formation was considered out of the question, even though two moles of aldehyde were required for condensation to proceed. The suggested reaction route involved hemiacetal-type intermediates.



Further study was made of the scope of the Rosenmund-Dornsaft route. Veratraldehyde or piperonal failed to condense with glycine ethyl ester (1). No satisfactory reaction of veratraldehyde and sarcosine nitrile or ethyl ester occurred under a wide variety of conditions (1,2). 3,4-Carbonyldioxybenzaldehyde reacted vigorously with glycine ester in absence of both ether and sodium: much heat was evolved and an orange resin formed (1). The two compounds in ether alone rapidly gave a turbid solution from which a yellow oil deposited. This was not further investigated.

The condensation of p-nitrobenzaldehyde with glycine ethyl ester was examined more closely (2). After four days

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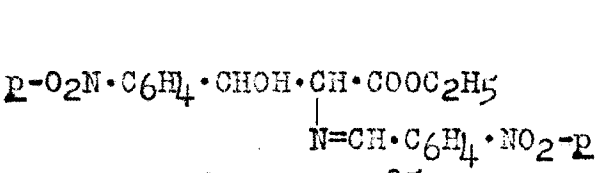
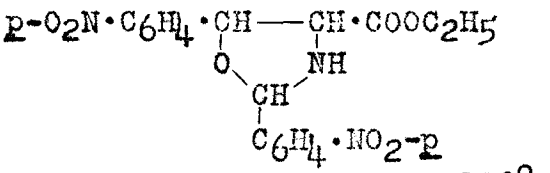
(1) C. E. Dalgliesh and F. G. Mann, J. Chem. Soc., 658 (1947).
(2) C. E. Dalgliesh, ibid., 90 (1949).

standing in presence of "molecular" sodium, compound A, considered to be a Schiff base, was isolated from the ether filtrate. This substance isomerized upon overnight storage to a possible oxazolidine structure, compound B (37% yield from



compound A, m.p. 85°



compound B, m.p. 139°

starting aldehyde). Refluxing B with dilute acetic acid decomposed it to p-nitrobenzaldehyde and glycine: by contrast, alcoholic hydrogen chloride at room temperature gave the expected p-nitrophenylserine ethyl ester hydrochloride, m.p. 185°, in 64% yield. This was saponified almost quantitatively by cold dilute alkali to p-nitrophenylserine, m.p. 188° (dec.), which was easily reduced by catalytic hydrogenation over palladium-charcoal to p-aminophenylserine, m.p. 205-207° (dec.). Both hydroxyamino acids were readily recrystallizable from water and gave strong ninhydrin reactions; the p-amino compound was described as being easily oxidized.

Attempts to condense p-nitrobenzaldehyde with glycine in aqueous alcohol under influence of sodium hydroxide were unsuccessful (1). Vigorous reaction occurred, with liberation

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(1) D. W. Woolley, J. Biol. Chem., 185, 293 (1950).

of ammonia and development of a dark red color. Acidification led to a product markedly low in nitrogen content, and which on working up, gave a small amount of still impure material with properties of an amino acid and high antibacterial activity. However, an R_F0.08 on paper chromatography in 0.1 N hydrochloric acid-phenol indicated p-nitrophenylserine (R_F0.68) was absent.

To obtain a compound of assured threo configuration, analogous to that of chloramphenicol, phenylserine was nitrated directly (1) by the procedure already found effective with phenylalanine (2). However, not only did the resultant monohydrated p-nitrophenylserine show the same melting range (188-195°) as the Dalgliesh (3) product (192-195°), but the absorption spectra curves were mutually superposable with identical maxima.

The view that an oxazolidine was not produced in condensation of p-nitrobenzaldehyde and glycine ethyl ester was expressed on the basis of infrared spectra determinations in which lines characteristic of a Schiff base were observed (4). However, which of the two Dalgliesh intermediates had been examined was not made clear.

An interesting new modification of the Erlenmeyer path

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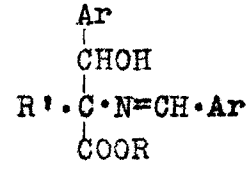
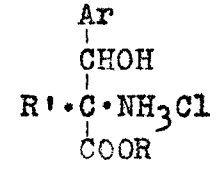
(1) D. Billet, Compt. rend., 230, 1358 (1950).
(2) E. Erlenmeyer, Sr., and A. Lipp, Ann., 219, 179 (1883).
(3) C. E. Dalgliesh, J. Chem. Soc., 90 (1949).
(4) E. D. Bergmann, M. Genas and H. Bendas, Compt. Rend., 231, 361 (1950).

to phenylserine was presented by Bergmann et al. (1). Quite surprisingly, condensation occurred when glycine esters and aromatic aldehydes in the correct proportions were merely dissolved in methanol or ethanol at room temperature, no other

Table 1

Products of Condensation Without Basic Catalyst

Substituents				
Ar	R	R'	M.p., °C.	M.p., °C.
Phenyl	methyl	hydrogen	-	185 (dec.)
p-Nitrophenyl	ethyl	hydrogen	148	190 (dec.)
p-Nitrophenyl	methyl	methyl	-	132
m-Nitrophenyl	methyl	hydrogen	-	131
p-Cyanophenyl	methyl	hydrogen	278 (dec.)	-
2,6-Dichloro-phenyl	methyl	hydrogen	128	198

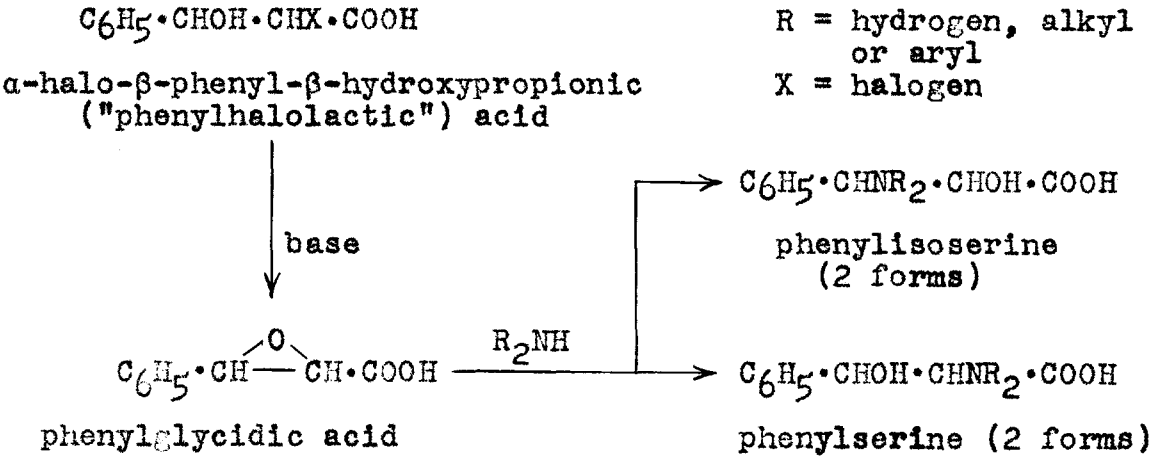
basic catalyst being required. Reaction was often manifested by a temperature rise. In some cases, intermediates precipitated out, which, upon careful hydrolysis with alcoholic hydrogen chloride, gave the ester hydrochlorides of the

(1) E. D. Bergmann, M. Genas, and H. Bendas, Compt. rend., 231, 361 (1950).

substituted phenylserines. Although neither analytical data nor procedural details were provided, the opinion was expressed that the various products were stereochemically alike. This method was significant in having led to an α-methylphenylserine derivative for the first time, presumably with alanine in place of glycine as a starting material.

2. Amination of phenylglycidic acid derivatives

The feasibility of preparing phenylserines by treating phenylglycidic acids with ammonia or amines has been investigated at periodic intervals. The value of studies made on this



approach has been diminished greatly by general use of isomerically impure starting materials. Consequently, the uncertainty was increased as to which of four possible racemic substances was produced. Furthermore, insufficient attention has been devoted to examination of products for presence of

more than a single isomer, or to rigid proof of structure.

Allophenylserine, reported as a minor product in glycine-benzaldehyde condensation, was also claimed to arise via the phenylglycidic acid route (1,2). While addition of ammonia in the cold to the sodium phenylglycidate derived from oily "phenylchlorolactic" acid gave only phenylisoserine, m.p. 220-221°, similar reaction starting from the solid "phenylchlorolactic" acid isomer yielded a new phenylisoserine diastereomer, m.p. 241°, as the main product, with recovery from the mother liquor of a compound apparently identical with allophenylserine. Surprisingly, repetition of the last reaction at higher temperatures produced only the phenylisoserine of m.p. 220-221°. Mere change of temperature thus influenced not only mode of epoxide ring opening, but also steric configuration of the product.

Addition of aniline, phenetidine, piperidine or phenylhydrazine to the two racemic sodium phenylglycidates and to one optically active form gave compounds which were formulated as phenylisoserine derivatives (2). By contrast, reaction of methylamine or dimethylamine with "phenylchlorolactic" acid supposedly led to substituted phenylserines (3). Treatment of various phenylglycidic esters with ammonia reportedly yielded

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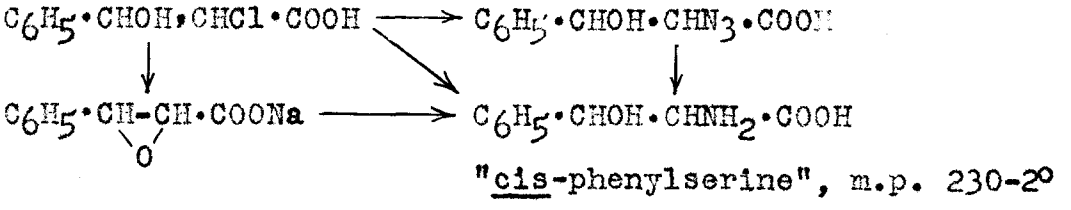
(1) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).
(2) E. Erlenmeyer, Jr., and C. Barkow, Ber., 39, 791 (1906).
(3) E. Fourneau, Bull. soc. chim. [4], 1, 549 (1907).

no product isolated in worthwhile quantity (1). Knoop clearly showed that "phenylchlorolactic" acid, or its acetyl derivative, reacted with methylamine to give in good yield not the anticipated N-methylphenylserine, but N-methylphenylisoserine (2). Phenylglycidic acid was suggested as a necessary intermediate. Proof of structure was afforded by acid permanganate oxidation to α-methylaminophenylacetic acid. Despite this indication of reaction course, this type of reaction was patented on the premise that nitrogen of the attacking amine became attached to the same carbon from which halogen was displaced (3).

Confusion over the course of the phenylglycidic route was heightened by Forster and Rao (4), who reported preparation of "cis-phenylserine" by ammonium sulfide reduction of "α-triazo-β-hydroxy-β-phenylpropionic" acid and by treatment of sodium phenylglycidate or cinnamic acid chlorhydrin with ammonia. The following reaction sequence (later proved erroneous) was presented:



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(1) K. W. Rosenmund and H. Dornsaft, Ber., 52, 1734 (1919): Arb. Pharm. Inst. Univ. Berlin, 12, 73 (1921).
(2) F. Knoop, Ber., 52, 2266 (1919).
(3) Etablissements Poulenc Frères. French Patent 532,465. Abstracted in C.A., 18, 989 (1924).
(4) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).

Synthesis of the corresponding methoxyamino acid in like fashion was also described. While several derivatives of each product were made, it was noteworthy that neither the free hydroxyamino acid nor its O-methyl analogue could be azlactonized, lack of such reaction being attributed to "cis" relationship of hydroxyl and amino groups within the molecule.

The structure of "cis-phenylserine" was formulated by Forster and Rao on the basis of questionable rationalization rather than experimental proof. Addition of hypohalous acids to cinnamic acid was taken to be unidirectional, effects of geoisomerism being neglected. Freedom of rotation around a single carbon-carbon bond was not considered. Conclusions as to reaction mechanism were based on faulty use of planar projection rather than three dimensional molecular models. The already contradictory reports in literature of the preceding two decades concerning the nature of products formed in phenylglycidic amination received no consideration.

The complaint by Forster and Rao that "Erlenmeyer's whole treatment of the subject is most bewildering" reflected gross errors in their interpretation of the paper by Erlenmeyer and Barkow (1), which they vigorously criticized. Erlenmeyer's use of individual "phenylchlorolactic" acid and sodium phenylglycidate isomers was overlooked. While their partial recognition of the effect of temperature on the

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(1) E. Erlenmeyer, Jr., and C. Barkow, Ber., 39, 791 (1906).

reaction course was apparent, formation of allophenylserine as a by-product was not mentioned. In one instance, an optically active phenylisoserine copper salt described by Erlenmeyer was taken to be a phenylserine compound. Such omissions were crystallized in the view (1) "we can only conclude that phenylisoserine (m.p. 220-221°) may be erased from the literature, the substance under that name being incompletely purified cis-phenylserine".

In repeating the work of Forster and Rao, Oesterlin (2) showed that since "cis-phenylserine" gave phenylglycine upon permanganate oxidation, it was in reality a β-amino acid, one of the two diastereomeric phenylisoserines, the second of which (m.p. 270-280°) Oesterlin reported he had also prepared for the first time. Phenylglycidic acid was considered a requisite intermediate in amination of "phenylhalolactic" acids. Accordingly, Fourneau's methylamino and dimethylamino hydroxy acids (3) were classified as phenylisoserine derivatives, but no oxidative proof of structure was mentioned for these particular substances. Without reference to Erlenmeyer's findings on the subject, Oesterlin stated that reaction always led to phenylisoserine derivatives, regardless of solvent or temperature, yet he described formation

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(1) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).
(2) M. Oesterlin, Metallbörse, 19, 1237 (1929).
(3) E. Fourneau, Bull. soc. chim., [4], 1, 549 (1907).

on one occasion from phenylglycidic acid and ammonia at 0° of an impure product which clearly contained an appreciable proportion of a phenylserine, judging from its behavior on permanganate oxidation. Lacking both analytical data and literature citations, Oesterlin's paper scarcely justified his concluding statement "so dass, jetzt sämtliche 4 Phenyloxyaminosäuren, entsprechend den beiden aliphatischen Serinen, bekannt sind".

In some instances, Oesterlin's work has escaped the attention of later writers, who have continued to refer to "cis-phenylserine" (1,2). Synthesis of compounds listed as phenylserine derivatives, by treatment of α-bromo-β-hydroxy-β-phenylpropionic acid or its p-nitro- analogue with various aliphatic amines, was described without structure proof or knowledge of earlier work being apparent (3).

The studies of Fourneau and Billeter (4) have partially resolved earlier contradictory findings in this area. It was reported that ammonia and primary aliphatic amines reacted with phenylglycidic ester in two well defined stages to give first of all phenylglycidic amides, then, by opening of the

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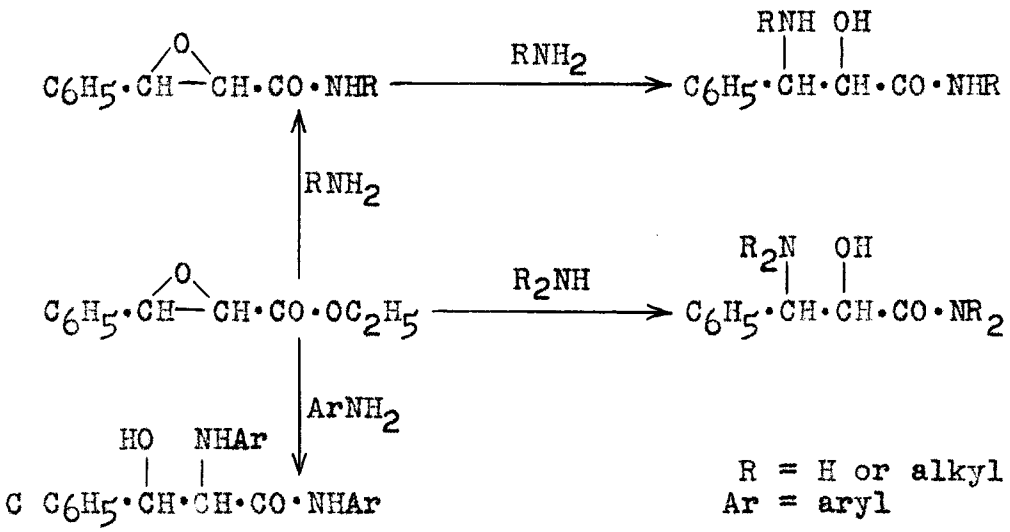
(1) H. D. Dakin, J. Biol. Chem., 140, 847 (1941).
(2) E. E. Howe, "Properties of Amino Acids", in Greenberg, ed., "Amino Acids and Proteins", Charles C. Thomas, Springfield, Illinois, c1951, p. 43.
(3) S. Bagchee, K. N. Gaind and J. N. Ray, J. Chem. Soc., 657 (1938).
(4) E. Fourneau and J. R. Billeter, Bull. soc. chim., [5], 7, 593 (1940).

ethylene oxide bridge, phenylisoserineamides. Secondary aliphatic amines behaved in similar fashion, but without an



observable intermediate stage. Aryl amines yielded N-substituted phenylserine esters, no amidation occurring. A number of N-arylated phenylserines prepared by this route are shown in Table 2.

Permanganate oxidation was found unsatisfactory as a method for distinguishing N-arylated phenylserines from phenylisoserines, both undergoing complete degradation of the side-chain. Replacement of hydroxyl with chlorine in amide derivatives, followed by hydrogenolysis, gave a phenylpropionamide, so that location of the amino group was not achieved. Attempted thermal decarboxylation proved no more indicative, deep-seated decomposition leading to phenylacetaldehyde with N-dimethylphenylisoserine, and

Table 2

N-Aryl Phenylserines

N-substituent	M.p., °C.	Ref.
Phenyl	156 (a), 158 (a), l-isomer 187 (a) ester 73-75, amide 206 methylamide 183	1 2 2
o-Tolyl	oil (b)	2
m-Tolyl	212-213	2
p-Tolyl	216-217	2
p-Ethoxyphenyl	187-188 (a), l-isomer 207 (a)	1
o-Methoxyphenyl	oil (b)	2
p-Methoxyphenyl	216-217	2
p-Carboxyphenyl	240 (b)	2
p-Acetamidophenyl	oil (b,c)	2
α-Naphthyl	unstable in air (b), amide 126	2
β-Naphthyl	204	2
Phenyl, methyl	oil (d), amide 171-172	2
6-Methoxy-8-quinolyl	120-130 (dec.) (b)	2
o-Aminophenyl	dihydrate 176 (dec.), ester 131 triacetate 189	3 3

(a) Originally described as phenylisoserines (1)
(b) Crystalline sodium salt prepared
(c) Deacetylated acid unstable in air
(d) Crystalline potassium salt

(1) E. Erlenmeyer, Jr., and C. Barkow, Ber., 39, 791 (1906).
(2) E. Fourneau and J. R. Billeter, Bull. soc. chim., [5], 7, 593 (1940).
(3) C. C. J. Culvenor, W. Davies, J. A. Maclaren, P. F. Nelson, W. E. Savige, J. Chem. Soc., 2573 (1949).

to acetophenone with N-phenylphenylserine. Phenylserine itself yielded benzaldehyde and methylamine, as well as carbon dioxide. Finally, constitution of one product in each series was demonstrated through catalytic reductive deamination to known compounds by refluxing with palladium in tetralin. It

$$C_6H_5 \cdot CHN(CH_3)_2 \cdot CHOH \cdot COOH \longrightarrow (CH_3)_2NH + C_6H_5 \cdot CH_2 \cdot CHOH \cdot COOH$$

$$C_6H_5 \cdot CHOH \cdot CHNHC_6H_5 \cdot COOH \longrightarrow C_6H_5 \cdot NH_2 + C_6H_5 \cdot CHOH \cdot CH_2 \cdot COOH$$

was presumed from the course of these reactions that other aliphatic aminations would give rise to N-alkylphenylisoserines, whereas aromatic amines would produce N-arylphenylserines.

The thought was apparently not entertained that amination of phenylglycidic compounds might actually involve parallel attack on both α- and β-carbon atoms, with formation of phenylserines and phenylisoserines in the same reaction, their proportion varying with reaction conditions. Nor was any attention paid to steric form of the starting phenylglycidic ester with its potential effect on production of diastereomers. The situation was aptly depicted by the statement (1), "Nos connaissances sur les acides phénylaminolactiques possibles sont encore fragmentaires, et la question mériterait une étude complète dont le présent travail n'est qu'une amorce".

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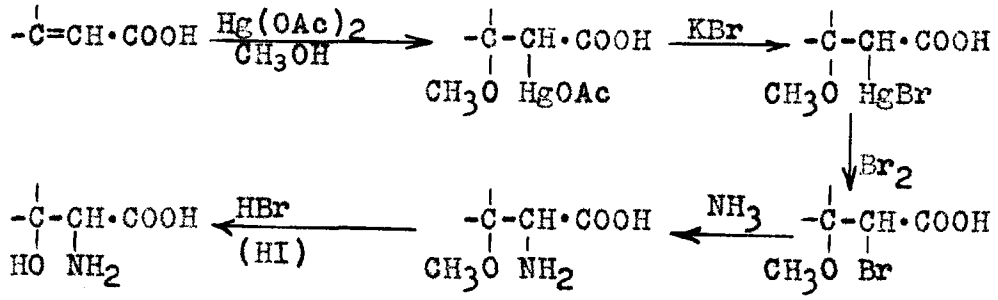
(1) E. Fourneau and J. R. Billeter, Bull. soc. chim., [5], I, 593 (1940).

Existing knowledge and uncertainty concerning the parent hydroxyamino acids was also summarized appropriately (loc. cit., p. 595):

> En résumé, on connaît d'une manière certaine l'une des phénylisosérines fondant à 230°, très probablement l'autre phénylisosérine fondant à 280°, et la phénylsérine fondant à 198-200°. Les phénylisosérines d'Erlenmeyer (F. 220° et 240°) sont vraisemblablement impures. Une autre phénylsérine d'Erlenmeyer, fondant à 189-190°, n'a pas été retrouvé ultérieurement.

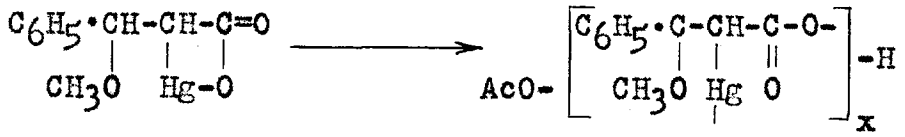
3. Mercuration of cinnamic acid derivatives

The apparent intermediacy of phenylglycidic acid structures in amination of "phenylhalolactic" acid derivatives, with consequent possible formation of phenylisoserines, as well as the desired phenylserines, has been noted. Procedural variations have been sought which would avoid the olefin oxide ring closure involved and resultant uncertainty over nature of the products. A promising approach to this end was based on a sequence of known reactions: α-mercuration of a suitable α,β-olefinic acid in alcoholic solution, replacement of mercury by halogen, amination, and finally cleavage of the ether group with hydrobromic or hydriodic acid.



Although successfully employed for preparation of aliphatic α-amino-β-hydroxy acids, this method proved unsatisfactory for aromatic analogues. Methyl cinnamate was easily converted to the corresponding α-bromo-β-methoxy ester, but hydrolysis of the latter to the parent acid was attended by troublesome side reactions (1). An isomeric α-bromo-β-methoxy acid, obtained with free cinnamic acid as the starting material, was aminated without difficulty, but cleavage of the product with hydrogen bromide or iodide was reported to give only cinnamic and bromohydrocinnamic acids, instead of a phenylserine. Additional interfering effects were encountered when p-methoxycinnamic ethyl ester was used in the sequence. Demercuration with bromine was accompanied by nuclear substitution. Although ring attack was avoided by use of iodine, the α-iodo acid obtained could not be reproducibly aminated.

Some discrepancies in the work of Schrauth and Geller were revealed by Van Loon and Carter (2). The initial mercuration reaction was shown to produce a cyclic mercuri-anhydride structure which was slowly transformed to a linear polymer.



(1) W. Schrauth and H. Geller, Ber., 55, 2783 (1922).
(2) E. J. Van Loon and H. E. Carter, J. Am. Chem. Soc., 59, 2555 (1937).

Regardless of which mercury adduct was brominated, mixtures of the two stereoisomeric α-bromo-β-methoxy acids were formed rather than a single isomer, although their proportion varied somewhat according to temperature and type of illumination used. Partial separation was effected on the basis of solubility differences in aqueous sodium carbonate. Both crude products were successfully aminated to give the two O-methylphenylserines (1). The less soluble isomer B, m.p. 253-254° (dec.), was obtained directly; isomer A, m.p. 231-233° (dec.), required extensive purification via its N-carbobenzoxy derivative (2). When these methoxyamino acids were treated with hydrogen bromide, β-phenylnaphthalene was produced, instead of either of the phenylserines (for details, see p. 40).

4. Reduction of oximino compounds

Synthesis of "erythro-β-phenylserine" was recently described, starting with α-oximinobenzoylacetic acid (3). This compound was hydrogenated in a reportedly stereospecific manner over platinum oxide in mixed hydrochloric-acetic acids

$$C_6H_5\cdot CO\cdot \underset{\underset{NOH}{\|}}{C}\cdot COOC_2H_5 \longrightarrow C_6H_5\cdot \underset{OH}{CH}\cdot \underset{NH_2\cdot HCl}{CH}\cdot COOC_2H_5 \longrightarrow C_6H_5\cdot \underset{OH}{CH}\cdot \underset{NH_2}{CH}\cdot COOH$$

(1) H. E. Carter and E. J. Van Loon, J. Am. Chem. Soc., 60, 1077 (1938).
(2) H. E. Carter and W. C. Risser, J. Biol. Chem., 139, 255 (1941).
(3) I. Elphimoff-Felkin and H. Felkin, Compt. rend., 232, 241 (1951).

at room temperature. The resultant ester hydrochloride, m.p. 186°, was converted by potassium carbonate solution to the free ester, m.p. 81-83°, which decomposed on standing with formation of benzaldehyde. The hydroxyamino acid, obtained by cold hydrolysis of the ester hydrochloride with alcoholic potassium hydroxide, was reported to melt at 260°, and to form a hydrochloride, m.p. 212°. Satisfactory identification was considered to have been achieved when it was found that heating of "erythro-β-phenylserine" with benzoyl chloride gave the same azlactone as arose from phenylserine similarly treated. An N,O-diacetyl ester, m.p. 126-127°, from action of hot acetic anhydride on the ester hydrochloride in presence of sodium acetate, and an N,O-dibenzoyl compound, m.p. 167-168°, were also mentioned. Derivatives of the erythrophenylserine were stated to be generally less stable than those of the well-known phenylserine. No experimental evidence for purity of the various compounds prepared was provided.

This route to the phenylserines has also attracted the attention of other investigators (1,2). However, data concerning efficiency of the various synthetic steps and the ratio of diastereomeric phenylserines formed have not yet become available.

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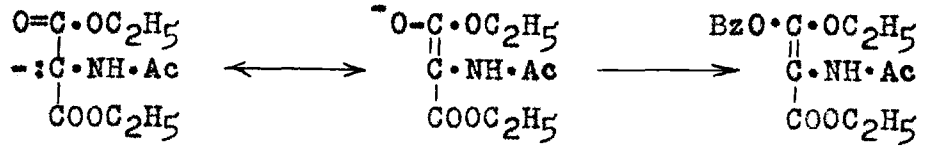
(1) W. G. Clark, T. A. Geissman and R. I. Akawie. Private communication, 1950.
(2) A. M. Mattocks and W. H. Hartung, J. Am. Pharm. Assn., 35, 18 (1946).
W. H. Hartung and Chang. Private communication, 1951.

5. Other methods

In an effort to circumvent low yields of the Roesnmund-Dornsaft approach, Dalgliesh tried to find entirely new paths to the phenylserines (1). Attempts to utilize the Erlenmeyer-Plöchl method of amino acid synthesis, with a view to effecting suitable addition reactions to the double bond of the product, were fruitless. Reactants tested unsuccessfully included sarcosine, its benzenesulfonyl derivative or its anhydride with veratraldehyde. Veratraldehyde condensed as anticipated with glycine anhydride and with creatinine, likewise 3,4-carbonyldioxybenzaldehyde with N-methylhydantoin, but addition to the resultant olefinic link could not be achieved.

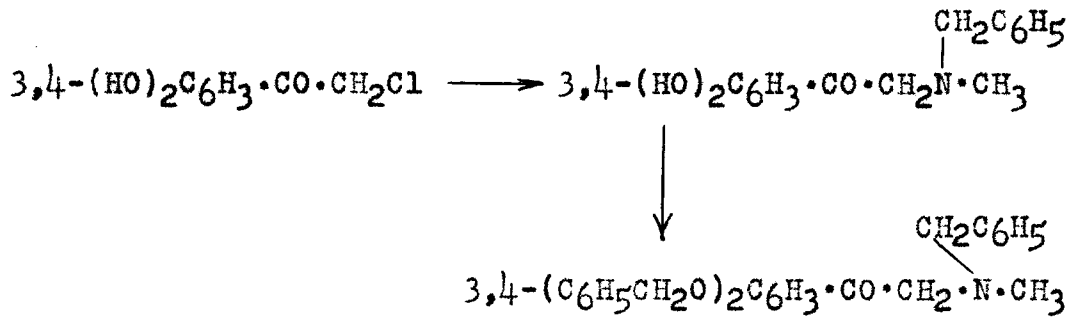
The possibility of a synthesis based on acetamidomalonic ester was also investigated, this compound having given good results with other amino acids. Although the reactants yielded no worthwhile product in organic media, benzoyl chloride reacted directly with the free sodio derivative to form a benzoylacetamidomalonic ester. That this was probably



(1) C. E. Dalgliesh, J. Chem. Soc., 90 (1949).

the unwanted O-benzoyl compound followed from lack of carbonyl reactivity and facile hydrolytic or hydrogenolytic cleavage to acetamidomalonic ester.

A promising route based on known adrenaline syntheses likewise proved abortive. Benzylmethylamine and 3,4-dihydroxyphenacyl chloride gave N-benzyladrenalone in high yield as a monoethanolate. This was converted to the gummy tribenzyladrenalone, isolated as a glassy hydrochloride and picrate, and as a crystalline oxalate diethanolate.



Bromination of tribenzyladrenalone gave an uncrystallized resin from which no crystalline product could be obtained after boiling with potassium cyanide.

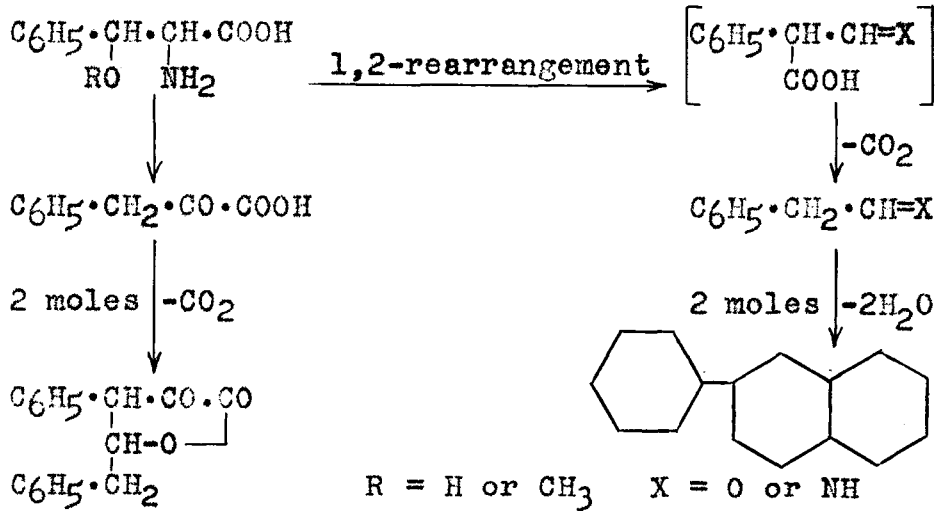
Dalgliesh remarked that the Strecker type synthesis appeared to be inapplicable to the phenylserines. The

requisite starting phenylglycolaldehyde was known to rearrange with ease to α-hydroxyacetophenone.

B. Chemical Reactions

1. Acid cleavage

Although it was known earlier that phenylserine (1) and p-methoxyphenylserine (2) were decomposed by strong acids, the first systematic study of cleavage products was made by Bettzieche (3). Ammonia, carbon dioxide, phenylacetaldehyde, phenylpyruvic acid and β-phenylnaphthalene were formed on refluxing phenylserine with 10% sulfuric acid, the last product in up to 65% yield when reaction was effected in a bomb at 160-170°. A reasonable reaction course was suggested by Carter and Van Loon (4), who investigated action of both



(1) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).
(2) K. W. Rosenmund and H. Dornsaft, Ber., 52, 1734 (1919): Arb. Pharm. Inst. Univ. Berlin, 12, 73 (1921).
(3) F. Bettzieche, Z. physiol. Chem., 150, 177 (1925).
(4) H. E. Carter and E. J. Van Loon, J. Am. Chem. Soc., 60, 1077 (1938).

sulfuric and hydrobromic acids on phenylserine and its two O-methyl derivatives. Isolation in small amounts of a new product, α-keto-β,δ-diphenyl-γ-valerolactone, the only one detectable on acid treatment of phenylpyruvic acid, showed that the latter was not a precursor of phenylacetaldehyde, as might otherwise be assumed, and that two distinct decomposition paths were involved, with the one leading to β-phenylnaphthalene preeminent.

Indication that the above sequence was not the only path of acid decomposition was recently provided (1). After refluxing phenylserine with glacial acetic acid for thirty minutes, 63% recovery of glycine was effected. No mention was made of other cleavage products simultaneously formed.

The hydrolysis of N-acetyl-p-nitrophenylserine has been effected by overnight refluxing with 6 N hydrochloric acid (2). A 71% yield of crude p-nitrophenylserine indicated that scission of the hydroxyamino acid did not occur to any appreciable extent. Whether this result was due to stabilization by the nuclear nitro group, or to the nature of the acid and reaction conditions employed was not investigated.

2. Alkaline cleavage

The reaction of phenylserine and its derivatives with

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(1) G. Weitnauer, Gazz. chim. ital., 81, 156 (1951).
(2) D. W. Woolley, J. Biol. Chem., 185, 293 (1950).

alkali has been studied in some detail. High recoveries of benzaldehyde and glycine were achieved when phenylserine was steam distilled with 10% sodium hydroxide (1). If escape of benzaldehyde was prevented by use of reflux conditions, N-benzalisodiphenylhydroxyethylamine, moderate amounts of oxalic acid, and traces of benzylamine were formed by further interaction of the two initial cleavage products. These findings were aptly in accord with Erlenmeyer's original reaction sequence for condensation of benzaldehyde with glycine (see p. 7).

Although free phenylserine was rapidly decomposed by hot sodium hydroxide, the N-tosylated derivative proved relatively stable. Ammonia, benzylamine, isodiphenylhydroxyethylamine, benzaldehyde, benzyl alcohol, p-toluenesulfonic, benzoic and phenylpyruvic acids, tosylglycine, glycine and unchanged N-tosylphenylserine were demonstrated in small amounts only after more drastic treatment in a bomb for five hours at 200° (2). Steam distillation of N-benzoylphenylserine with 8% sodium hydroxide gave only benzoic acid, benzaldehyde and glycine as well as unchanged starting material, whereas N-hippurylphenylserine boiled with 15% sodium hydroxide also yielded traces of hippuric acid.

Phenylserine phenylhydantoin and its derivatives were

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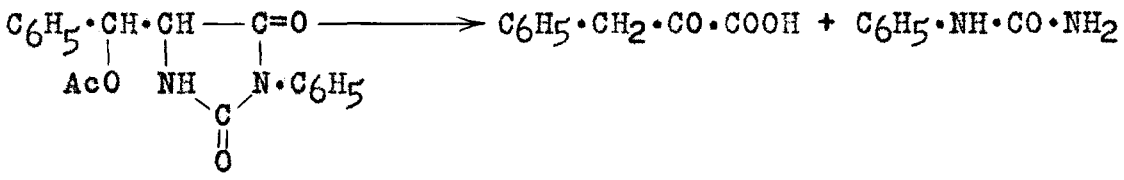
(1) F. Bettzieche, Z. physiol. Chem., 150, 177 (1925).
(2) F. Bettzieche and R. Menger, ibid., 172, 56 (1927).

decomposed in comparable fashion by 4 N sodium hydroxide at 70° (1). Only small amounts of benzaldehyde were obtained from



saturated phenylhydantoin, whereas from its O-acetyl derivative or the related benzalphenylhydantoin were recovered 80% of the calculated quantity of phenylurea and 70% of phenylpyruvic acid.

3. Direct esterification

The scant attention given in early work to esterification of phenylserines was reflected in brief mention of only the ethyl ester hydrochloride, which was not characterized with certainty (2,3,4). Interest in phenylserine derivatives which could be used for chloramphenicol synthesis has led recently to preparation of several esters and their salts. In general, the well-known Fischer technique, with minor

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(1) M. Bergmann and D. Delis, Ann., 458, 76 (1927)
(2) E. Abderhalden and S. Buadze, Fermentforschung, 8, 487 (1926).
(3) F. Bettzieche and R. Menger, Z. physiol. Chem., 172, 64 (1927).
(4) D. G. Doherty, J. E. Tietzman and M. Bergmann, J. Biol. Chem., 147, 617 (1943).

variations, has been employed to secure the ester hydrochlorides, which were isolated and characterized in some cases. In other instances, the ester hydrochlorides were converted by mild alkaline treatment directly to the free esters (Table 3). Without specifying method of resolution, Vogler (1) has described conversion of L-phenylserine, m.p. 183-186° (dec.) ($[\alpha]_D^{20}$ = -50.2 ± 2°, c = 2 in 6 N hydrochloric acid, -32 ± 2° in water), to L-phenylserine ethyl ester hydrochloride, m.p. 88-90° ($[\alpha]_D^{18}$ = -30.2 ± 2°, c = 2.009 in water), in 90% yield. This was treated with ammonium hydroxide to obtain an 83% yield of the free L-ester, m.p. 62-63° ($[\alpha]_D^{18}$ = + 15.6 ± 1°, c = 2.109 in methanol).

4. Azlactonization

Azlactonization of phenylserine by heating with acetic or benzoic anhydrides was effected by numerous investigators after Erlenmeyer. Reaction of the amino acid with acetic anhydride to give a 75% yield of α-acetaminocinnamic acid azlactone, m.p. 151°, was found to require twenty-four hours at room temperature, as opposed to fifteen minutes at 70° (2). The same compound was obtained when phenylserine was heated with acetic anhydride and pyridine, under which treatment

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(1) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).
(2) M. Bergmann and D. Delis, Ann., 458, 76 (1927).

Table 3

Phenylserine Esters and Ester Hydrochlorides

Ester	Hydrochloride		Free Base		Ref.
	% Yield	M.p., °C.	% Yield(a)	M.p., °C.	
Methyl	-	156	75	62	(1)
Ethyl	-	137-139	-	-	(2)
	-	-	86	84 (b)	(3)
	-	138 (c)	73	86	(1)
	91	136-137	-	-	(4)
n-Propyl	-	129	72	57	(1)
n-Butyl	-	116	81	52	(1)
n-Dodecyl	-	110	67	50	(1)

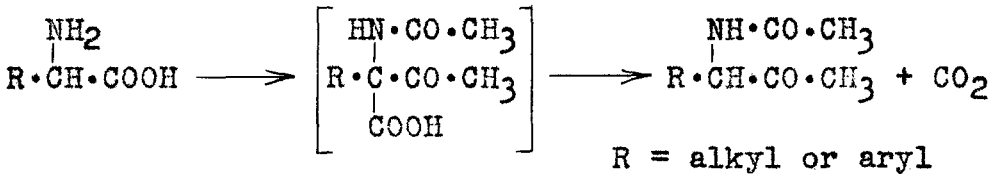
(a) Based on starting phenylserine

(b) Acid oxalate, m.p. 136°

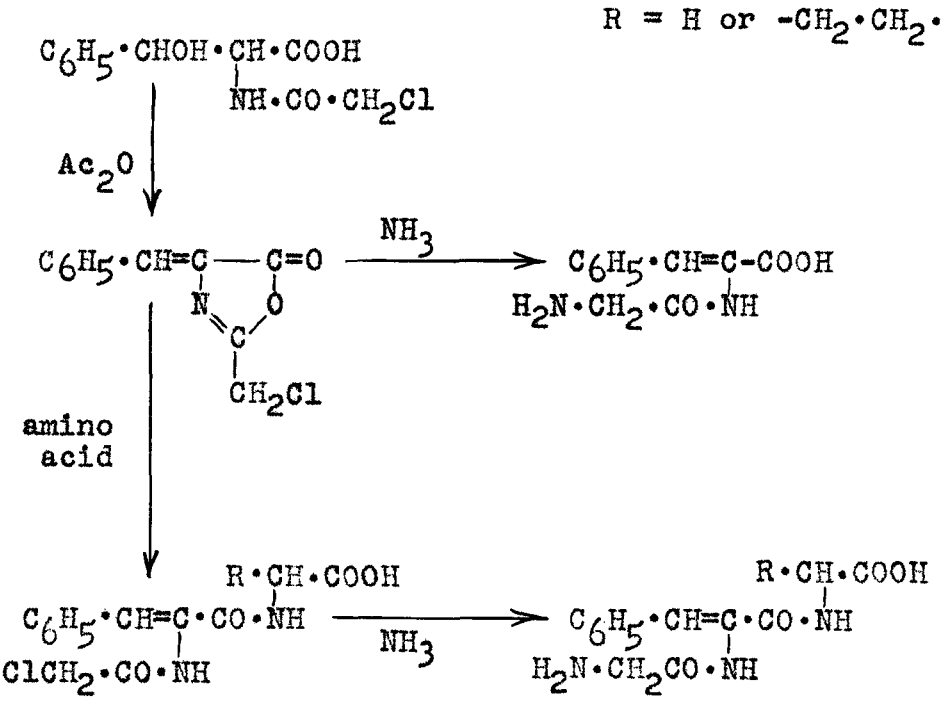
(c) Initial formation of phenylserine hydrochloride, m.p. 157°, was noted.

(1) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).
(2) D. G. Doherty, J. E. Tietzman and M. Bergmann, J. Biol. Chem., 147, 617 (1943).
(3) C. G. Alberti, B. Asero, B. Camerino, R. Sannicolò and A. Vercellone, Chimica e industria (Milan), 31, 357 (1949).
(4) K. Hayes and G. Gever, J. Org. Chem., 16, 269 (1951).

other α-amino acids were converted to α-acetamino ketones (1).



Heating N-chloroacetylphenylserine with acetic anhydride produced chloroacetaminocinnamic acid azlactone, m.p. 114°, which was of use for synthesizing various dehydropeptides (2).



The problem of geoisomerism in azlactonization of phenylserines received no attention prior to the work of Carter and

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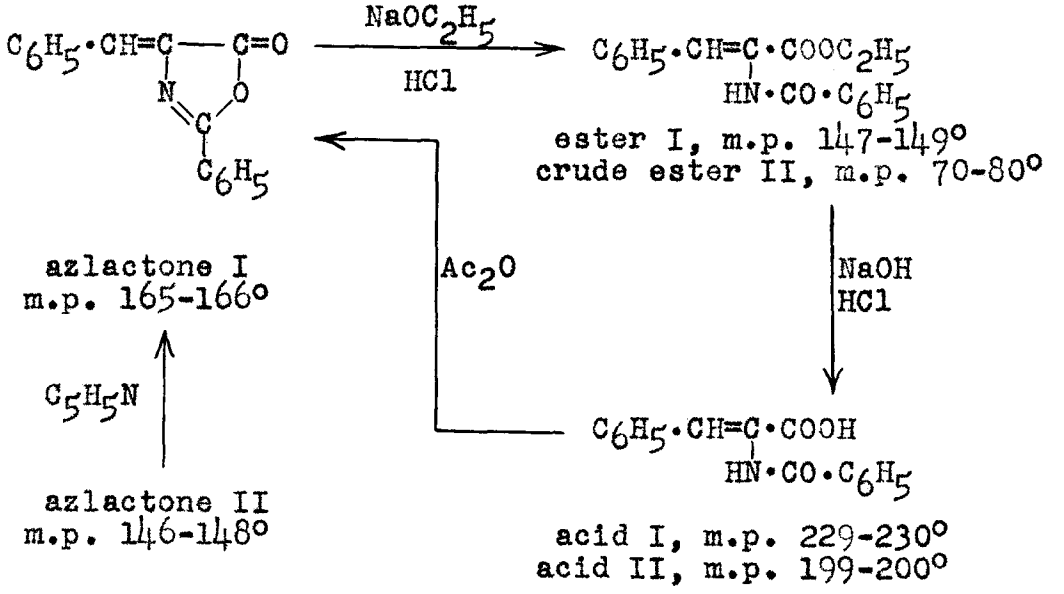
(1) H. D. Dakin and R. West, J. Biol. Chem., 78, 745 (1928).
(2) M. Bergmann, V. Schmitt and A. Miekeley, Z. physiol. Chem., 187, 264 (1930).

Risser (1). Regardless of which O-methylphenylserine was heated with acetic anhydride (fifteen minutes at 100°), the product crystallizing out on cooling consisted of the well-known α-benzamidocinnamic acid azlactone I. A new less stable isomeric azlactone II was obtained in crude state upon dilution of the mother liquor with ice water, and was purified via the corresponding α-benzamidocinnamic acid II.



Only azlactone I was produced when benzoyl chloride or acetic anhydride plus pyridine was used for cyclization, or in the event of prolonged heating.

Acetic anhydride treatment has been used to synthesize other azlactones from phenylserine derivatives (Table 4). Azlactone formation has served as the basis of a qualitative

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(1) H. E. Carter and W. C. Risser, J. Biol. Chem., 139, 255 (1941).

Table 4

Azlactones from Acylated Phenylserine Peptides

Azlactones	M.p., °C.	Ref.
Carbobenzoxyglycyldehydrophenylalanine	141-142	(1)
Benzoyldehydrophenylalanyldehydrophenylalanine	189-190	(1)
Acetyldehydrophenylalanyldehydrophenylalanine	184-186 (a) 191-193 (dec.)	(1)
Acetylbis(dehydrophenylalanyl)-dehydrophenylalanine	233-235 (dec.)	(1)
Acetyltris(dehydrophenylalanyl)-dehydrophenylalanine	247-249 (dec.)	(1)
α-Chloropropionyldehydrophenylalanine	syrup	(2)

(a) Geoisomers

(1) D. G. Doherty, J. E. Tietzman and M. Bergmann, J. Biol. Chem., 147, 617 (1943).
(2) J. P. Greenstein, V. E. Price and F. M. Leuthardt, J. Biol. Chem., 175, 953 (1948).

test for β-hydroxy-α-amino acids (1). The sample was heated briefly with benzoic anhydride, the product dissolved in aqueous alcohol and treated dropwise with 0.5% aqueous permanganate. Instant decolorization constituted a positive test.

5. N-Substitution: peptide synthesis

N-Acyl derivatives were effectively employed to separate the diastereomeric O-methylphenylserines (2,3). Reversal of isomer solubility order was encountered in passing from the

Table 5

N-Acyl-O-methylphenylserines

Acyl Group	Steric Form (a)	Free Acid M.p., °C.	β-Phenylethylamine salt M.p., °C.
N-Benzoyl	A	166-167	184-188
N-Benzoyl	B	220-222	169-171
N-Carbobenzoxy	A	103-105	132-135
N-Carbobenzoxy	B	140-142	80-86

(a) Arbitrarily designated diastereomers, relative configuration unknown.

(1) M. M. Botvinnik, G. Ya. Gaukhman and I. S. Severin, Doklady Akad. Nauk. S.S.S.R., 63, 269 (1948). Original not seen. Abstracted in C.A., 43, 2124 (1949).
(2) H. E. Carter and E. J. Van Loon, J. Am. Chem. Soc., 60, 1077 (1938).
(3) H. E. Carter and W. C. Risser, J. Biol. Chem., 139, 255 (1941).

acylated acids to the corresponding phenylethylamine salts. Since the same phenomenon was observed with N-acylated O-methylthreonines, threonines and α-amino-β-thiol-n-butyric acids, it appeared that phenylethylamine might be generally applicable in separation and purification of such isomeric pairs.

Numerous N-acyl derivatives of phenylserine have been synthesized (Table 6), in many cases by use of the Schotten-Baumann technique. The influence of acylation conditions on the nature of the product was reflected in early literature. Benzoylation of phenylserine in sodium carbonate solution was stated to give predominantly α-benzamidocinnamic acid azlactone, along with a small quantity of material, m.p. 160°, which may have been N-benzoylphenylserine (1). Attempts to prepare N,O-dibenzoylphenylserine, by treating the N-benzoyl compound with benzoyl chloride in sodium bicarbonate solution, were fruitless, only the azlactone being formed in minor amounts (2).

Uncertainty as to reaction course in acylation of phenylserine has been eliminated in part by recent studies of Weitnauer (3). Only a single product, the yellow α-acetamidocinnamic acid azlactone, m.p. 153°, was obtained when acetic anydride was used under anhydrous conditions, regardless of

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(1) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).
(2) F. Bettzieche and R. Menger, Z. physiol. Chem., 172, 56 (1927).
(3) G. Weitnauer, Gazz. chim. ital., 81, 156 (1951).

Table 6

N-Acyl Derivatives of Phenylserine

Acyl Group	% Yield	M.p., °C.	Ref.
N-p-Toluenesulfonyl	88	191-192	(1)
N-α-Bromoisocaproyl	75	115-120	(2)
N-Chloroacetyl	85	155-157	(2)
	-	167	(3)
N-α-Chloropropionyl	25	161	(4)
N-Benzoyl	65	158	(5)
	-	156	(6)
	-	160-161	(7)
N-Acetyl	65	-	(6)
	-	152	(7)
N,O-Diacetyl	-	141-142	(7)
N-Dichloroacetyl	37	170	(8)
	73	164	(6)

(1) F. Bettzieche, Z. physiol. Chem., 150, 177 (1925).
(2) E. Abderhalden and S. Buadze, Fermentforschung, 8, 487 (1926).
(3) M. Bergmann, V. Schmitt and A. Miekeley, Z. physiol. Chem., 187, 264 (1930).
(4) J. P. Greenstein, V. E. Price and F. M.Leuthardt, J. Biol. Chem., 175, 953 (1948).
(5) F. Bettzieche and R. Menger, Z. physiol. Chem., 172, 56 (1927).
(6) D. W. Woolley, J. Biol. Chem., 185, 293 (1950).
(7) G. Weitnauer, Gazz. chim. ital., 81, 156 (1951).
(8) D. Billet, Compt. rend., 231, 295 (1950).

temperature or whether pyridine or other anhydrous solvents were present. In aqueous solution under Schotten-Baumann conditions, a white substance was produced in small yield (1,2). Despite the fact that this material displayed the same chemical and physical properties, including melting point alone and mixed, as the azlactone, it was formulated as a "lactimide", although no evidence was advanced to support this obsolete structure. From earlier preparation of the azlactone in colorless form under anhydrous conditions (3), and from easy decolorization of the yellow form by recrystallization from methanol, Alberti and Vercellone have concluded that the "lactimide" and the azlactone are identical (4). Treatment of phenylserine in aqueous medium with acetic anhydride under alkaline, neutral or acid conditions gave, as well as the "lactimide", a mixture of N-acetyl and N,O-diacetyl derivatives in good yield, the relative proportion varying with conditions (1). An interesting exemplary procedure described formation of 51 grams of crude acetyl compound, m.p. 138-140°, from addition of acetic anhydride to a boiling aqueous solution of 50 grams of the hydroxyamino acid, the monosubstituted product crystallizing readily from water and the diacetyl

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(1) G. Weitnauer, Gazz. chim. ital., 81, 156 (1951).
(2) G. Carrara and G. Weitnauer, Ibid., 79, 856 (1949).
(3) M. Bergmann and D. Delis, Ann., 458, 76 (1927).
(4) C. G. Alberti and A. Vercellone, Chimica e industria (Milan), 33, 359 (1951).

derivative being worked up from the mother liquor through dioxane-ether. When phenylserine was refluxed with glacial acetic acid, decomposition occurred.

Benzoylation proceeded along similar lines. Only α-benzamidocinnamic acid azlactone, m.p. 167-168°, was produced by action of benzoyl chloride on phenylserine in presence of pyridine. Schotten-Baumann conditions gave almost exclusively N-benzoylphenylserine. The N,O-dibenzoyl compound was reportedly obtained in an impure state, but procedural details were lacking. Weitnauer concluded that in strongly alkaline media monoacylation prevailed, whereas mostly diacyl derivatives formed under weakly alkaline, neutral or acidic conditions.

N-Acylphenylserines are of some interest as a potential source of oxazolones, which would be of use in effecting epimerization, i.e. proceeding from the phenylserine to the allophenylserine series. Oxazolone formation was not noted in treatment of N-benzoylphenylserine in ether with thionyl chloride, only the unsaturated azlactone being produced. Similar negative results were reported in another investigation (1). In this case, thionyl chloride acting on L-N-acetylphenylserine ethyl ester was believed to give chiefly the corresponding β-chloro compound.

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(1) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).

Various acyl derivatives of phenylserine ethyl ester (Table 7) have been prepared. These were of interest both as synthetic intermediates and as compounds of possible chemotherapeutic value.

Particular attention has been given to N-dichloroacetyl-p-nitrophenylserine, which differs from chloramphenicol only in having a terminal carboxyl instead of a hydroxymethyl group. The same product resulted from Schotten-Baumann dichloroacetylation of p-nitrophenylserine, regardless of whether the latter was obtained by direct nitration of phenylserine, or by the Rosenmund-Dornsaft route (1). Direct

$$p\text{-}O_2N\cdot C_6H_4\cdot CHOH\cdot CHNH_2\cdot COOH \longrightarrow p\text{-}O_2N\cdot C_6H_4\cdot CHOH\cdot \underset{\displaystyle NH\cdot CO\cdot CHCl_2}{\underset{|}{CH}}\cdot COOH$$

m.p. 176°: 27% yield

nitration of N-dichloroacetylphenylserine gave only amorphous products (2). Better results were reported from cold fuming

$$C_6H_5\cdot CHOH\cdot \underset{\displaystyle NH\cdot Ac}{\underset{|}{CH}}\cdot COOH \longrightarrow p\text{-}O_2N\cdot C_6H_4\cdot CHOH\cdot \underset{\displaystyle NH\cdot Ac}{\underset{|}{CH}}\cdot COOH$$

m.p. 186-187°: 62% yield

$$p\text{-}O_2N\cdot C_6H_4\cdot CHOH\cdot \underset{\displaystyle NH\cdot CO\cdot CHCl_2}{\underset{|}{CH}}\cdot COOH \longleftarrow p\text{-}O_2N\cdot C_6H_4\cdot CHOH\cdot \underset{\displaystyle NH_2}{\underset{|}{CH}}\cdot COOH$$

m.p. 158-162° : ?% yield m.p. ? : 71% yield

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(1) D. Billet, Compt. rend., 231, 293 (1950).
(2) D. W. Woolley, J. Biol. Chem., 185, 293 (1950).

Table 7

Phenylserine Ethyl Ester Acyl Derivatives

Acyl Group	% Yield	M.p., °C.	Method	Ref.
L-N-Acetyl (a)	86	120-122	Ac_2O, HOAc, room temp.	(1)
N,O-Diacetyl (b)	-	168-169	unspecified	(2)
	91	169-170	Ac_2O, heat or C_5H_5N	(3)
N-Dichloroacetyl	96	153-154	acid chloride, ether	(4)
	86	149-150	Schotten-Baumann	(5)
	65	150	methyl dichloroacetate	(6)
N-Dichloroacetyl-O-acetyl	54	186-187	Ac_2O, C_5H_5N, reflux	(4)
	80	183-185	Ac_2O, C_5H_5N, room temp.	(5)
N-p-Nitrobenzoyl	71	119	Schotten-Baumann, Me_2CO	(6)
N-p-Aminobenzoyl	76	195	nitro compound reduction	(6)
N-p-Toluenesulfonyl	54	103-104	Schotten-Baumann, Me_2CO	(6)
N-3,5-Dinitrobenzoyl	84	149	Schotten-Baumann, Me_2CO	(6)
N-p-Chlorobenzoyl	55	106-107	Schotten-Baumann, Me_2CO	(6)
N-p-Methoxybenzoyl	75	131	Schotten-Baumann, Me_2CO	(6)
N-2-Chlorocinchoninyl	67	178	Schotten-Baumann, Me_2CO	(6)

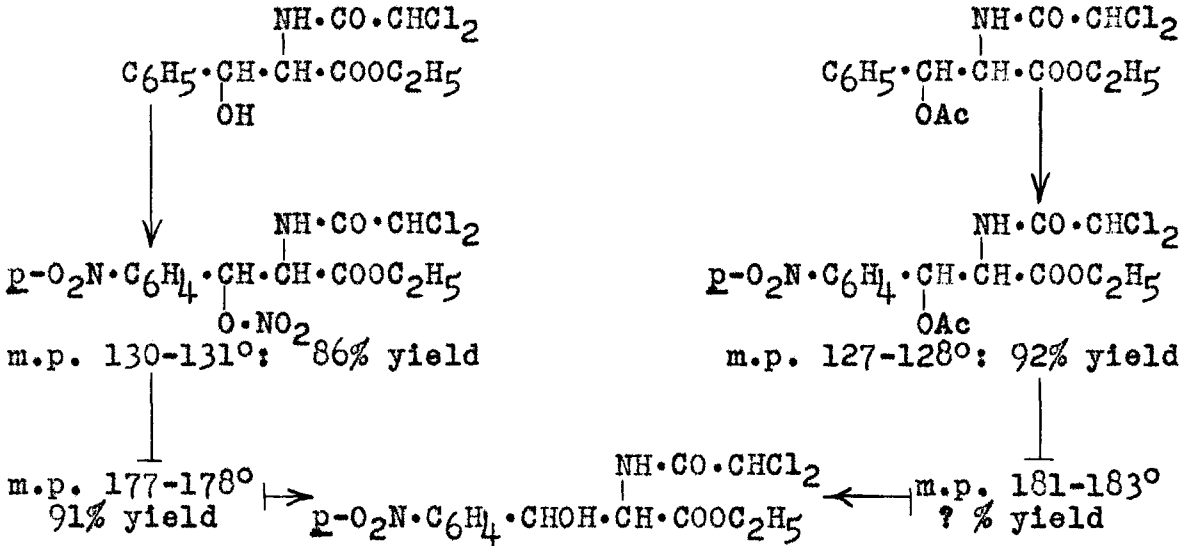
(a) $[\alpha]_D^{20} = 0 \pm 1°$ (c = 2.006 in methanol)

(b) Fuming nitric acid gave N,O-diacetyl-p-nitrophenylserine, m.p. 122-124° (2).

(1) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).
(2) C. G. Alberti, B. Asero, B. Camerino, R. Sannicolò and A. Vercellone, Chimica e industria (Milan), 31, 357 (1949).
(3) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).
(4) G. Carrara, F. M. Chiancone, V. D'Amato, E. Ginouhliac, C. Martinuzzi and G. Weitnauer, ibid., 80, 709 (1950).
(5) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).
(6) J. Büchi, S. Contini and R. Lieberherr, Helv. Chim. Acta, 34, 274 (1951).

nitric acid treatment of crude N-acetylphenylserine, hydrolysis of the nitro product by refluxing with 6 N hydrochloric acid and final Schotten-Baumann dichloroacetylation.

An alternative approach has involved ethyl ester derivatives. N-dichloroacetylphenylserine ethyl ester with fuming



nitric acid at -30° underwent simultaneous ring and side-chain nitration, hydrolysis according to the selective Kunz technique being used to remove the β-nitrate radical to give N-dichloroacetyl-p-nitrophenylserine ethyl ester (1). To demonstrate that no epimerization had occurred, this compound was also prepared by similarly nitrating N-dichloroacetyl-O-acetyl-p-nitrophenylserine ethyl ester, with Kunz hydrolysis as before

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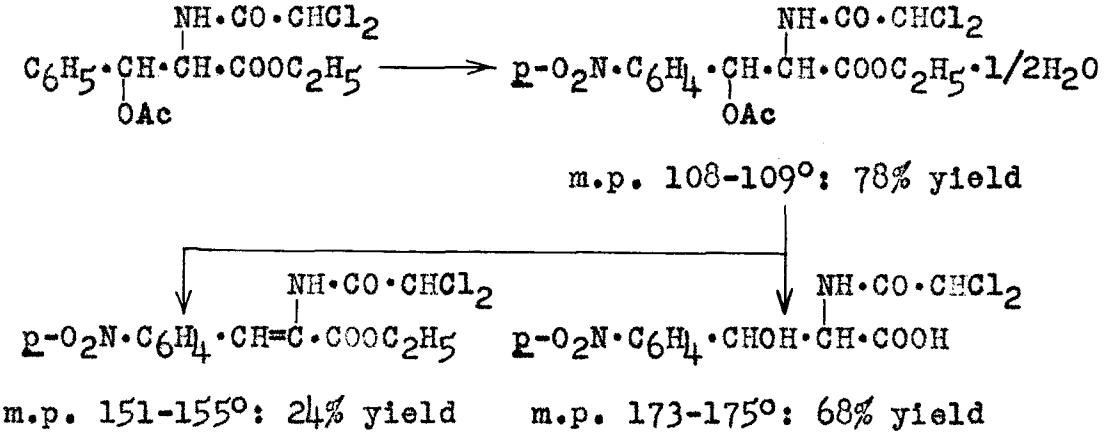
(1) G. Carrara, F. M. Chiancone, V. D'Amato, E. Ginouhliac, C. Martinuzzi and G. Weitnauer, Gazz. chim. ital., 80, 709 (1950).

to cleave the O-acetyl group. Somewhat different conditions were employed in another study (1). N-Dichloroacetyl-O-acetylphenylserine ethyl ester was nitrated at 0° with fuming nitric-sulfuric acids to give a hemihydrated product, which, upon hydrolysis with cold 1 N methanolic sodium hydroxide, yielded not only the desired N-dichloroacetyl-p-nitrophenylserine, but also α-dichloroacetamido-p-nitrocinnamic acid ethyl ester, the latter with interesting acidic properties,



due to conjugative effects. Attempts to effect acid hydrolysis of N-dichloroacetyl-p-nitrophenylserine were unsuccessful. Huebner and Scholz attributed the differences which they observed between their N-dichloroacetyl-p-nitrophenylserine and Woolley's preparation, in respect to crystal form and melting point (mixed m.p. 158-165°), to the presence of impurities in the latter instance.

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(1) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).

Table 8

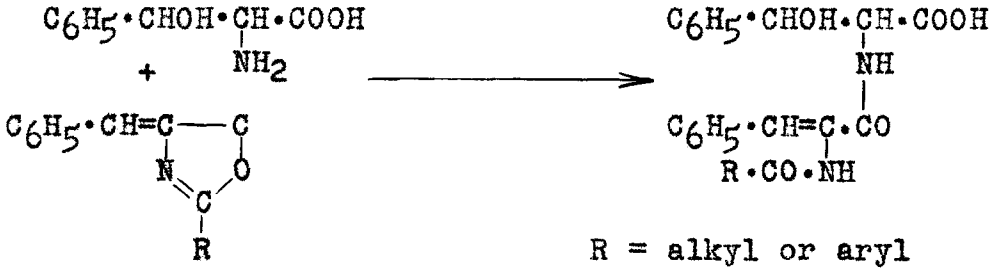
Phenylserine Peptides

Peptide	% Yield	M.p.,°C.	Route	Ref.
Glycylphenylserine	80	188 (dec.)	amination	(1)
DL-Leucylphenylserine	61	206 (dec.)	amination	(1)
N-Benzoylglycylphenylserine	16	143	Schotten-Baumann	(2)
N-Carbobenzoxyglycylphenylserine ethyl ester	-	149-151	Schotten-Baumann	(3)
N-Carbobenzoxyglycylphenylserine	-	161-163	hydrolysis	(3)
N-Carbobenzoxyglycyldehydrophenylalanylphenylserine	-	168-170	azlactone	(3)
N-Benzoyldehydrophenylalanylphenylserine	-	180 (dec.)	azlactone	(3)
N-Acetyldehydrophenylalanylphenylserine	-	226-228 (dec.)	azlactone	(3)
N-Acetylbis(dehydrophenylalanyl)phenylserine	-	223-225 (dec.)	azlactone	(3)
N-Acetyltris(dehydrophenylalanyl)phenylserine monohydrate	-	199 (dec.)	azlactone	(3)

(1) E. Abderhalden and S. Buadze, Fermentforschung, 8, 487 (1926).
(2) F. Bettzieche and R. Menger, Z. physiol. Chem., 172, 56 (1927).
(3) D. G. Doherty, J. E. Tietzman and M. Bergmann, J. Biol. Chem., 147, 617 (1943).

This group of studies indicates that the short route followed by Billet to N-dichloroacetyl-p-nitrophenylserine or the parent acid is the most practical. Protection of side-chain functional groups during nitration is not essential, reaction occurring without appreciable epimerization.

Phenylserine peptides and acyl peptides listed in Table 8 were prepared by amination of the corresponding α-haloacyl compounds, by use of the Schotten-Baumann technique, or via the azlactone method.



R = alkyl or aryl

Reaction of phenylserine with phenylisocyanate, under conditions similar to those of Schotten-Baumann acylation, gave a 75% yield of the N-phenylureido derivative, m.p. 194-195°, which cyclized almost quantitatively with hydrochloric acid to the corresponding saturated hydantoin, m.p. 220° (1). Acetic anhydride in pyridine thence produced in good yield the O-acetyl derivative, m.p. 166-167°, which brief treatment with ammonium hydroxide converted quantitatively to benzalphenylhydantoin, m.p. 255°, readily

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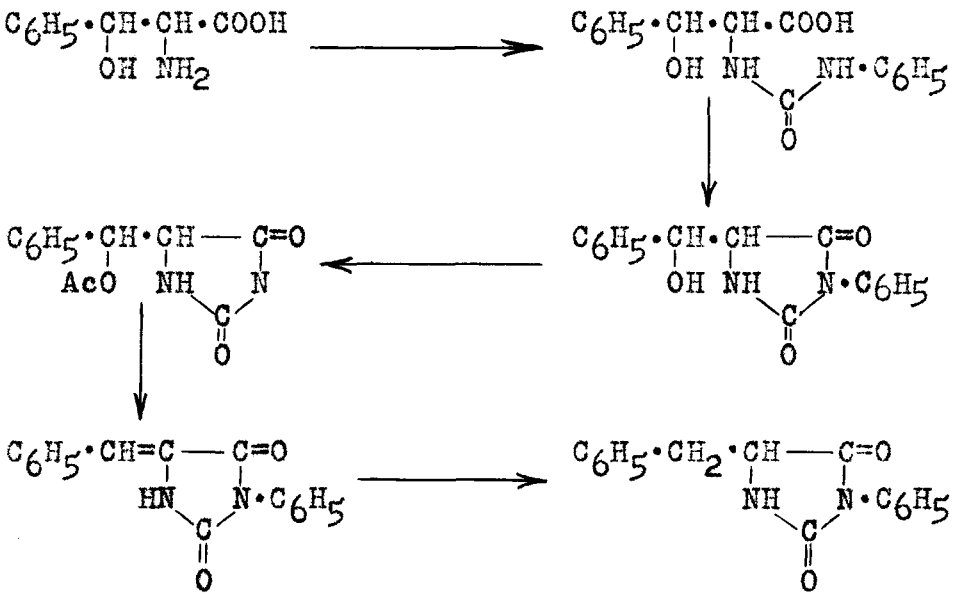
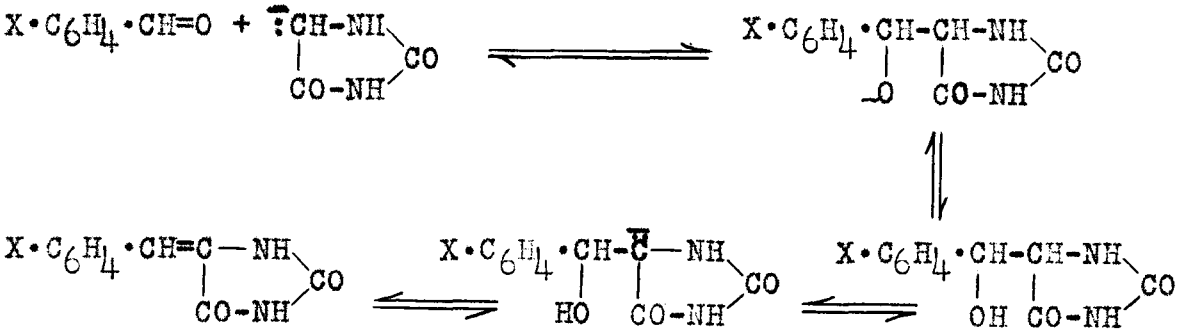
(1) M. Bergmann and D. Delis, Ann., 458, 76 (1927).



characterized by facile catalytic hydrogenation to the phenylhydantoin of phenylalanine.

Several phenylserine hydantoins were prepared by direct condensation of aromatic aldehydes with hydantoin, in the course of a recent study designed to correlate structure with reactivity of aromatic aldehydes (1). The nature of the



phenylserine hydantoin

(1) A. P. Phillips and J. G. Murphy, J. Org. Chem., 16, 954 (1951).

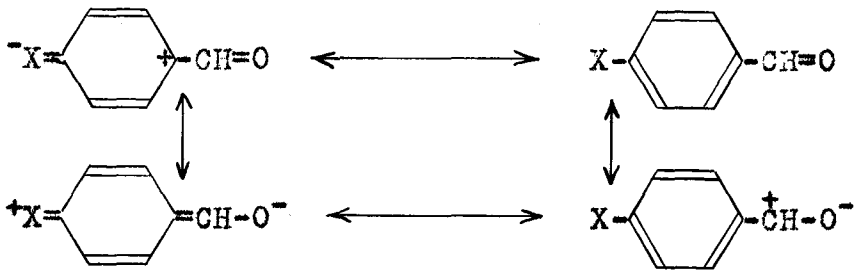
Table 9

Phenylserine Hydantoins

X	% Yield	% Aldehyde recovered	M.p., °C.
p-NO_2	87	11	253-254
m-NO_2	79	21	216-217
H	22	77	213-214 183-185 (a)

(a) Low melting diastereomer crystallizes more slowly from water.

nuclear substituent affected both the yield and type of product formed, by its influence on the positive character of the carbonyl group and consequent susceptibility of the latter to nucleophilic attack. The following order of reactivity was observed: p-NO_2 $\rangle$ m-NO_2 $\gg$ H $\gg$ p-CH_3O $\rangle$ p-$(CH_3)_2N$. With aldehydes containing the last two groups, no phenylserine derivative was produced, indeed, only negligible amounts of unsaturated hydantoin. Results were explained on the basis of resonance and electronic effects in both the starting aldehyde and intermediates formed: for example, greater



contribution by form A with p-nitrobenzaldehyde would enhance susceptibility of the carbonyl group to nucleophilic attack and also stabilize the aldol product, whereas with p-dimethylaminobenzaldehyde the influence of form D would effectively block reaction. Triethylamine and piperidine were equally effective as basic catalysts. Aldol formation was facilitated by mild reaction conditions, greater amounts of unsaturated product forming with increasing reaction time and concentration of base employed. The interpretations of this study are of significance insofar as they can be extended to other condensation reactions whereby phenylserine derivatives are produced.

Treatment of phenylserine with dimethyl sulfate resulted in deep-seated decomposition (1). Apparently, no simple N- or O-methyl derivative was formed. Isolation of ordinary betaine and tetramethylammonium salts, as well as a little cinnamic acid, from the reaction mixture showed that cleavage of both C-C and C-N bonds had occurred instead. The erroneous interpretation was recently made (2) that Dakin had prepared a true betaine of phenylserine.

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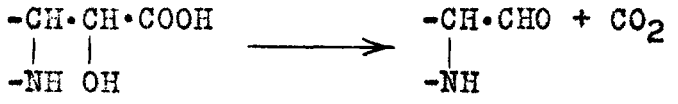
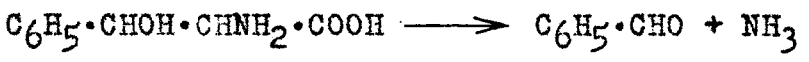
(1) H. D. Dakin, J. Biol. Chem., 140, 847 (1941).
(2) E. E. Howe, "Properties of Amino Acids", in Greenberg, ed., "Amino Acids and Proteins", Charles C. Thomas, Springfield, Illinois, c1951, p. 43.

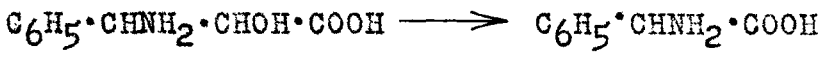
6. Oxidation

Isoserines have been distinguished from serines by treatment of their N-acyl derivatives with lead tetraacetate (1). Under the conditions employed only the N-acylisoserines were cleaved. Unreacted oxidant was estimated iodometrically.



Oxidation with potassium permanganate was used by Oesterlin (2) to differentiate phenylserine from the phenylisoserines. The former gave only benzaldehyde and ammonia, whereas the latter were degraded to phenylglycine.





The method proved inapplicable to N-aryl or N-alkyl derivatives, with benzoic acid and benzaldehyde the only products in either series (3).

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(1) F. Knoop, F. Ditt, W. Hecksteden, J. Maier, W. Merz, R. Härle, Z. physiol. Chem., 239, 30 (1936).
(2) M. Oesterlin, Metallbörse, 19, 1237 (1929).
(3) E. Fourneau and J. R. Billeter, Bull. soc. chim., [5], 7, 593 (1940).

Chloramine T was found to be a useful agent for oxidative decarboxylation of phenylserine (1). With the D-antipode of the hydroxyamino acid in citrate buffer at pH 4-5, L-mandelic acid could be obtained, via the intermediate mandelonitrile,

$$C_6H_5 \cdot CHOH \cdot CHNH_2 \cdot COOH + 2Ts.NCl \cdot Na \rightarrow C_6H_5 \cdot CHOH \cdot CN + CO_2 + 2Ts \cdot NH_2 + 2NaCl$$

providing that the latter was speedily extracted from the medium to minimize racemization.

7. Reduction: chloramphenicol synthesis

An early instance of reduction of phenylserine derivatives involved treatment of the ethyl ester hydrochloride with Grignard reagents (2). Phenyl magnesium bromide gave

$$C_6H_5 \cdot CHOH \cdot CHNH_2 \cdot COOC_2H_5 \xrightarrow{RMgBr} C_6H_5 \cdot CHOH \cdot CHNH_2 \cdot CR_2OH$$

$$R = C_6H_5 \text{ or } C_6H_5 \cdot CH_2$$

1,1,3-triphenyl-2-amino-1,3-propanediol, m.p. 154-155°, in 40% yield. Benzyl magnesium bromide was less effective, yielding only 13% of 1,1-dibenzyl-2-amino-3-phenyl-1,3-propanediol, m.p. 126-127°.

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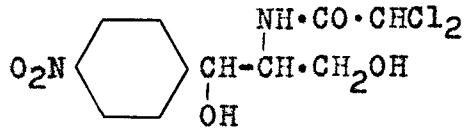
(1) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).
(2) F. Bettzieche and R. Menger, Z. physiol. Chem., 172, 64 (1927).

Revelation of the structure of chloramphenicol (1) focused attention on the possibility of using phenylserine as



Chloramphenicol

a synthetic precursor for the antibiotic. Accordingly, in a number of independent investigations, phenylserine esters were reduced in up to 76% yield with lithium aluminum hydride in anhydrous ether (2,3,4,5,6,7) to phenylserinol. This was

$$C_6H_5 \cdot CHOH \cdot CHNH_2 \cdot COOC_2H_5 \longrightarrow C_6H_5 \cdot CHOH \cdot CHNH_2 \cdot CH_2OH$$

converted to chloramphenicol by methods already described (8).

Catalytic hydrogenation of phenylserine ethyl ester over Raney nickel was described in detail in a recent patent (9). Similar reduction was claimed not only for esters of ring

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(1) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, Abstracts of Papers, 115th Am. Chem. Soc. Meeting, p. 9K (1949).
(2) C. G. Alberti, B. Asero, B. Camerino, R. Sannicolò and A. Vercellone, Chimica e industria (Milan), 31, 357 (1949).
(3) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).
(4) K. N. F. Shaw and S. W. Fox, Abstracts of Papers, 118th Am. Chem. Soc. Meeting, p. 28N (1950).
(5) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).
(6) K. Hayes and G. Gever, J. Org. Chem., 16, 269 (1951).
(7) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).
(8) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., ibid., 71, 2463 (1949).
(9) G. W. Moersch (to Parke, Davis and Co.). U. S. Patent 2,538,792. January 23, 1951.

substituted phenylserines, but also for various allophenylserine esters. No information was provided on the physical properties of products or starting materials, or on the origin of the latter.

The feasibility of improving efficienty or of curtailing the number of steps from phenylserine to chloramphenicol by use of an appropriately substituted derivative has also been studied. N,O-diacetylphenylserine ethyl ester, with lithium aluminum hydride in ether-chloroform, gave, among other products, one melting at 132-134°, which did not depress the melting point of authentic N-acetylphenylserinol (1). Treated in similar fashion, N-dichloroacetyl-p-nitrophenylserine gave, after Alorco chromatography, a non-crystalline gum in which the antibiotic, in racemic form, was probably the predominating constituent, as indicated by Shigella paradysenteriae assay (2). The selectivity of lithium aluminum hydride in reducing such polyfunctional compounds was established by Felkin (3).

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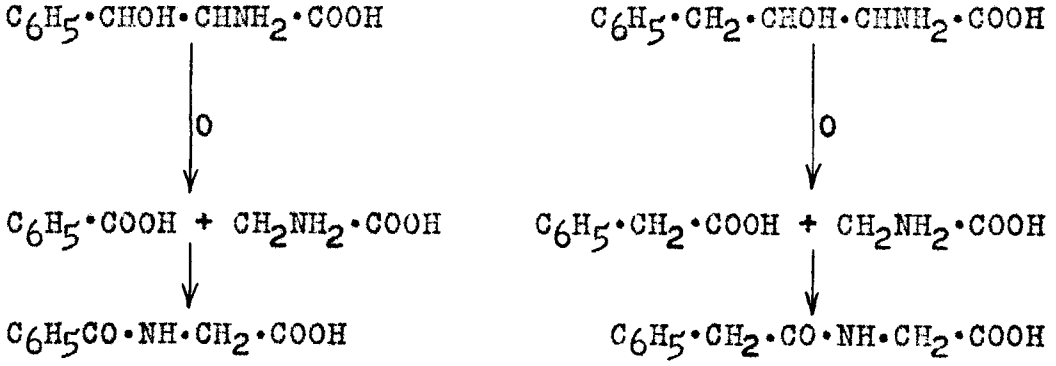
(1) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).
(2) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).
(3) H. Felkin, Compt. rend., 230, 304 (1950).

C. Biochemistry

1. Animal feeding experiments

Study of the biochemical properties of phenylserine was initiated by Dakin (1). Substantial amounts of hippuric acid were recovered from the urine of cats after subcutaneous injection of solutions of the hydroxyamino acid, with no other product demonstrated. Similar results were obtained upon feeding phenylserine (2), or its higher homologues (3), γ-phenylthreonine and δ-phenyl-β-hydroxy-norvaline to dogs. On the basis of these findings, β-oxidation was postulated as



the cardinal feature in metabolism of α-amino-β-hydroxy acids, with concurrent liberation of glycine in the intermediate step.

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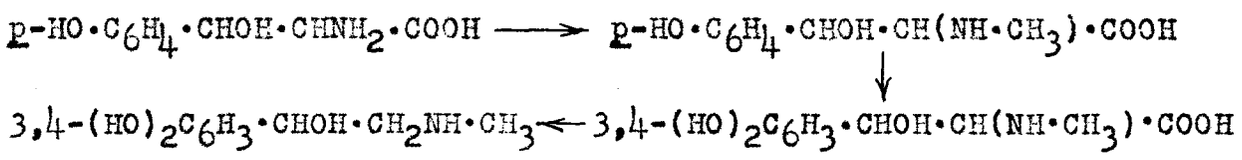
(1) H. D. Dakin, J. Biol. Chem., 6, 235 (1909).
(2) F. Knoop, Z. physiol. Chem., 89, 151 (1914).
(3) F. Knoop, F. Ditt, W. Hecksteden, J. Maier, W. Merz, R. Märle, ibid., 239, 30 (1936).

Armstrong and Lewis (1) pointed out that, in earlier animal feeding experiments of Dakin and of Knoop, recoveries of hippuric acid were sufficiently low to leave uncertain whether all phenylserine was metabolized to benzoic acid. Using synthetic diets with which it was possible to detect formation of either phenylalanine or tyrosine from possible dietary precursors, they found that not only did phenylserine have no growth promoting activity for young white rats, but that it was actually slightly toxic as part of the limited ration. It was stressed, however, that these results did not preclude the possibility that allophenylserine, which these investigators had unsuccessfully tried to synthesize, might serve in lieu of phenylalanine or tyrosine in the diet.

2. Intermediary metabolism: relation to adrenaline biosynthesis

It was first pointed out by Friedmann (2), who established the structure of adrenaline, that some proteins might conceivably contain p-hydroxyphenylserine or its N-methyl derivative. Nuclear oxidation of the latter *in vivo* would give



(1) M. D. Armstrong and J. D. Lewis, J. Biol. Chem., 186, 849 (1950).
(2) E. Friedmann, Beitr. chem. Physiol. Path., 8, 95 (1906).

"Adrenalinsaure", from which the hormone itself could arise by enzymatic decarboxylation. However, the apparent susceptibility of phenylserine to β-oxidation led Knoop to feel that the hydroxyamino acid was unlikely to be the biological adrenaline precursor (1).

The alternative view that adrenaline might derive from tyrosine was regarded poorly by Rosenmund and Dornsaft (2), because of known facile metabolic side-chain degradation with the latter, and because its addition to macerated adrenals failed to increase their vasopressor activity. Two new possible routes of adrenaline synthesis were presented, with phenylserine, or its 3,4-dihydroxy derivative (DOPS) as hypothetical intermediates. The first started from phenylalanine, as shown in Fig. 1, whereas the second path proceeded from 3,4-dihydroxyphenylalanine (DOPA) in similar fashion. It was further speculated that both DOPA and DOPS might be normal constituents of proteins, but not easily demonstrated due to lability under usual conditions of isolation and protein hydrolysis.

While welcoming synthesis of DOPS as a potential contribution to knowledge of the mode of adrenaline formation, Knoop criticized the scheme of hypothetical precursors outlined above, both for want of direct experimental evidence,

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(1) F. Knoop, Z. physiol. Chem., 89, 151 (1914).
(2) K. W. Rosenmund and H. Dornsaft, Ber., 52, 1734 (1919): Arb. Pharm. Inst. Univ. Berlin, 12, 73 (1921).

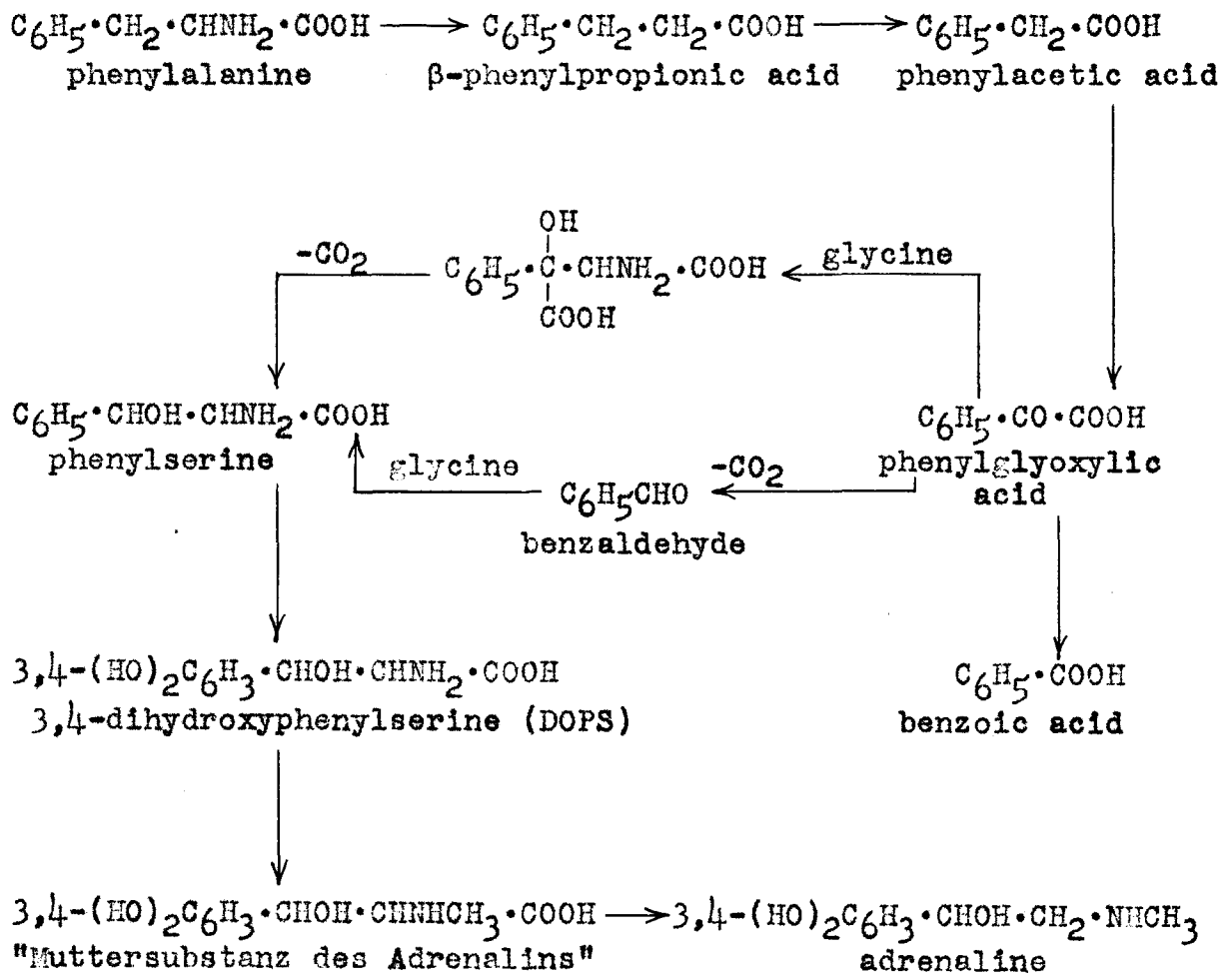

Fig. 1 Rosenmund-Dornsaft Scheme for Adrenaline Biosynthesis

and for its failure to consider such known facts as the β-decarboxylative degradation of fatty acids, absence of phenylpropionic acid from animal metabolism, and stability of phenylacetic acid toward further biological degradation (1). Rosenmund and Dornsaft (2) then defended their hypothesis on the grounds that adrenaline was not necessarily the sequel of a normal amino acid degradation, but more probably arose by an anomalous path more akin to those encountered in bacterial metabolism. Low hormone content of the adrenal medulla favored such a view. Knoop closed the argument (3) by underlining the need for further investigation rather than hypothesization to clarify the picture, but simultaneously speculated that tyrosine might yet prove to be the biological adrenaline precursor, since its failure to increase the vasopressor activity of macerated adrenals would not of necessity parallel its action in the intact organ, nor was its β-oxidation in the side-chain improbable.

During succeeding decades, little attempt was made experimentally to verify or to discredit the Rosenmund-Dornsaft hypothesis for adrenaline biosynthesis, partly due to accumulation of evidence which seemed to support alternative routes. An isolated report (4) noted that subcutaneous

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(1) F. Knoop, Ber., 52, 2266 (1919).
(2) K. W. Rosenmund and H. Dornsaft, Ber., 53, 317 (1920).
(3) F. Knoop, Ber., 53, 716 (1920).
(4) M. Guggenheim. "Die biogenen Amine", Karger, Basle and New York, 1940, p. 431.

injection into rabbits of DOPS up to 100 mg. per kilogram was without effect on arterial blood pressure or blood sugar level. Recent resurgence of interest has resulted in an extensive series of papers dealing with this phase of phenylserine biochemistry.

The decomposition of phenylserine and p-hydroxyphenylserine by animal tissues has been investigated by Werle and his group (1,2). Under aerobic conditions, oxygen uptake, but no ammonia evolution, was observed with both compounds in presence of guinea-pig liver or kidney slices at pH 9.0-9.4, but not at pH 8. Organ extracts were more active than slices in attacking p-hydroxyphenylserine, but hardly affected phenylserine, thereby suggesting two distinct enzymes to be involved. Although no products were isolated, β-oxidative cleavage according to Knoop's scheme was considered to have occurred. Under a nitrogen atmosphere, phenylserine showed no sign of degradation. By contrast, p-hydroxyphenylserine was rapidly but incompletely decarboxylated at pH 7.1 by extracts from guinea-pig or rabbit kidney, beef kidney, pancreas or adrenals, and horse adrenals. Dialysis of material precipitated by ammonium sulfate achieved some concentration and purification of the enzyme, which differed sharply from other mammalian

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(1) E. Werle, S. Brüninghaus, Chang-Tok, O. Ehrismann, E. v. Pechmann, W. Peschel and A. Zabel, Angew. Chem., 60, 51 (1948).
(2) E. Werle and W. Peschel, Biochem. Z., 320, 1 (1949).

amino acid decarboxylases in its loss of activity upon standing for a few hours, and in its stimulation by ascorbic acid, or by 10^{-3} M cyanide (10^{-2} M inhibited; 10^{-4} M had no effect). On the basis of these findings, the question of p-hydroxyphenylserine or DOPS decarboxylatability in relation to adrenaline formation was deemed worthy of reinvestigation.

At that time, DOPS attracted the interest of Blaschko and co-workers, in the course of their studies on amino acid decarboxylases. With acetone-dried preparations of Streptococcus faecalis R, which possessed L-tyrosine decarboxylase activity, carbon dioxide was slowly evolved under anaerobic conditions to an extent corresponding to 47% DOPS decarboxylation (1,2). Pharmacological assay of the incubated solution on the arterial blood pressure of the spinal cat and on rat uterus muscle showed that l-noradrenaline had been simultaneously produced in ammounts corresponding closely to the carbon dioxide liberated. By contrast, DOPS, alternatively named noradrenalinecarboxylic acid, was reported to be unaffected by extracts of guinea-pig adrenals or kidney, the latter containing L-DOPA decarboxylase. This negative finding was interpreted to mean that DOPS was unlikely to be a precursor of adrenaline or of noradrenaline. The N-methyl derivative,

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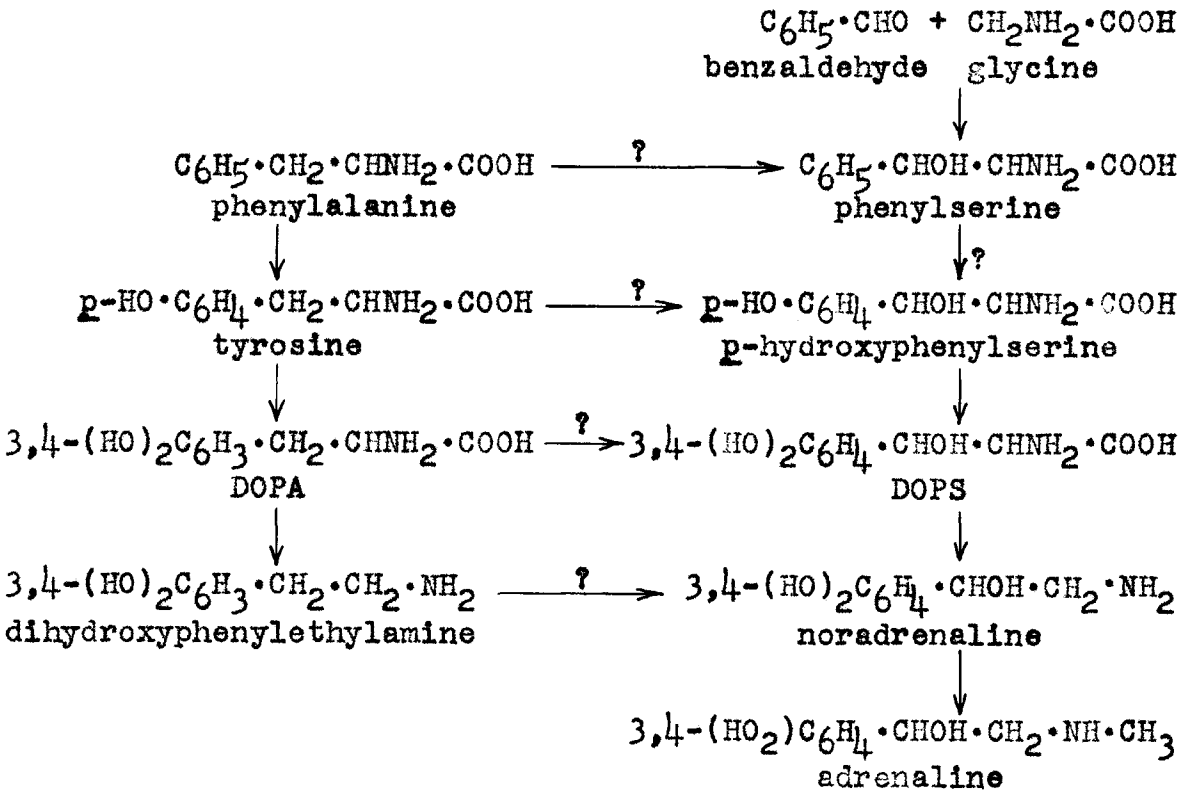
(1) H. Blaschko, P. Holton and G. H. S. Stanley, Biochem. J., 42, xlviii (1948).
(2) H. Blaschko, P. Holton and G. H. S. Stanley, Brit. J. Pharmacol., 3, 315 (1948).

adrenalinecarboxylic acid, was not decarboxylated by a number of mammalian tissue extracts, nor by bacterial enzyme preparations (1,2). The general failure of N-methylamino acids to serve as substrates was attributed to their inability to combine with the codecarboxylase, pyridoxal phosphate, to give the Schiff base seemingly involved in enzymic decarboxylation.

Alternative schemes for adrenaline biosynthesis have been presented by Beyer (3), with detailed discussion of each step



(1) H. Blaschko, Biochim. et Biophys. Acta, 4, 130 (1950).
(2) H. Blaschko, J. H.Burn and H.Langemann, Brit. J. Pharmacol., 5, 431 (1950).
(3) K. H. Beyer, Advances in Chem. Ser. No. 2, 37 (1950).

in relation to supporting experimental evidence. New significant experiments concerning the phenylserines were also described. The oxidation of p-hydroxyphenylserine to DOPS was observed to occur rapidly in presence of phenol oxidase. Contrary to the findings of the Blaschko group, it was noted that phenylserine, p-hydroxyphenylserine and DOPS were decarboxylated by guinea-pig, cat or dog kidney under anaerobic conditions, at speeds paralleling that for DOPA. At pH 6.5, with dog kidney homogenate, rates were in the order phenylserine > DOPA > DOPS. With guinea-pig kidney homogenate, where DOPS was decarboxylated least, addition of the corresponding amines caused inhibition of degradation for both DOPA and DOPS. Injection of DOPS into the artificially ischemic kidney in a cat, followed by release of circulation, resulted in a well-sustained sharp rise in blood pressure. After return to a low level, further intravenous injection of DOPS caused a still greater blood pressure increase. Upon intravenous injection in dogs, phenylserine, p-hydroxyphenylserine, DOPS and adrenaline were increasingly active in raising blood pressure, whereas DOPA had no action. These results were taken to be good presumptive avidence that the observed vasopressor effects were due to decarboxylation of the phenylserines.

In favoring the phenylserine route to adrenaline, Beyer considered biological condensation of benzaldehyde and glycine not to be unlikely, although other paths to the various

possible phenylserine precursors were readily conceivable. The feasibility of this step has become more worthy of consideration with the discovery in liver and kidney of various animals of an enzyme, named glycinogenase, which hydrolytically cleaved β-hydroxyamino acids (1). The facts that disruption

$R' = H$ or CH_3 $\quad R'' = H$, CH_3, C_2H_5 or C_6H_5

proceeded at the same rate under aerobic or anaerobic conditions, and that β-hydroxyvaline was among the compounds dissociated by the enzyme, tended to contradict Knoop's β-oxidation hypothesis for hydroxyamino acids. It is of interest that allothreonine was attacked as well as threonine, suggesting lack of enzyme stereospecificity with respect to the β-carbon atom. Glycine was determined by a selective colorimetric micromethod (2). The ketonic cleavage products from the various hydroxyaminoacids were individually identified: in the case of phenylserine, benzaldehyde was trapped in the Conway diffusion apparatus as the p-nitrophenylhydrazone (3). It was observed

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(1) A. E. Braunshtein and G. Ya. Vilenkina, Doklady Akad. Nauk S.S.S.R., 66, 243 (1949).
(2) B. Alexander, G. Landwehr and A. M. Seligman, J. Biol. Chem., 160, 51 (1945).
(3) G. Ya. Vilenkina, Doklady Akad. Nauk S.S.S.R., 69, 385 (1949).

that activity of the enzyme was sharply lowered by carbonyl reagents, but was unaffected by experimental B_6-avitaminosis in the rat liver.

In the light of Beyer's findings, the effect of fresh guinea-pig kidney extract at pH 7.4 on DOPS under anaerobic conditions was reinvestigated (1,2). Carbon dioxide was liberated slowly, with concurrent formation of an equivalent quantity of l-noradrenaline, which was determined pharmacologically by its pressor action and its differential effect on normal or denervated nictitating membrane in the spinal cat. Extracts of guinea-pig liver, dog liver and kidney showed progressively less decarboxylative power. Failure in earlier runs was attributed to use of small amounts of tissue extracts, too short an incubation time to observe carbon dioxide formation and to not having tested for pressor activity. In a summary of substrate specificity requirements for amino acid decarboxylases (3), DOPS, 3,4-dihydroxyphenylethylamine and p-hydroxyphenylethanolamine were briefly considered as possible adrenaline precursors. However, in a more extended review on biosynthesis of the hormone (4), Blaschko favored

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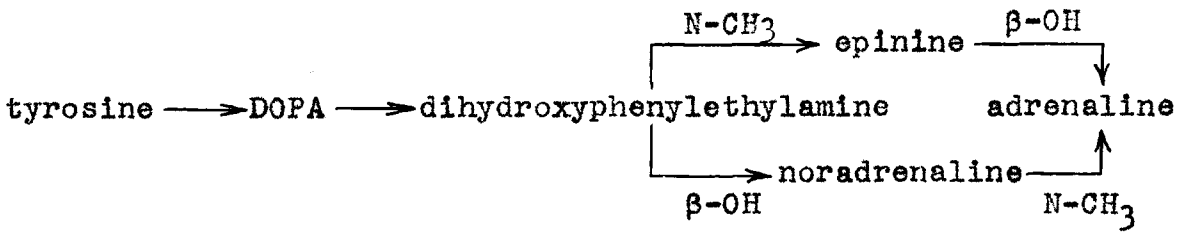
(1) K. H. Beyer, H. Blaschko, J. H. Burn and H. Langemann, Nature, 165, 926 (1950).
(2) H. Blaschko, J. H. Burn and H. Langemann, Brit. J. Pharmacol., 5, 431 (1950).
(3) H. Blaschko, Proc. Roy. Soc. (London), B137, 307 (1950).
(4) H. Blaschko, "Biosynthesis of Adrenaline", in Pincus and Thimann, eds. "The Hormones", Academic Press, New York, 1950, vol. 2, p. 617.

a specific path which excluded DOPS, because of its slow rate



of enzymic decarboxylation compared to DOPA, although he recognized that there was no experimental evidence for occurrence of β-hydroxylation after decarboxylation, nor for participation of epinine in the sequence presented.

The latest publication by the group at Oxford described demonstration of noradrenaline in the urine of rabbits intravenously injected with DOPS, after establishment of diuresis by an initial dose of water (1). The transformation still occurred in adrenalectomized animals. No adrenaline was eliminated in either case. Pressor substances were not obtained from urine of normal animals, nor from that collected after injection of adrenalinecarboxylic acid. These findings provide further support for the implication of earlier in vitro studies that DOPS may be the natural precursor of noradrenaline.

Another recently tested approach involved partial exhaustion of the glandular adrenaline store in fasted rats by subcutaneous injection of subconvulsive doses of insulin (2).

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(1) C. G. Schmiterlöw, Brit. J. Pharmacol., 6, 127 (1951).
(2) C. G. Van Arman, Am. J. Physiol., 164, 476 (1951).

Although glucose was fed after a definite interval, spontaneous recovery of the adrenaline store did not occur within six hours. Phenylserine, phenylalanine, tyrosine, tyramine, synephrine, epinine, noradrenaline and adrenaline did not permit regeneration of the adrenaline store during the six hour recovery period, whereas partial recovery was induced by a complete diet, an amino acid mixture or DOPA. Toxic symptoms were observed both with phenylserine and DOPA in the doses used. Further study was deemed necessary to learn whether the six hour recovery period was sufficiently long for the various compounds to show their effect.

The fact that none of the phenylserines have yet been found in proteins or other naturally occurring compounds constitutes a major argument against their postulation as intermediates in adrenaline biosynthesis. Even if this objection is overcome, the problem of firmly establishing the site and nature of each chemical step leading to the hormone is complicated by the diverse intermediary metabolic paths which phenylserines appear to follow, as well as by the complexity of the enzyme preparations used for in vitro investigation.

3. Microbiological activity

Study of the action of phenylserine on microorganisms commenced with the work of Beerstecher and Shive (1), who

(1) E. Beerstecher, Jr., and W. Shive, J. Biol. Chem., 164, 53 (1946).

observed that the compound competitively inhibited utilization of phenylalanine in Escherichia coli, Lactobacillus arabinosus 17-5, and Streptococcus faecalis R, with antibacterial indices of 1000, 200 and 200 respectively. Toxic effects of greater than 3-10 mg. of phenylserine per ml. of medium to E. coli were only incompletely reversed by phenylalanine. Tryptophan was one tenth as effective as an antagonist of phenylserine or β-2-thienylalanine, with progressively lesser effect at higher inhibitor levels, and no effect at high phenylalanine concentrations. This suggested that tryptophan was a precursor of phenylalanine in the organism. Toxicity of phenylserine was one tenth that of β-2-thienylalanine.

Tyrosine was more active than phenylalanine in reversing β-2-thienylalanine inhibition of E. coli growth, but in no way decreased phenylserine toxicity. Phenylpyruvic acid interfered with neither antimetabolite. It thus appeared that while β-2-thienylalanine prevented direct irreversible oxidation of phenylalanine to tyrosine in the organism, phenylserine interfered with a different unknown metabolic pathway (1). Upon daily transfer in a medium containing phenylalanine, E. coli became increasingly sensitive to tyrosine, until one part in thirty million completely blocked

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(1) E. Beerstecher, Jr., and W. Shive, J. Biol. Chem., 167, 49 (1947).

growth (1). In levels required for normal development of other species, phenylalanine completely and non-competitively eliminated tyrosine toxicity, but phenylserine enhanced it. Thus, tyrosine had a controlling role in biosynthesis of phenylalanine, whose utilization was disturbed by phenylserine.

A mutant strain of E. coli was unable to utilize glycylphenylserine in place of its required exogenous phenylalanine (2). The peptide likewise failed to display any sparing action on the natural amino acid requirement.

Phenylserine has been found to cause 50% inhibition of Pseudomonas aeruginosa growth at a level of one mg. per ml. (3). This was of chemotherapeutic interest, since few compounds have been found with pronounced inhibitory action on this infective organism.

Interest in antimetabolite activity of phenylserine sharpened when announcement of the structure of chloramphenicol (4) showed the similarity of the two compounds. Extensive investigation of the joint effects of the antibiotic, phenylserine and other amino acids followed (See Table 10).

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(1) E. Beerstecher, Jr., and W. Shive, J. Biol. Chem., 167, 527 (1947).
(2) S. Simmonds, E. L. Tatum and J. S. Fruton, ibid., 169, 91 (1947).
(3) G. J. Martin and J. N. Moss, Am. J. Pharm., 121, 169 (1949).
(4) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and C. R. Bartz, Abstracts of Papers, 115th Am. Chem. Soc. Meeting, p. 9K (1949).

Table 10

Combined Action of Chloramphenicol, Phenylserine and Other Amino Acids on *E. coli*

	Cysteine	Serine	Phenyl-serine	β-2-Thienyl-alanine	Chloram-phenicol
Glycine	X(6)	X(4,5)	X(5)	X(6)	X(5)
Aspartic Acid	+(6)	+(4,5)	+(5)	X(6)	+(5)
Alanine		X(4,5)	0(5)		0(5)
Phenylalanine		0(4)	X(1)	X(1)	0(6)
Tryptophan		0(4)	X(1)	X(1)	0(5)
Tyrosine		0(4)	+(3)	X(2)	0(6)
Phenylserine	+(6)	+(6)		X(6)	+(5)
β-2-Thienylalanine			X(6)		+(6)
Cysteine			+(6)		+(6)
Chloramphenicol	+(6)	+(5)	+(5)	+(6)	

X = antagonism, + = synergism, 0 = no modification of toxicity

(1) E. Beerstecher, Jr., and W. Shive, J. Biol. Chem., 164, 53 (1946).
(2) E. Beerstecher, Jr., and W. Shive, ibid., 167, 49 (1947).
(3) E. Beerstecher, Jr., and W. Shive, ibid., 167, 527 (1947).
(4) B. D. Davis and W. K. Maas, J. Am. Chem. Soc., 71, 1865 (1949).
(5) C. Mentzer, P. Meunier, L. Molho-Lacroix, Compt. rend., 230, 241 (1950).
C. Mentzer, P. Meunier, L. Molho-Lacroix and D. Billet, Bull. soc. chim. biol., 32, 55 (1950).
(6) D. Molho and L. Molho-Lacroix, ibid., 32, 680 (1950).

Other noteworthy relationships, not apparent in Table 10, were also discovered (1,2). Inhibitory power diminished in the series chloramphenicol, β-2-thienylalanine, cysteine, serine and phenylserine. Although β-2-thienylalanine, cysteine and serine each strongly fortified action of the antibiotic, phenylserine was so synergistic with chloramphenicol that growth did not occur at all. Methionine failed to modify toxicity of chloramphenicol, showing behavior of cysteine was not due merely to its sulfur content. Phenylalanine antagonism to chloramphenicol was reported only for E. coli which had previously developed some tolerance for the antibiotic. Growth curves obtained with the amino acids were similar to those of controls, whereas chloramphenicol markedly modified both slope and height. Data were insufficient to judge whether antagonisms were competitive or not.

Implications of the findings were discussed at length. The effect of structural modification on properties of a normal metabolite was remarkable. A single change produced an antagonist, whereas two simultaneous changes led to nullification of interaction. Thus, serine was antagonized by alanine, but not by phenylalanine, while phenylserine was antagonized

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(1) C. Mentzer, P. Meunier, L. Molho-Lacroix, Compt. rend., 230, 241 (1950).
C. Mentzer, P. Meunier, L. Molho-Lacroix and D. Billet, Bull. soc. chim. biol., 32, 55 (1950).
(2) D. Molho and L. Molho-Lacroix, ibid., 32, 680 (1950).

by phenylalanine, or the isosteric thienylalanine, but not by alanine. Serine and cysteine were qualitatively alike. Chloramphenicol was apparently too far removed from alanine or phenylalanine to be antagonized thereby.

Chloramphenicol was considered correlated best with D-serine and least with β-2-thienylalanine with respect to similarity in relations of antagonism and synergism. In this regard, phenylserine was intermediate between the antibiotic and β-2-thienylalanine. The fact that chloramphenicol showed synergism with the amino acids was not taken to mean that mechanisms of biological action were the same, since two antimetabolites acting on different enzyme systems could readily display additive effects. In fact, unlike character in growth curves and interactions with other amino acids favored dissimilar mechanisms. However, two distinct modes of biological functionality were clearly evident, although others may well remain to be elucidated. One was associated in some unknown way with β-chalcogen substitution, as reflected by similarity of chloramphenicol, cysteine, serine and phenylserine as regards glycine antagonism and aspartic acid synergism. The other, typified with β-2-thienylalanine, which lacks β-hydroxyl or sulfhydryl, involved direct interference with phenylalanine metabolism. Phenylserine was considered to participate in both mechanisms, by virtue of its β-hydroxyl, and as a phenylalanine antimetabolite.

The critical importance of strain and species of organism, composition of medium, and type of datum assembled in respect to experimental conclusions drawn was apparent in an investigation designed to show whether antibacterial properties of chloramphenicol derived from antimetabolite action (1). Whereas phenylalanine, and, to a lesser degree, tryptophan and tyrosine non-competitively overcame toxic effects on E. coli of up to one microgram of chloramphenicol per ml. of a glucose-salts medium, antagonism disappeared when larger amounts of antibiotic or a synthetic amino acid medium was used. With a strain of L. casei requiring both phenylalanine and tyrosine on synthetic amino acid medium, only the former compound antagonized chloramphenicol. Phenylserine showed anomalous behavior in that, at low levels, it inhibited growth of an E. coli mutant which specifically required exogenous phenylalanine or phenylpyruvic acid, but, with increasing concentrations, actually stimulated growth. This unusual effect was encountered only when suboptimal amounts of phenylalanine were present and not with the wild type E. coli, nor with L. casei. Woolley stressed the fact that results were most understandable when the organism employed specifically required the metabolite under study.

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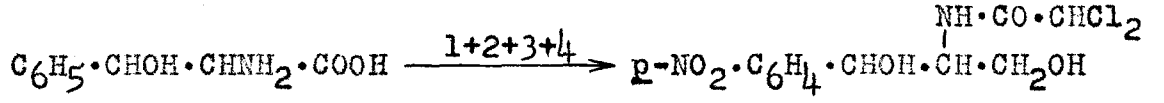
(1) D. Woolley, Federation Proc., 9, 249 (1950); J. Biol. Chem., 185, 293 (1950).

The effect of stepwise alteration in chemical structure on antibacterial activity and phenylalanine antagonism was revealed in an examination of compounds ranging from phenylalanine through various phenylserine derivatives to chloramphenicol (Table 11).



1 = α-CH_2OH for α-COOH
2 = N-Cl_2OH·CO for N-H
3 = β-OH for β-H
4 = p-NO_2 for p-H

It was noteworthy that phenylserine was the only compound involving a single structural change which showed antimetabolite activity. With progressively greater alteration in the phenylalanine molecule, non-competitive and frequently irreversible antagonism emerged, along with increasing antibacterial potency.

Woolley showed some doubt concerning steric purity of the phenylserine derivatives used, particularly in relation to the observed bacteriological results. The uncertainty was subsequently increased by the differences observed in properties of his N-dichloroacetyl-p-nitrophenylserine preparation and one synthesized by another route (1). Other investigators have reported no antibacterial activity for p-nitrophenyl-

(1) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).

Table 11

Toxicity and Phenylalanine Antagonism of Compounds Intermediate in Structure Between Chloramphenicol and Phenylalanine

Compound	Change	E.coli			
		Phenylalanineless		Wild type	
		Toxicity	Antagonism	Toxicity	Antagonism
N-Dichloroacetyl-phenylalanine	2	replaces phenylalanine		0	0
Phenylalaninol	1	0	0	+	slight NC
p-Nitrophenylalanine	4	0	0	++	slight NC
Phenylserine	3	++	C	++	C
N-Dichloroacetyl-phenylalaninol	1+2	0	0		
p-Nitrophenylserine	3+4	++	NC	++	NC
N-Dichloroacetyl-p-nitrophenylalanine	2+4	+	NC	0	0
Phenylserinol	1+3	0	0	0	0
N-Dichloroacetyl-phenylserine	2+3	0	0	0	0
N-Dichloroacetyl-phenylserinol	1+2+3	+++	CI	+++	slight NC
N-Dichloroacetyl-p-nitrophenylserine	2+3+4	++	C	+	slight NC
p-Nitrophenylserinol	1+3+4	++	CI	++	0
N-Dichloroacetyl-p-nitrophenylserinol (Chloramphenicol)	1+2+3+4	++++	NC		

+ = toxic, 0 = non-toxic, C = competitive, NC = non-competitive

CI = competitive at low, irreversible at high concentration

serine prepared by two different routes (1), N-dichloroacetyl-p-nitrophenylserine (2) and the ethyl ester of the latter (3).

Phenylserine was most recently used as an antimetabolite in an investigation of the tryptophan-nicotinic acid path in E. coli (4). Nicotinic acid was found to be very weakly antagonistic, while ornithine did not affect phenylserine inhibition.

Studies have been made on the metabolism of chloramphenicol by E. coli, Bacillus mycoides, Bacillus subtilis, and Proteus vulgaris (5). With each organism, both p-aminophenylserine and p-nitrophenylserine were reported among eighteen decomposition products demonstrated by paper chromatography and by chemical techniques. Five biological degradation pathways appeared to be involved, the relative importance of each varying with the organism.

In all biochemical work with phenylserine and its derivatives, insufficient attention has been paid to the relation between either optical or steric form and biological activity. Possible presence of allophenylserine in preparations used,

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(1) D. Billet, Compt. rend., 230, 1358 (1950); 231, 293 (1950).
(2) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).
(3) G. Carrara, F. M. Chiancone, V. D'Amato, E. Ginouhliac, C. Martinuzzi and G. Weitnauer, Gazz. chim. ital., 80, 709 (1950).
(4) C. Marnay, Bull. soc. chim. biol., 33, 174 (1951).
(5) G. N. Smith and C. S. Worrel, Arch. Biochem., 28, 1, 232 (1950).

while deemed doubtful, is not to be excluded. The report that inhibitory properties of D-serine were not connected with L-serine metabolism, insofar as the latter did not antagonize the former, is significant (1). This finding may point the way to an explanation of the seemingly diverse biological activity of phenylserine. The possibility that anti-phenylalanine activity may well reside specifically in L-phenylserine, with only the D-form behaving in the same unknown fashion as D-serine, has not been tested. Nor has the effect of either enantiomorph on L-serine metabolism been investigated. Studies have yet to be extended to pure allo-phenylserine, to various heterologues, to the isosteric β-phenylcysteine, preparation of one diastereomer of which was recently described (2), or to the thio analogues of chloram-phenicol. It would also be of interest to investigate whether phenylserine, its immediate derivatives, or analogues could be effectively employed in culturing Streptomyces venezuelae to throw light on the path of chloramphenicol biosynthesis, or to secure new antibiotic substances.

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(1) B. D. Davis and W. K. Maas, J. Am. Chem. Soc., 71, 1865 (1949).
(2) A. H. Cook, G. Harris, I. Heilbron, J. Chem. Soc., 1060 (1948).

4. Enzymic peptide cleavage

While investigating whether enzymes could hydrolyze peptides containing amino acids not found in nature, Abderhalden and Buadze (1) incubated glycylphenylserine and DL-leucylphenylserine with yeast juice at pH 8 and 37°. Development of slight negative optical rotations, constant for the former substrate after two and one-half hours, and for the latter after twelve and one-quarter hours, suggested that enzymatic cleavage had indeed occurred. Further confirmation was provided by isolation of glycine as its ethyl ester hydrochloride from the first hydrolysate, together with small amounts of material which elementary analyses and low optical activity suggested may have been slightly resolved phenylserine ethyl ester hydrochloride.

D. Steric Configuration

The configuration of phenylserine obtained from benzaldehyde-glycine condensation became of concern with the demonstration that only one of the four stereoisomers of chloramphenicol possessed antibacterial activity (2). Although earlier investigators had considered the hydroxyamino acid

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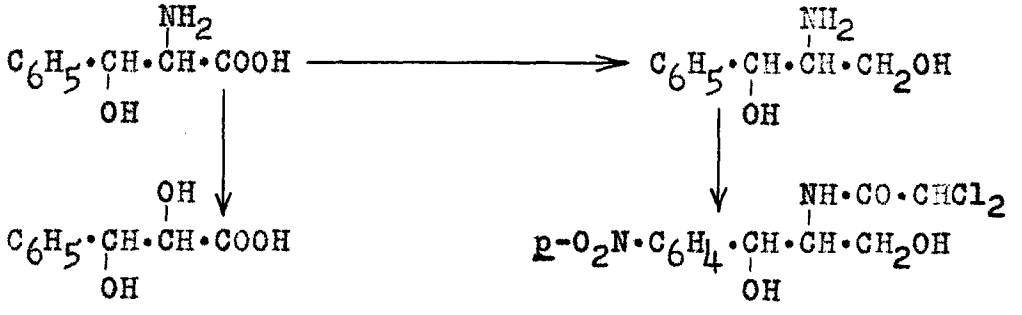
(1) E. Abderhalden and S. Buadze, Fermentforschung, 8, 487 (1926).

(2) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, Abstracts of Papers, 115th Am. Chem. Soc. Meeting, p. 9K (1949).

thus formed to be a single racemic isomer, Woolley presumed that the material was a diastereomeric mixture, since it was formed from two optically inactive substances (1). Efforts to establish whether his various derivatives were sterically pure met with failure. Oxidation of chloramphenicol to N-dichloroacetyl-p-nitrophenylserine for comparison with synthetic material could not be effected. Successful transformation of phenylserine into a chloramphenicol intermediate of known steric form (2,3,4,5,6,7) gave the first experimental proof of its threo configuration, which had been stipulated by Erlenmeyer on less firm grounds long years before (8). Supporting



evidence was provided by conversion to threo-phenylglyceric

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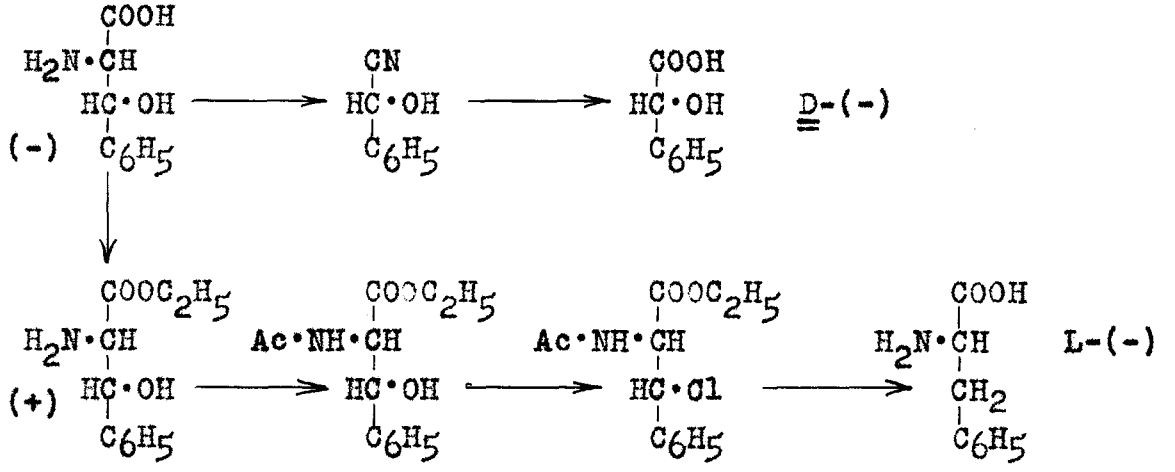
(1) D. W. Woolley, J. Biol. Chem., 185, 293 (1950).
(2) C. G. Alberti, B. Asero, B. Camerino, R. Sannicolò and A. Vercellone, Chimica e industria (Milan), 31, 357 (1949).
(3) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).
(4) K. N. F. Shaw and S. W. Fox, Abstracts of Papers, 118th Am. Chem. Soc. Meeting, p. 28N (1950).
(5) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).
(6) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).
(7) K. Hayes and G. Gever, J. Org. Chem., 16, 269 (1951).
(8) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).

acid on treatment with nitrous acid (1). Studies with individual optical isomers have thoroughly confirmed the fact that phenylserine is effectively a structural analogue of threonine (2). D-Phenylserine reacted with chloramine T to give mandelonitrile, from which L-mandelic acid was obtained by acid hydrolysis. Thionyl chloride acting on the N-acetyl ethyl ester



of L-phenylserine gave the corresponding β-chloro derivative, which, upon catalytic hydrogenation and subsequent acid hydrolysis, was transformed to L-phenylalanine, characterized further as the nasylate. Thus, configuration was established unequivocally through the use of reactions which avoided attack on the asymmetric center under study in a given sequence.

To date, yields from the Rosenmund-Dornsaft route have been too low to permit study of the steric course of the

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(1) D. Billet, Compt. rend., 230, 1074 (1950).
(2) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).

reaction. No more than a single racemic form was encountered with any of the ring substituted phenylserines thus prepared (1,2). When p-nitrophenylserine obtained by this method failed to exhibit antibacterial activity, it was thought that possibly the substance was of the erythro configuration, i.e. that it might be p-nitroallophenylserine (3). It was further hypothesized that spatial arrangement of groups would favor the erythro configuration in the intermediate oxazolidine structure postulated by Dalgliesh. This view was subsequently criticized on the basis that neither theoretical nor practical grounds would exclude formation of an oxazolidine from a threo precursor (4).

The steric form of DOPS, prepared according to the Rosenmund-Dornsaft procedure has been related to noradrenaline in an interesting manner (5). It was reported that the hydroxyamino acid preparation behaved like a single diastereomer, i.e. Dd-Ll or Dl-Ld*. In view of enzymic stereospecificity observed in all cases thus far with amino acid

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* Capital letters refer to configurations about the α-carbon atom; small letters designate the β-carbon atom.

(1) C. E. Dalgliesh and F. G. Mann, J. Chem. Soc., 658 (1947).
(2) C. E. Dalgliesh, ibid., 90 (1949).
(3) D. Billet, Compt. rend., 230, 1358 (1950).
(4) E. D. Bergmann, M. Genas and H. Bendas, ibid., 231, 361 (1950).
(5) H. Blaschko, P. Holton and G. H. S. Stanley, Biochem. J., 42, xlviii (1948); Brit. J. Pharmacol., 3, 315 (1948).

decarboxylases, only the Ll or Ld forms were considered open to attack by tissue preparations. Since l-noradrenaline was produced almost quantitatively, it was reasoned that the starting DOPS was the Dd-Ll antipodal mixture exclusively. It was suggested that the prefix "allo" be reserved for naming the still unknown Dl-Ld isomer. Further correlation with known reference compounds was not achieved, the relative configuration of l-noradrenaline being unknown. Similarity of results with DOPS preparations from different laboratories indicated their probable identity of configuration (1,2).

Little is known concerning the stereochemistry of phenylserines prepared by other methods. The new Bergmann approach (3) has interesting possibilities as a source of erythro as well as threo compounds, in view of the particularly mild reaction conditions. The configuration of N-arylphenylserines, the only class of products currently obtainable by amination of phenylglycidic acid derivatives, is yet obscure. Although both diastereomeric O-methylphenylserines have been produced via the mercuration method, neither has been correlated with known reference compounds. Catalytic reduction of α-oximinobenzoylacetic acid was recently reported to lead

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(1) K. H. Beyer, H. Blaschko, J. H. Burn and H. Langemann, Nature, 165, 926 (1950).
(2) H. Blaschko, J. H. Burn and H. Langemann, Brit. J. Pharmacol., 5, 431 (1950).
(3) E. D. Bergmann, M. Genas and H. Bendas, Compt. rend., 231, 361 (1950).

preferentially into the erythro series (1), but further experimental data are needed to substantiate this claim.

E. Heterocyclic Analogues

With the finding that phenylserine was a satisfactory starting point for synthesis of chloramphenicol, interest has understandably been given to the prospect of preparing hydroxyamino acids wherein the phenyl group was replaced by an isosteric heterocyclic nucleus. Such compounds would be worthy of investigation not only as potential sources of new antibiotic analogues, and adrenaline-type compounds, but also as likely antimetabolites in themselves.

The first phenylserine heterologue to be synthesized was β-2-furylserine (2). No product was obtained when furfural and glycine were subjected to action of sodium hydroxide in water or aqueous alcohol. However, treatment with potassium hydroxide in cold ethanol gave a clear pink solution from which the expected Schiff base salt precipitated slowly during the ensuing 24-72 hours refrigeration. Acetic acid cleaved this intermediate to furfural and furylserine, which was presumed to possess the threo configuration found in phenylserine similarly prepared. The maximum yield attained of 48%

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(1) I. Elphimoff-Felkin and H. Felkin, Compt. rend., 232, 241 (1951).
(2) K. Hayes and G. Gever, J. Org. Chem., 16, 269 (1951).

involved a three day condensation period at 10°C. Attempts to run the reaction in methanol, in which the Schiff salt was soluble, failed. Treatment of furylserine with benzoyl chloride in presence of alkali gave an azlactone, m.p. 170°.

Esterification of furylserine was possible only under closely controlled conditions. Decomposition occurred when ethanol saturated with hydrogen chloride or when temperatures greater than 30° were used. Likewise, unrecrystallized furylserine gave only an oily ester in low yield. A successful approach involved allowing furylserine to stand in dilute alcoholic hydrogen chloride solution for five days at room temperature. The ester hydrochloride was not obtained, decomposition occurring upon attempted concentration. However, neutralization of the ester hydrochloride solution with sodium ethoxide permitted isolation of the free ester in 73% yield. Although this compound decomposed slowly, its acid oxalate proved quite stable.

Furylserine ethyl ester was readily reduced by lithium aluminum hydride to furylserinol, in the manner employed with phenylserine ethyl ester. The aminediol was totally acylated without difficulty, then nitrated smoothly in good yield. Although the resultant nitro compounds gave satisfactory analytical data and ultraviolet absorption spectra closely related to those of 5-nitro-2-furfuryl esters, they could not be induced to crystallize. Further processing was not

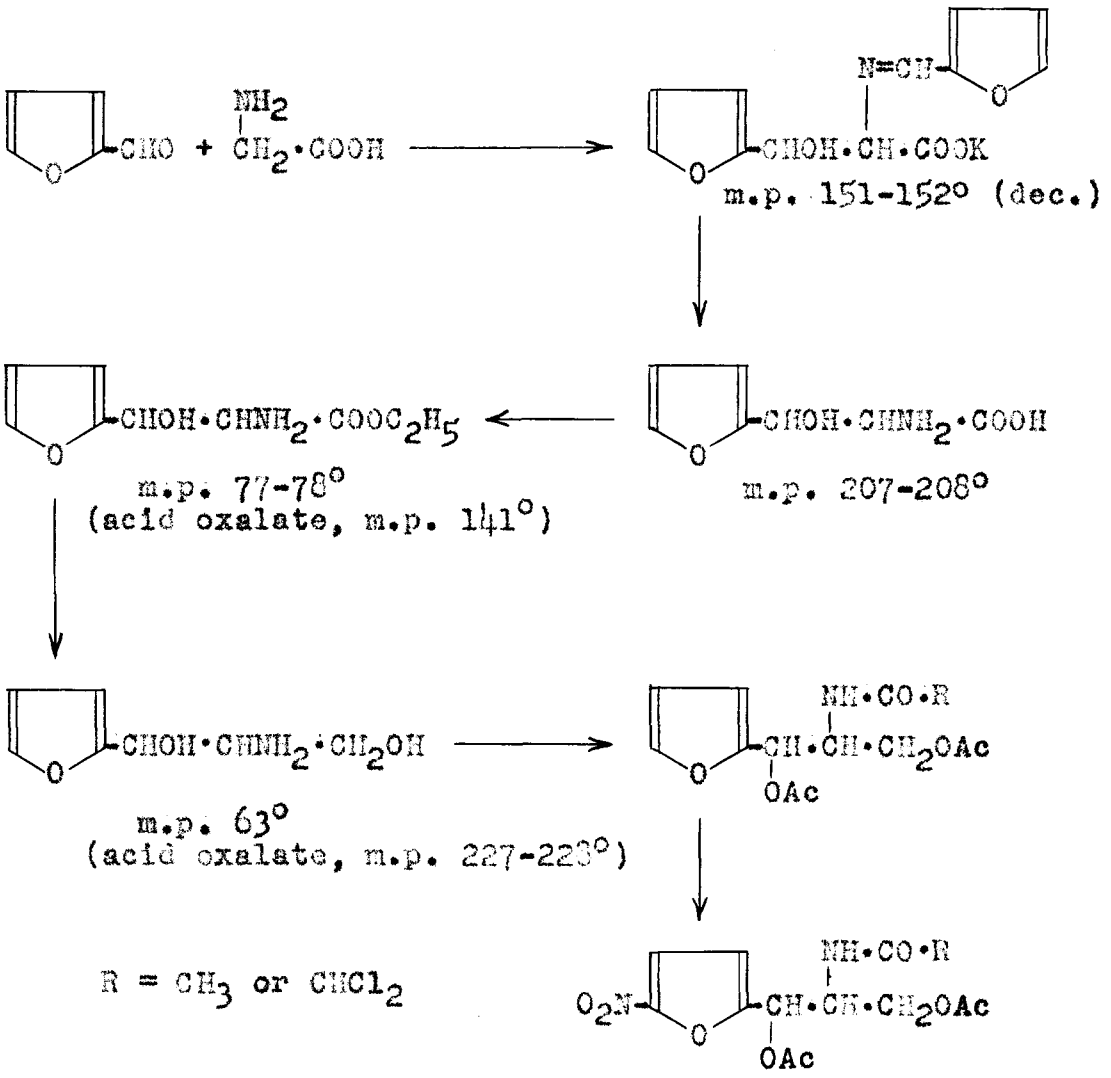

Fig. 3 Synthesis of β-2-Furylserine and Derivatives

described, nor was any mention made of biological tests with the various intermediates. The over-all reaction sequence is summarized in Fig. 3.

By use of the German patent procedure for phenylserine synthesis (1), the thiophene analogue has been obtained with relative ease (2). Glycine condensed rapidly with 2-thienaldehyde in presence of aqueous sodium hydroxide at room temperature, precipitation of the Schiff base salt commencing after half an hour. After overnight standing, trituration of the solid cake with concentrated hydrochloric acid produced white crystals of 2-thienylserine, m.p. 178-179°, in 79% yield. As with furylserine, a threo configuration was presumed. No derivatives of the new hydroxyamino acid were reported, synthesis of the thiophene analogue of chloramphenicol having already been achieved by another route (3).

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(1) Ges. für Kohlentechnik m.b.H. German Patent 632,424. July 8, 1936.
(2) G. Weitnauer, Gazz. chim. ital., 81, 162 (1951).
(3) G. Carrara and G. Weitnauer, Ibid., 81, 142 (1951).

III. EXPERIMENTAL PROCEDURES AND RESULTS*

A. Preparation of Phenylserine by Condensation of Benzaldehyde and Glycine

1. Condensation in ethanolic sodium hydroxide

Condensation was effected according to the method of Forster and Rao (1). To a mechanically stirred mixture of 18.7 g. (0.25 mole) of glycine and 53.0 g. (0.50 mole) of benzaldehyde, from a freshly opened bottle, in 150 ml. of 33% ethanol at 10° was slowly added a cold solution of 35.0 g. (0.87 mole) of sodium hydroxide in 100 ml. of water. Agitation was continued for twenty minutes, during which the turbid emulsion became homogeneous and clear. Upon standing for forty minutes, a white pasty material precipitated, leaving a syrupy yellow supernatant. During further agitation for ten minutes, the entire reaction mixture set to a viscous paste.

After overnight standing at room temperature, the condensation product was filtered off and washed with four 125 ml. portions of cold ethanol, a rubber dam being employed each time to press the cake as dry as possible. Each filtration required from one to three hours, with No. 1 or No. 50

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* All melting points are uncorrected.

(1) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).

Whatman paper and regardless of whether the mixture was at room temperature or prechilled in an ice bath. The pH of the fourth alcoholic wash still exceeded 10.

The filter cake was dried in air, then extracted with four 100 ml. portions of boiling water, with filtration, to leave only a small residue of N-benzaldiphenylhydroxyethylamine. The filtrate was cooled to room temperature, acidified with 35 ml. of glacial acetic acid and extracted with three 100 ml. volumes of ether to remove liberated benzaldehyde. The aqueous layer was concentrated in vacuo. Four crystal crops were filtered off during distillation to curtail troublesome bumping. The final filtrate, which approximated 50 ml. in volume and smelled strongly of acetic acid, was discarded.

The four crops of crystals were combined, dissolved in 175 ml. of boiling water, and, after filtration, treated with 175 ml. of ethanol. Following storage at 5° for two hours, the resultant iridescent crystals were filtered off, washed with several portions of ethanol and dried in air to give 18.6 g. (37% yield) of phenylserine monohydrate, dec. pt. 195°, 197° (separate determinations: bath initially at 191°, 3 minutes heating). Two further crops of crystals were obtained from the alcoholic filtrate upon concentrating, each amounting to only 0.9 g. (2% yield) and each decomposing in the same range as the first crop.

2. Condensation in aqueous potassium hydroxide

To a mechanically stirred solution of 375 g. (5.0 moles) of glycine and 420 g. (7.5 moles) of potassium hydroxide in 1250 ml. of water at 20° was added 1061 g. (10.0 moles) of benzaldehyde from a freshly opened bottle. Temperature of the reaction mixture rose to a maximum of 30° in fifteen minutes, and fell gradually thereafter. Turbidity disappeared within thirty minutes. During a four hour period of agitation, the viscosity of the dark yellow liquid increased substantially, but only a small amount of insoluble suspensoid was visible. After overnight standing at room temperature, the mixture was a thick pale yellow paste.

The mechanically agitated paste was treated with 500 ml. of concentrated hydrochloric acid in several small portions. Temperature rose to 43° momentarily. The observed pH 4.7 was adjusted to 5.7 by addition of 30 ml. of concentrated ammonium hydroxide. Mixing was continued with ice bath cooling until the temperature had fallen to 5°. The resultant granular slurry was suction-filtered, and a further 250 ml. of water was passed through the cake to partly displace the dark orange filtrate, which was surmounted by a layer of benzaldehyde cleaved from the condensation intermediate by action of the acid.

The pale orange filter cake from acid treatment was washed by mixing well with four 1250 ml. portions of boiling

95% ethanol, suction-filtration being employed each time. The now faintly colored cake was simmered briefly with 2000 ml. of boiling water and suction-filtered; approximately one fifth of the material failed to dissolve. This residue was treated with a further 1000 ml. of boiling water as before, but it hardly diminished in quantity. The water-insoluble residue was discarded, since its ready solubility in methanol, whence it was precipitated anew on addition of water, suggested diphenylhydroxyethylamine rather than phenylserine.

The combined hot aqueous extracts, wherein precipitation of crude phenylserine had already commenced, were stored at 5° for three days, then suction-filtered with the aid of a rubber dam to give a yellow filtrate and a buff-colored filter cake which was somewhat sticky and smelled of benzaldehyde. This cake was washed by simmering with 500 ml., then two 250 ml. portions of methanol with suction-filtration each time. The first hot methanol wash, orange in color, dissolved almost half of the cake; the other methanol washes, almost colorless, had scant apparent effect. The methanol-insoluble creamy powder was taken up in 1500 ml. of boiling water; the resultant pale yellow solution was suction-filtered to remove dust, reheated to 90° and treated with an equal volume of boiling ethanol.

After overnight storage at 5°, white lustrous crystalline plates were filtered from the 50% ethanol slurry, and

washed first with 500 ml. of 50% ethanol, then 250 ml. of 95% ethanol. Thus were obtained, after drying in air to constant weight, 168 g. (17% yield) of phenylserine monohydrate first crop.

The orange benzaldehyde-water filtrate from the acid treatment yielded only red tars upon concentration, as well as light colored powders which, by their infusibility, appeared to be mainly potassium chloride. Likewise, working up the 95% ethanol washings failed to give any more phenylserine.

Stepwise concentration <u>in vacuo</u> of the aqueous filtrate from crude phenylserine, the methanol washings and the 50% ethanol filtrate from the first crop, respectively, led to seven crystalline fractions, totalling 148 g., which were combined and recrystallized from 50% ethanol in the manner described above for obtaining the first crop of phenylserine. This gave 99 g. (10% yield) of second crop then, after sub-zero storage of the filtrate therefrom, 24 g. (2.4% yield) of third crop, as white microcrystalline powders.

Decomposition points were determined simultaneously for the above first crop material, for a mixture of this with first crop phenylserine monohydrate obtained by the Forster and Rao procedure, and for the latter substance alone as a control. Triads involving the above second and third crops were run similarly. All samples decomposed in the range

190-193°, with initial bath temperatures of 174-179°.

Samples of first crop phenylserine monohydrate were desiccated over anhydrous magnesium perchlorate at 10-15 mm. and 50-60° to constant weight during twenty-four hours. Dehydration was accompanied by some loss of lustre and shrinkage.

Anal. Calcd. for $C_9H_{11}O_3N \cdot H_2O$: H_2O, 9.05
Found: H_2O, 9.4, 8.8

3. Condensation in aqueous sodium hydroxide

Condensation was effected according to the German patent procedure (1), with minor extensions. To a mechanically stirred solution of 30.0 g. (0.40 mole) of glycine and 24.0 g. (0.60 mole) of sodium hydroxide in 100 ml. of water at 15° was added 84.9 g. (0.80 mole) of benzaldehyde, from a freshly opened bottle, with external water bath cooling at 15°. Temperature rose to a maximum of 21° in seven minutes, and declined gradually thereafter. The emulsified reaction mixture became homogeneous and clear in 12-13 minutes. Viscosity increased steadily and formation of suspensoid particles commenced. Rapid gelation and final solidification to a cheesy mass occurred within 45 minutes.

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(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

After standing overnight at room temperature, the solid condensation cake was fragmented, and, with steady agitation and external cooling at 15°, 49 ml. (0.60 mole) of concentrated hydrochloric acid was slowly added. The lumps disintegrated as a white precipitate of phenylserine was gradually thrown down. During mixing for one hour, the phenylserine agglomerated into oily pellets approximating 1-2 mm. in diameter.

Following overnight storage at 5°, the crude phenylserine was filtered off and sucked as dry as possible with the aid of a rubber dam. The straw-colored filtrate, at pH 3.8, was separated from the surmounting benzaldehyde layer and treated with an equal volume of ethanol. When overnight refrigeration failed to induce further deposition of phenylserine, this material was discarded. The filter cake was fragmented and washed by mixing well with three 250 ml. portions of boiling 95% ethanol, the resultant thin slurry being suction-filtered each time. The white microcrystalline phenylserine thus obtained was dried over anhydrone at 50-60° and 10-15 mm. to a constant weight of 50.5 g. (70% yield).

The ethanol-washed phenylserine was recrystallized from 1000 ml. of hot 50% aqueous methanol in the manner described in earlier runs (45.3 g., 90% recovery) and then from 900 ml. of hot 50% aqueous ethanol to give finally 42.8 g. (85% recovery from the original 50.5 g.) of anhydrous phenylserine as shimmering plates. The combined alcoholic recrystallization

filtrates were concentrated in vacuo to a moist residue, which was taken up in 80 ml. of boiling water and treated with the same volume of hot dioxane. Overnight refrigeration, filtration, washing and desiccation led to 9.0 g. (18% recovery) more of phenylserine as fine white microcrystals (dec. pt. 195°, bath initially at 177°).

The combined 95% ethanol washings from the crude phenylserine were concentrated in vacuo to 100 ml., then treated with 100 ml. of ether. Filtration, ether washing and drying of the precipitate thus formed gave 11.2 g. of white powdery crystals which showed a strongly positive ninhydrin test. This material, upon recrystallization from hot 66% aqueous dioxane, yielded a further 2.3 g. (5% yield) of anhydrous phenylserine (dec. pt. 189°, bath initially at 177°).

4. Attempted synthesis of β-2-furylserine

The condensation procedure was modelled on that of the German patent method for preparation of phenylserine (1). To a mechanically stirred solution of 15.0 g. (0.20 mole) of glycine and 12.0 g. (0.30 mole) of sodium hydroxide in 50 ml. of water at 15° was added 38.4 g. (0.40 mole) of freshly distilled colorless furfuraldehyde, with water bath cooling at 15°. Temperature rose to a maximum 25° in two minutes and

(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

declined gradually thereafter. The emulsified reaction mixture clarified completely in the same time interval and assumed an orange color which increased in intensity to brown during fifteen minutes. No precipitation was observed during three and one-half hours of agitation. After storage at 5° for 42 hours, the reddish brown syrup was still clear. It was therefore discarded.

B. Preparation of Compounds Concerned in Transformation of Phenylserine to Chloramphenicol

1. Phenylserine methyl ester hydrochloride

A vigorous stream of dry hydrogen chloride was passed through a suspension of 9.0 g. (0.045 mole) of monohydrated phenylserine (recrystallized first crop material) in 90 ml. of absolute methanol for two hours. Speedy solution of the amino acid was accompanied by evolution of sufficient heat to promote gentle refluxing, with temperature falling slowly thereafter. No crystallization occurred upon overnight storage at -15°. The clear colorless solution was concentrated *in vacuo* to a crystalline residue which was dissolved in 50 ml. of warm methanol. Upon treatment of the methanol solution with 300 ml. of ether, two layers formed with rapid crystallization ensuing at the interface. Overnight storage at -15°, filtration, double washing with ether and drying at 50-60° gave 8.6 g. (81% yield) of phenylserine methyl ester

hydrochloride as white iridescent flakes, m.p. 160° (dec.), unchanged by recrystallization from methanol-ether. Analytical data were obtained on a larger batch similarly prepared.

Anal. Calcd. for $C_{10}H_{14}O_3NCl$: N, 6.05; Cl, 15.30
Found: N*, 6.11, 6.08; Cl (gravimetric), 15.31, 15.38, 15.33

2. Phenylserine ethyl ester hydrochloride

Synthesis of this compound was effected in the manner described for the methyl homologue, starting with absolute ethanol. An 85% yield of product was obtained, m.p. 140°, unchanged by recrystallization from ethanol-ether. Analytical data were obtained on a larger batch similarly prepared.

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: N, 5.70; Cl, 14.43
Found: N, 5.61, 5.70; Cl (gravimetric), 14.32, 14.32, 14.42

3. Phenylserine methyl ester

A vigorous stream of dry ammonia was passed through a suspension of 11.5 g. (0.050 mole) of phenylserine methyl ester hydrochloride in 200 ml. of ether for fifteen minutes. The ester hydrochloride dissolved, and temperature fell

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* N analyses in this investigation were effected by Mr. J.A. McMillan, the Kjeldahl micromethod being employed in all cases.

rapidly due to evaporation effects. The well granulated ammonium chloride which precipitated was removed readily by passage through a fluted filter, the salt being freed of adherent ester by flushing with several portions of warm ether. Evaporation of the combined ether filtrate and washings in a dry air stream gave 9.4 g. (97% yield) of crude phenylserine methyl ester, m.p. 60-61°, as white granular crystals. Recrystallization from a 1:4 mixture of hot ether-cyclohexane yielded long white needles, m.p. 62°.

Anal. Calcd. for $C_{10}H_{13}O_3N$: N, 7.17
Found: N, 7.14, 7.20

4. Phenylserine ethyl ester

Phenylserine ethyl ester hydrochloride (12.3 g., 0.050 mole) was treated in the manner described for preparation of phenylserine methyl ester to give 9.9 g. (95% yield) of crude phenylserine ethyl ester, m.p. 82-83°, as large white plates. Recrystallization from hot cyclohexane steadied the melting point to 83°, with decline occurring to 81-82°, after storage for ten weeks in a desiccator.

Anal. Calcd. for $C_{11}H_{15}O_3N$: N, 6.69
Found: N, 6.53, 6.65

5. Phenylserine ethyl ester directly from phenylserine

In one run, phenylserine monohydrate was treated in ethanol with dry hydrogen chloride in the fashion described earlier. The residue remaining after distillation of the ethanolic hydrogen chloride was taken up in ether and converted to the free ester with ammonia in the manner already described. Yield of several crystal crops, melting in the range 82-84°, totalled 91%.

6. Phenylserinol from phenylserine methyl ester

In an oven-dried all-glass assembly consisting of a 500 ml. three-necked flask fitted with dropping funnel, oil-sealed Hershberg stirrer and reflux condenser with attached drying tube, 2.28 g. (0.060 mole) of lithium aluminum hydride was suspended in 50 ml. of sodium-dried ether. With steady agitation, a solution of 3.90 g. (0.020 mole) of phenylserine methyl ester in 225 ml. of dry ether was added slowly via the dropping funnel. Thirty minutes after evolution of hydrogen from the faintly greenish-yellow turbid reaction mixture had ceased, excess lithium aluminum hydride was destroyed by slow addition of 200 ml. of ether saturated with water, followed by 10 ml. of water. After mixing for a further fifteen minutes, the creamy slurry was suction-filtered to give a colorless ether filtrate and white powdery cake.

The ether filtrate was concentrated *in vacuo* below 40° to a yellow oil, which was taken up in 10 ml. of hot ethyl acetate, suction-filtered to remove dust, warmed almost to the boiling point and then treated with 10 ml. of cyclohexane to produce a faint turbidity. A yellow oily layer separated on cooling. The mixture was seeded with phenylserinol, rapid deposition of a flocculent precipitate ensuing upon further cooling to -10°. Suction-filtration, washing with three 2 ml. portions of ethyl acetate-cyclohexane (1:1) and drying at 50-60° yielded 1.37 g. (41% yield) of phenylserinol as a white powder, m.p. 88-90°. Thrice recrystallized *dl-threo*-1-phenyl-2-amino-1,3-propanediol has been reported to melt at 86-87° (1).

The white filter cake obtained after the reduction was extracted with three 100 ml. portions of boiling ethanol. Concentration of the extracts *in vacuo* yielded a yellow viscous oil. Working this material successively in chloroform, ethanol-ether, and ethyl acetate gave only orange gums and infusible residues from which no more phenylserinol was obtained.

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., *J. Am. Chem. Soc.*, 71, 2463 (1949).

7. Phenylserinol from phenylserine ethyl ester

In an oven-dried all-glass assembly consisting of a 500 ml. three-necked flask fitted with an oil-sealed Hershberg stirrer and Soxhlet extraction unit surmounted by a reflux condenser with attached drying tube, 5.93 g. (approximately 0.16 mole) of finely powdered lithium aluminum hydride was suspended in 250 ml. of sodium-dried ether. After a Soxhlet cup lined with No. 1 Whatman paper to reduce porosity and containing 10.46 g. (0.0500 mole) of phenylserine ethyl ester had been placed in the extraction chamber, agitation and heating over a water bath were initiated in such a way that siphoning occurred at roughly four-minute intervals. Hydrogen evolution diminished after 75 minutes. The water bath was removed at the close of two hours and, to destroy excess lithium aluminum hydride, ten 1.0 ml. portions of water were introduced via the Soxhlet cup, with allowance for two siphonings between additions. A further 100 ml. of ether was added to compensate for evaporation loss. After the deposit adhering to the flask wall had been loosened with the aid of a stiff wire, refluxing with agitation was effected for a further half-hour, then 250 ml. of 10% aqueous sodium hydroxide was slowly added, and mixing was continued for five minutes longer.

The basic emulsion was cleaved in a separatory funnel, and the aqueous layer was extracted with five more 250 ml.

portions of ether. The combined ether extracts were concentrated *in vacuo* to a straw-colored syrupy gum, which was taken up in 37 ml. of hot ethyl acetate and treated with 25 ml. of cyclohexane to produce a faint turbidity. By cooling at 5° for three hours, washing twice with fresh solvent mixture and drying at 50-60°, 4.12 g. (49% yield) of phenylserinol was obtained as snow-white microcrystals, m.p. 88-89°, unchanged by recrystallization from ethyl acetate-cyclohexane.

The ether-extracted alkaline aqueous layer was treated with 50 ml. of 50% sodium hydroxide, the concentration of base thus being raised to approximately 16%, in an endeavor to secure more effective solution of aluminum hydroxide present. When gelatinous emulsions were encountered upon shaking with ether, the mixture was diluted with 100 ml. of water. Six more extractions with 250 ml. portions of ether were carried out, and the combined ether extracts were treated as before to give a further 2.16 g. (26% yield) of microcrystalline phenylserinol, m.p. 88-89°.

The basic layer was diluted with water to 500 ml. and shaken with 200 ml. of methylene dichloride. The resultant stiff emulsion showed little sign of cleavage on standing and was accordingly discarded. Concentration of the ethyl acetate-cyclohexane filtrates to a small amount of oil, which was worked up with ethyl acetate and cyclohexane in the described fashion, gave 0.12 g. (1.4% yield) of less pure phenylserinol, m.p. 86-88°.

8. N-Acetylphenylserinol and N,O-diacetylphenylserinol

Phenylserinol was acetylated in the manner outlined by the Parke, Davis and Co. group (1). A sample of 1.07 g. (0.0064 mole) of phenylserinol was heated with 10 ml. of acetic anhydride in a water bath at 70 ± 2° for fifteen minutes. Acetic anhydride was removed in vacuo to give a clear pale yellow viscous syrup which was taken up in 10 ml. of hot ethanol and again concentrated to dryness. Rubbing with 10 ml. of hot cyclohexane gave a white gummy paste which was dissolved by addition of 10 ml. of hot ethanol. After filtering off dust, cooling at 0° for 30 minutes, and again filtering, 0.43 g. (27% yield) of crude N,O-diacetyl compound, m.p. 161-164°, was obtained. Recrystallization from boiling water gave N,O-diacetylphenylserinol, as glittering clusters of large white polyhedra, m.p. 168-169°. Twice recrystallized from ethanol, dl-threo-N,O-diacetyl-1-phenyl-2-amino-1,3-propanediol has been reported to melt at 168-169° (1). Treatment of the ethanol-cyclohexane filtrate with 10 ml. of cyclohexane, followed by cooling and filtration led to a second 0.12 g. (7% yield) crop of less pure N,O-diacetyl derivative, m.p. 154-164°.

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

Addition of a third 10 ml. volume of cyclohexane to the ethanol-cyclohexane filtrate produced 0.10 g. (7% yield) of a fine white powder, m.p. 128-130°, containing a few large colorless dense crystals, m.p. 134-135°. Recrystallization of this material from hot ethyl acetate gave small white thin parallelopipeds, m.p. 134-135°. Two forms of dl-threo-N-acetyl-1-phenyl-2-amino-1,3-propanediol have been reported (1), melting at 136-137° and 144-145° respectively.

9. Triacetylphenylserinol

This compound was prepared by a modified Parke, Davis and Co. procedure (1). A solution of 7.91 g. (0.047 mole) of phenylserinol in 60 ml. of acetic anhydride and 60 ml. of pyridine was allowed to stand at room temperature for 48 hours, with occasional shaking, then was concentrated in vacuo to a brown syrup. This residue was taken up in 100 ml. of warm ethyl acetate, the resultant solution being washed successively with 35 ml. of 5% hydrochloric acid to remove pyridine, 15 ml. of water and 25 ml. of 5% aqueous sodium bicarbonate. The washes were back-extracted in order with 25 ml. of ethyl acetate. The combined ethyl acetate solutions were dried over anhydrous sodium sulfate, then

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

concentrated *in vacuo* to a pale amber gum from which solvent vapor bubbles had ceased to emerge. A solution of the gum in 50 ml. of hot ethyl acetate was treated with 80 ml. of hot Skelly B. As temperature declined, the mixture clouded and split out a lower pale orange oily layer, which set to a hard crystalline mass upon overnight storage at 5°.

The crystal cake was fragmented, filtered, washed with cold solvent and dried to give 10.39 g. (75% yield) of triacetylphenylserinol as long blunt-ended white needles, m.p. 80-81°. Concentration of the filtrate *in vacuo* and working up the gum as before yielded a further 1.89 g. (14% yield), m.p. 80-81°, and 0.57 g. (4% yield), m.p. 79-80°, as second and third crops. Twice recrystallized from ethanol-petroleum ether, *dl-threo*-triacetyl-1-phenyl-2-amino-1,3-propanediol has been stated to melt at 79-80° (1).

10. *Triacetylphenylserinol directly from phenylserine ethyl ester*

With the Soxhlet technique described for preparation of phenylserinol, 4.18 g. (0.020 mole) of phenylserine ethyl ester was reduced with 2.28 g. (0.060 mole) of lithium aluminum hydride. To the reaction mixture quenched with 5 ml. of water was added 100 ml. of acetic anhydride and

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., *J. Am. Chem. Soc.*, *71*, 2463 (1949).

100 ml. of pyridine. After most of the ether had been drawn off *in vacuo*, the mixture was allowed to stand at room temperature, with occasional shaking, for 42 hours.

The gelatinous mixture was suction-filtered with some difficulty during nine hours. The residual bluish paste, after double washing with acetic anhydride-pyridine (1:1), was discarded when qualitative tests showed it to be predominantly aluminum acetate. The pale brown filtrate was concentrated *in vacuo* to a heavy syrup. Addition of 100 ml. of hot ethyl acetate, followed by five days standing at room temperature, threw down more aluminum acetate, which was again removed by filtration. When Skelly B added to an aliquot of filtrate produced two layers and no crystals, even after prolonged cooling, *in vacuo* concentration was again effected to an orange gummy syrup, in which the odor of pyridine had greatly diminished. The gum was dissolved in 50 ml. of hot ethyl acetate, and, after removal of 0.24 g. of infusible material, believed to be lithium acetate on the basis of a brilliant brick-red flame test, 100 ml. of cyclohexane was added. Further filtration at this point eliminated 0.1 g. of brown gum, leaving a straw-colored filtrate, which became turbid upon addition of 100 ml. of Skelly B.

Overnight refrigeration at 5° gave a gummy yellow crystalline mass surmounted by needles 5 mm. or more in length. The latter were carefully suspended without disturbing

the gum, filtered, washed twice with cold solvent mixture and dried at 50-60° to give 1.26 g. (22% yield) of triacetyl-phenylserinol as snow-white needles, m.p. 81-82°, undepressed upon admixture with material prepared from phenylserinol. A further 1.54 g. (26% yield), m.p. 79-81°, was worked up from the filtrate of the first crop, but the gum from which the latter had been skimmed was discarded.

11. Triacetyl-p-nitrophenylserinol

Processing here was according to the Parke, Davis and Co. method (1). With steady agitation and temperature maintained at 18-20°, 2.93 g. (0.010 mole) of triacetyl-phenylserinol was fed in small portions during eight minutes into 8 ml. of fuming nitric acid (d. 1.46) previously decolorized with urea. After 30 minutes total mixing time, 2 volumes of ice were added to arrest reaction, then sodium bicarbonate in small portions until evolution of carbon dioxide ceased. Four extractions with 25 ml. volumes of ethyl acetate were performed, and the combined extracts were washed with water, dried over anhydrous sodium sulfate, then concentrated *in vacuo* to a pale yellow gum. This gum was dissolved in 10 ml. of hot ethyl acetate, treated with the same volume of Skelly B and stored at 5° overnight.

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

By rubbing with a rod to accelerate crystallization, filtering, washing twice with cold solvent mixture and drying, 1.23 g. (36% yield) of crude triacetyl-p-nitrophenylserinol, m.p. 125-132°, was obtained as a faintly yellow powder. By merely washing this product with hot ether, the melting range was raised to 138-141° or alternatively, to 140-143° by a single recrystallization from benzene-ether, in either case a white granular solid being produced. Recrystallized from acetone-petroleum ether, then twice from ethanol, dl-threo-triacetyl-1-p-nitrophenyl-2-amino-1,3-propanediol was reported to melt at 140-143°, and, with two more recrystallizations from water, at 146-147° (1).

12. p-Nitrophenylserinol

Nitration of 2.93 g. (0.010 mole) of triacetylphenylserinol was repeated according to the procedure described in the preceding section, with advantageous replacement of ethyl acetate by methylene dichloride for extraction of the neutralized nitration mixture. The gum obtained by in vacuo concentration of the water washed, sodium sulfate-dried methylene dichloride extracts was hydrolyzed by heating with 45 ml. of concentrated hydrochloric acid on a boiling water bath for two and one-half hours. After being washed with

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

ether, the acid solution was concentrated *in vacuo* to a pale yellow gum which was dissolved in 6 ml. of boiling water and treated with solid sodium bicarbonate (0.5 g.) in small portions until carbon dioxide evolution ceased and the pH approximated 8. After overnight storage at 5°, the clear aqueous solution was extracted with three 5 ml. volumes of ethyl acetate. Working up this series of extracts in the usual manner led only to a small amount of gum, which was discarded.

The aqueous layer was adjusted to pH 11 by dropwise addition of 50% sodium hydroxide and extracted three times with ethyl acetate as before. Flocculation commenced in the sodium sulfate-dried yellow extracts upon standing at room temperature. After overnight storage at 5°, filtration, washing with a little cold ethyl acetate and drying at 50-60°, 0.63 g. (30% yield) of crude *p*-nitrophenylserinol was recovered as a white powder, m.p. 138-140°. Recrystallization from 15 ml. of hot water gave 0.48 g., m.p. 140-141°. A superficial yellow to orange color slowly developed in the material upon exposure to light. Recrystallized from water, *dl-threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol was reported to melt at 139-141°, and, with further recrystallization from water, at 141.5-142.5° (1).

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

13. N-Dichloroacetyl-p-nitrophenylserinol (racemic chloramphenicol): synthesis and bioassay

Dichloroacetylation was carried out according to the Parke, Davis and Co. method (1), with only slight modification. With a gentle stream of dry air drawn through the reaction mixture to sweep out methanol formed, 0.212 g. (0.0010 mole) of p-nitrophenylserinol was heated with 3.0 ml. of methyl dichloroacetate on a boiling water bath for two hours. After removal of excess ester in vacuo, the orange-yellow gum produced was washed by simmering with three 10 ml. volumes of Skelly B, then dissolved in 3.0 ml. of hot ethyl acetate and treated with 3.6 ml. of Skelly B. When refrigeration at 5° for two hours gave only oil, methanol was added to effect solution, a small amount of insoluble matter was filtered off and the filtrate was concentrated to dryness in vacuo. The orange residual oil was taken up again in 3.0 ml. of hot ethyl acetate, the slightly turbid solution being stored at 5° overnight. Filtration, dropwise washing with 1.0 ml. of cold ethyl acetate and drying at 50-60° led to 0.142 g. (44% yield) of N-dichloroacetyl-p-nitrophenylserinol in the form of hard snow-white microcrystals, m.p. 150-151°. Mixture with an authentic sample of racemic chloramphenicol (m.p. 151-152°) did not depress the melting point. Twice

(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

recrystallized from ethyl acetate-petroleum ether and twice from water, dl-threo-N-dichloroacetyl-1-p-nitrophenyl-2-amino-1,3-propanediol was stated to melt at 150.5-151.5° (1).

Table 12

Microbiological Assay Results (2)

Organism	DL-Chloramphenicol KS-II-87	D-Chloramphenicol (Parke Davis X-2791)
E. coli (26)	5.0 (a)	2.5
S. aureus (FDA-209)	5.0	2.5
B. subtilis (III)	3.3	1.25
K. pneumoniae (PCI-602)	1.7	1.0
P. vulgaris (8427)	1.7	1.0
M. tuberculosis (607)	17.0	10.0

(a) Expressed as micrograms of antibiotic required to inhibit the test organism

The results of microbiological assay, presented in Table 12, showed that the product was approximately one half as active as the D form of the antibiotic.

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).
(2) D. R. Colingsworth, The Upjohn Co. Private communication, 1950.

C. Characterization of Allophenylserine

1. Allophenylserine ethyl ester hydrochloride

A mixture of 23.5 g. of third crop and 30.9 g. of second crop phenylserine from potassium hydroxide-catalyzed glycine-benzaldehyde condensation was suspended in 540 ml. of absolute ethanol in an all-glass assembly consisting of a 1000 ml. three-necked flask surmounted by a gas inlet tube and reflux condenser with attached drying tube. A vigorous stream of dry hydrogen chloride was passed through the suspension during four hours. Complete solution occurred during twenty minutes, and sufficient heat was generated to induce gentle refluxing. As temperature gradually declined to room level, precipitation of fine white crystals set in.

After refrigeration at 5° for three hours, suction-filtration, washing with cold ethanol, then ether, and drying at 50-60°, 24.7 g. (34% yield) of allophenylserine ethyl ester hydrochloride, m.p. 175-176° (dec.), was obtained as glittering fluffy white flakes. After two recrystallizations from absolute ethanol, the material decomposed sharply at 176°.

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: N, 5.70; Cl, 14.43
Found: N, 5.73, 5.77; Cl (gravimetric), 14.36, 14.39, 14.40

Concentration of the ethanolic hydrogen chloride filtrate and treatment with ether led to a 11.9 g. (16% yield) second

crop, m.p. 140-150°, and a 12.6 g. (17% yield) third crop, m.p. 135-141°, of mixed ethyl ester hydrochlorides. Similar processing of tail crops of crude phenylserine prepared according to the German patent method (1) also led to varying amounts of allophenylserine ethyl ester hydrochloride.

2. Allophenylserine ethyl ester

Allophenylserine ethyl ester hydrochloride (12.3 g., 0.050 mole) was treated in ether with ammonia gas in the manner described for preparation of phenylserine methyl ester. After removal of ammonium chloride, stepwise evaporation of the ether solution gave a 3.9 g. (37% yield) first crop, as long white needles, m.p. 86°, a 3.1 g. (30% yield) second crop, m.p. 86°, and a 2.6 g. (25% yield) residue, m.p. 85-86°. Recrystallization of the three crops from 250 ml. of cyclohexane led to 8.7 g. (91% recovery) of small shimmering white crystal flakes, m.p. 83-84°, falling to 82-83° after storage in a desiccator for a week. A mixture of phenylserine and allophenylserine ethyl esters melted in the range 63-71°.

Anal. Calcd. for $C_{11}H_{15}O_3N$: N, 6.69
Found: N, 6.64, 6.66

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(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

3. Allophenylserinol

By using the Soxhlet technique described for preparation of phenylserinol from phenylserine ethyl ester, 4.18 g. (0.020 mole) of allophenylserine ethyl ester was reduced with 2.28 g. (0.060 mole) of lithium aluminum hydride. The reaction mixture, quenched with 10 ml. of water, was filtered through a Soxhlet extraction cup, and the filtrate with a further 100 ml. of ether was used to effect extraction of the insoluble residue in the standard Soxhlet manner for one hour. The ether extract was concentrated in vacuo and the yellow oil taken up in 10 ml. of ethyl acetate. Crystallization commenced upon addition of 0.25 ml. of cyclohexane, 0.25 ml. of chloroform and 0.5 ml. of Skelly D. By overnight storage at 5°, filtration, cold ethyl acetate washing and drying at 50-60°, 0.98 g. (30% yield) of allophenylserinol was obtained as glittering white thin plates, m.p. 104-105°. Concentration of the filtrate in vacuo, solution in chloroform and precipitation with Skelly D gave 0.14 g. (4% yield) of a less pure product, m.p. 99-102°. Recrystallized from chloroform, dl-erythro-1-phenyl-2-amino-1,3-propanediol was stated to melt at 104-105° (1).

A second Soxhlet extraction of the reduction residue with 200 ml. of ether for two hours, with subsequent processing

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

in similar fashion via ethyl acetate and Skelly D, led to a first crop of 0.47 g. (14% yield), m.p. 105-106°, and a second crop of 0.19 g. (6% yield), m.p. 102-104°. A third extraction of the residue, effected by thorough mixing with 150 ml. of ether, followed by filtration, and processing of the extract as before, yielded a further 0.20 g. (6% yield), m.p. 103-104°. Only a small amount of gum was obtained from further extraction of the reduction residue with hot chloroform.

4. N-Acetylallophenylserinol

Monoacetylation of allophenylserinol was effected according to the Parke-Davis method (1). A sample of 0.310 g. (0.0019 mole) of allophenylserinol was shaken with 1.0 ml. of acetic anhydride. Spontaneous warming, but no crystallization occurred on subsequent cooling. The clear gum produced by in vacuo removal of acetic anhydride was taken up in ethanol and again evaporated to dryness. Crystallization of the residue from ethyl acetate-cyclohexane gave a first crop of 0.233 g. (60% yield), m.p. 106-108°, and a second crop of 0.053 g. (14% yield), m.p. 107-108°. Recrystallization of the combined crops from ethyl acetate produced 0.205 g. (80% recovery) of N-acetylallophenylserinol as clustered elongated white tablets, m.p. 108°. After recrystallization from ethyl

(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

acetate, dl-erythro-N-acetyl-1-phenyl-2-amino-1,3-propanediol was reported to melt at 106.5-107° (1).

5. N,O-Diacetylallophenylserinol and triacetylallophenylserinol

Processing was in accord with Parke-Davis methods (1). A solution of 0.658 g. (0.0039 mole) of allophenylserinol in 2.0 ml. of acetic anhydride was heated at 100° on a water bath for thirty minutes. The pale brown gum remaining after *in vacuo* concentration to dryness was dissolved in 10 ml. of hot ethyl acetate, then treated with 30 ml. of ether and 90 ml. of cyclohexane. After cooling to 15°, filtering, washing with cold solvent mixture and drying at 50-60°, 0.550 g. of cream-colored slightly sticky powder, m.p. 98-111°, was obtained. Recrystallization twice from benzene and once from ethyl acetate finally produced 0.288 g. (29% yield) of N,O-diacetylallophenylserinol, as square or octagonal tablets, m.p. 109-110°. Twice recrystallized from ethyl acetate, dl-erythro-N,O-diacetyl-1-phenyl-2-amino-1,3-propanediol was stated to melt at 110-111° (1).

All solutions and residues from the diacetyl derivative were collected in methanol, concentrated to dryness *in vacuo*, and allowed to stand in 2.0 ml. of acetic anhydride plus 2.0 ml. of pyridine for four days. The gum remaining after

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

removal of the acetylating agents *in vacuo* was crystallized from 10 ml. of ethyl acetate to give 0.349 g. (30% yield) of triacetylallophenylserinol in the form of long thin needles, m.p. 116°. Treatment of the filtrate with Skelly D yielded a 0.183 g. (16% yield) second crop, m.p. 115-116°. After two recrystallizations from ethanol, *dl-erythro*-triacetyl-1-phenyl-2-amino-1,3-propanediol was reported to melt at 115-116° (1).

6. *Allophenylserine*

Hydrolysis of 9.828 g. (0.0400 mole) of allophenylserine ethyl ester hydrochloride was effected by addition of 41.00 ml. of 1.95 *N* (0.0800 mole) sodium hydroxide and 2.0 ml. of ethanol, with standing at room temperature and intermittent shaking during 90 minutes. The clear solution was treated with 3.37 ml. (*ca.* 0.040 mole) of concentrated hydrochloric acid to give *ca.* pH 5, rapid crystallization occurring during the operation. After overnight storage at 5°, suction-filtration, two washings with 10 ml. volumes of ice water and drying in air at room temperature to constant weight, 5.583 g. (77% yield) of allophenylserine was obtained as an interwoven fibrous mat of long slender thread-like needles, with texture comparable to asbestos.

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., *J. Am. Chem. Soc.*, *71*, 2463 (1949).

No significant decline in weight occurred upon desiccation over anhydrone at 50-60° and ca. 0.1 mm. during 16 hours. Upon recrystallization for analysis from hot water with slow cooling, the same crystal form was obtained.

Anal. Calcd. for $C_9H_{11}O_3N$: N, 7.73

Found: N, 7.64

Samples from different preparations decomposed in the range 189-193°, depending on heating rate and initial bath temperature. No depression was noted upon admixture with phenylserine.

D. Partial Separation of Phenylserine and Allophenylserine via Ethyl Ester Hydrochlorides

1. Effect of recrystallization on ratio of phenylserine diastereomers

Crude phenylserine was prepared according to the German patent (1), with two steps in the procedure changed. Acidification of the intermediate condensation product with concentrated hydrochloric acid was effected after only four hours standing, instead of the 24 hours specified. After three hot ethanol washes in the already described manner, the crude phenylserine was dried in air at 50-60°, rather than over anhydrone in vacuo.

Solution of 5.00 g. portions of crude phenylserine was effected in 25 ml. volumes of water by gentle simmering. To

(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

each hot solution was added 25 ml. of hot organic solvent, the mixture being allowed to cool slowly to room temperature before overnight storage at 5°. Filtration and drying in air at 50-60° led to data assembled in Table 13.

Table 13

Recovery of Crude Phenylserine on Recrystallization

Solvent added	Recovered phenylserine g.
None	3.26
Methanol	4.44
Ethanol	4.72
Acetone	4.50
Dioxane	5.16

A vigorous stream of dry hydrogen chloride was bubbled through a suspension of 2.500 g. of the crude phenylserine noted in the preceding section in 25 ml. of absolute ethanol, until the initially rising temperature had again fallen to room levels. The solution was then stored at 5° for 24 hours, with occasional agitation. Suction-filtration, washing with a little cold ethanol and drying gave 0.432 g. (13% yield) of impure allophenylserine ethyl ester hydrochloride, m.p. 166-168° (dec.). Storage of the filtrate for nine days more at 5° led to a further 0.236 g. (7% yield), m.p. 174° (dec.).

Under these conditions, material obtained by recrystallization of crude phenylserine from water alone yielded only 0.024 g. (0.7% yield) of crude allophenylserine ethyl ester hydrochloride, m.p. 168-169° (dec.). Similar processing of phenylserine, obtained by recrystallization of crude product (42% recovery) first from 20 ml. of 50% methanol per gram, then from 20 ml. of 50% ethanol per gram, gave no allophenylserine ethyl ester hydrochloride. In this case, phenylserine ethyl ester hydrochloride, m.p. 139°, was worked up from the clear solution in 89% yield.

2. Solubilities of diastereomeric ethyl ester hydrochlorides

Solubilities of these compounds in absolute ethanol were estimated approximately from observations made in the course of recrystallization operations. With dioxane (refluxed and distilled over sodium) and acetone (distilled from potassium permanganate and dried over potassium carbonate), procedure was more specific. Solutions containing excess free solute were held at the specified temperatures with frequent shaking for at least one hour, after which measured volumes of clear supernatant were withdrawn, evaporated to dryness at 100° and the residues weighed. A different approach was necessary with phenylserine ethyl ester hydrochloride in dioxane at 95°, solubility being so great that high viscosity of the solution precluded accurate volumetric transfer. In this case,

Table 14

Solubilities of Diastereomeric Ethyl Ester Hydrochlorides (a)

Solvent	Temperature °C.	Ethyl ester hydrochloride g./100 ml.	
		Phenylserine	Allophenylserine
Ethanol	80	>100	5.0
	5	> 13	1.2-1.5
Acetone	55	22	0.06
	5	7.5	0.04
Dioxane	95	160	0.15
	14	4.7	0.05

(a) Method of determination is described in text.

weighed portions were added to a measured volume of solvent until a trace of undissolved material still remained one hour later.

3. Attempted separation of ethyl ester hydrochlorides via dioxane

Second and third crops of ethyl ester hydrochlorides from the run in which allophenylserine ethyl ester hydrochloride was first encountered were thoroughly mixed. A sample of 12.0 g. was simmered with 15 ml. of anhydrous dioxane. Complete solution occurred within 30 minutes. Passage of the solution through a steam-jacketed sintered glass pressure filter was initially very slow and ceased entirely with occurrence of crystallization on the under side of the sintered plate.

4. Partial separation of ethyl ester hydrochlorides via acetone

A sample of 12.8 g. of the mixture noted in the preceding section was simmered with 100 ml. of anhydrous acetone. An appreciable proportion remained undissolved after ten minutes. The mixture was easily passed through a steam-jacketed pressure filter, whose inner chamber was held at approximately 80° by drawing steam through the outer jacket under reduced pressure. Washing of undissolved material was effected by similar passage of two 25 ml. volumes of boiling acetone.

The insoluble residue was dissolved by passing 50 ml. of hot ethanol in three portions through the filter under pressure. The pale yellow solution, a large part of which was accidentally lost, was treated with 25 ml. of ether and stored overnight at 5°. Filtration, washing and drying yielded 0.20 g. of allophenylserine ethyl ester hydrochloride, m.p. 177° (dec.).

The pale yellow acetone solution was treated with 75 ml. of ether and stored overnight at 5°. Filtration, washing and drying produced 9.13 g. of slightly sticky crystals, m.p. 96-139°, smelling of mesityl oxide. Recrystallization from 15 ml. of hot ethanol with addition of 150 ml. of ether led to 7.91 g. of crude phenylserine ethyl ester hydrochloride as white odorless crystals, m.p. 137-139°.

Similar processing through acetone and ethanol was used to split allophenylserine ethyl ester hydrochloride from several other batches of crude phenylserine ethyl ester hydrochloride.

Table 15

Partial Separation of Ethyl Ester Hydrochlorides

Starting material		Allophenylserine Et ester HCl		Impure phenylserine Et ester HCl	
Wt., g.	M.p., °C.	Wt., g.	M.p., °C.	Wt., g.	M.p., °C.
37.3	138-141	3.6	177	30.9	138-140
1.98	138-157	0.62	177	1.03	137-139
15.7	135-150	1.2	175	11.9	137-140

5. Effect of phenylserine ethyl ester hydrochloride on solubility of allophenylserine ethyl ester hydrochloride in acetone

Phenylserine ethyl ester hydrochloride (1.000 g.) was dissolved in 8.0 ml. of dry acetone by simmering in a reflux assembly. Allophenylserine ethyl ester hydrochloride (0.100 g.) was added, and simmering was continued for a further five minutes. The hot mixture was suction-filtered, 1.0 ml. cold dry acetone being used to complete transfer of the undissolved allo compound to the filter funnel. Drying gave 0.026 g. and 0.030 g. of allophenylserine ethyl ester hydrochloride undissolved in two such runs.

E. Paper Chromatography

1. Phenylserine and allophenylserine

Paper chromatography was studied as a means of detecting, roughly estimating and distinguishing the phenylserine diastereomers. The capillary ascent technique of Williams and Kirby (1) was employed, with some modifications. Aqueous solutions generally 0.1% (W/V) in either isomeric hydroxyamino acid, alone or mixed, were used in exploratory runs, other concentrations being used as subsequent occasion demanded. These were stored at 5° when not in use, to minimize growth of microorganisms. Finely drawn pipettes, into which the test solutions had been allowed to rise by capillarity, were touched to points at least 1.4 cm. apart along a line 3.0 cm. from the narrow side of a 20 x 27 cm. rectangle of No. 4 Whatman filter paper. Care was taken to place only a single drop of solution per spot, and to hold spot diameter in the range of 0.5-0.7 cm. Filter papers were cylinderized by stapling, with the long sides not quite touching. The cylinders were stood upright, sample spots near the bottom, in screw-capped wide-mouthed chemical jars (5 in. diameter, 12 in. tall) containing 125-150 ml. of solvent, consisting of the clarified upper water-poor layer of various two-phase

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(1) R. J. Williams and E. Kirby, Science, 107, 481 (1948).

mixtures described subsequently. When the solvent front had approached the upper edge of the cylinder, a line was pencilled across it; the cylinders were then inverted and dried in air at room temperature (one hour) or under an infrared lamp at 50-60° (20 minutes). Stables were clipped and the opened sheet was sprayed uniformly with 0.2% (W/V) ninhydrin solution in n-butanol saturated with water. Final oven drying was effected at 80° for 10-15 minutes.

The ethyl ester hydrochlorides, while giving good ninhydrin tests in aqueous solution, failed to respond on papergrams. In this case, only a single trial was made with the upper layer from a mixture of 600 ml. of Skelly D, 350 ml. of methanol and 50 ml. of acetone.

A number of solvent mixtures tested for ability to separate the free hydroxyamino acids showed little promise (Table 16). Although partial separation was achieved by n-butanol-hydrochloric acid-acetone, the mixture was not investigated further, since corrosion of stables necessitated thread-stitching of the paper cylinder. While neither acetone nor concentrated ammonia alone in butanol-water were effective, striking difference in the extent of migration of phenylserine and allophenylserine was observed with a mixture of the four components. The proportion of each solvent necessary for optimum separation was determined by testing various acetone and ammonia levels (Table 17). In subsequent use of paper

Table 16

Ineffective Solvent Mixtures in Paper Chromatography of Phenylserine and Allophenylserine (a)

Components of mixture, ml.									100 R_F (b)
n-BuOH	H_2O	HOAc	Me_2CO	C_5H_5N	Con. NH_4OH	5% NaOH	Et Bu-CO	Dioxane	
250	50								14-16
200	250	50							33-40
200	150	50							47-50
200	100	100							52-55
200	150	25	25						36-39
200	140	30	30						45-49
200	150		50						34-37
200	150		25	25					24-28
200	150			50					25-27
200					100				27-28
	100	100					200		3-4
200						200			14-16
200			35			165			18-22
200	160				20		20		11-15
200	175							25	26-27
200	150				25			25	30-32

n-BuOH	5% HCl	Me_2CO	100R_F	
			Phenyl-serine	Allophenyl-serine
200	200		39-40	49-52
200	160	40	60-64	65-68

(a) Single sheet runs with duplicate spots of each isomer in 0.1% solution, the last seven runs also including a mixed solution 0.1% in each isomer.

(b) R_F = distance from starting line to centre of spot / distance traversed by solvent front from starting line

Table 17

Effect of Acetone and Ammonia Levels in Paper Chromatography of Phenylserine and Allophenylserine

Components of mixture, ml.					$100R_F$		
No. of samples (a)	n-BuOH	H_2O	Me_2CO	Con. NH_4OH	Phenyl-serine	Allophenyl-serine	Δ(b)
6(2)	200	190	5	5	21-26	15-21	5-8
3(1)	200	185	5	10	23-24	16-18	6-7
3(1)	200	185	10	5	28	18-21	7-10
6(2)	200	180	10	10	28-30	19-20	8-10
3(1)	200	170	10	20	28-30	18-20	8-11
3(1)	200	170	15	15	33-35	21-24	10-14
3(1)	200	170	20	10	34-35	22-23	11-13
6(2)	200	160	20	20	35-39	23-26	11-15
3(1)	200	140	20	40	47-50	33-34	14-16
2(1)	200	170	25	5	40-41	28-29	12
5(2)	200	150	25	25	41-45	28-32	13-14
2(1)	200	100	25	75	51-54	40-44	10-11
2(1)	200	50	25	125	58-61	48-53	8-10
3(1)	200	140	30	30	42-43	28-30	12-15
3(1)	200	130	35	35	47-48	34-36	11-13
3(1)	200	140	40	20	52-53	43-44	8-10
10(4)	200	120	40	40	54-58	42-45	10-12

(a) Value outside parentheses applies to number of samples of each isomer: solutions 0.1% in each isomer alone and mixed were used; value inside parentheses indicates the number of sheets and different batches of solvent mixture tested.

(b) Difference in $100R_F$ values for adjacent spots of the two isomers

chromatography as an analytical tool, the upper layer from a mixture of 200 ml. of n-butanol, 150 ml. of water, 25 ml. of acetone and 25 ml. of concentrated ammonium hydroxide was the solvent of choice, with 3-6 hours allowed for ascent. A photograph of a typical papergram is presented in Fig. 3.

In preparing paper sheets for chromatography, several features were found to be of concern. Growth of microoragnisms in the amino acid solutions led to appearance of extra spots and streaks on the developed paper grams. No lateral interference effects were observed with as many as thirteen samples per sheet. Use of more than a single drop of solution per spot resulted in irregularly shaped final spots. Rough standardization of initial spot diameter was essential for effective comparison of solutions of known concentration with unknowns. When stapling the paper into cylindrical form, care was taken that edges did not contact or overlap, otherwise the solvent front became bowed on climbing. Unless the solvent mixture was clarified by addition of a few ml. of appropriate organic solvent, varying amounts of aqueous layer settled out, with resultant irregular spot migration.

Points of technique in final development of the chromatograms also proved to be important. Spots were lighter in hue, with poorer border definition, when wet paper cylinders were dried at 100° instead of at lower temperatures. Sensitivity in respect to detectable amino acid concentration was

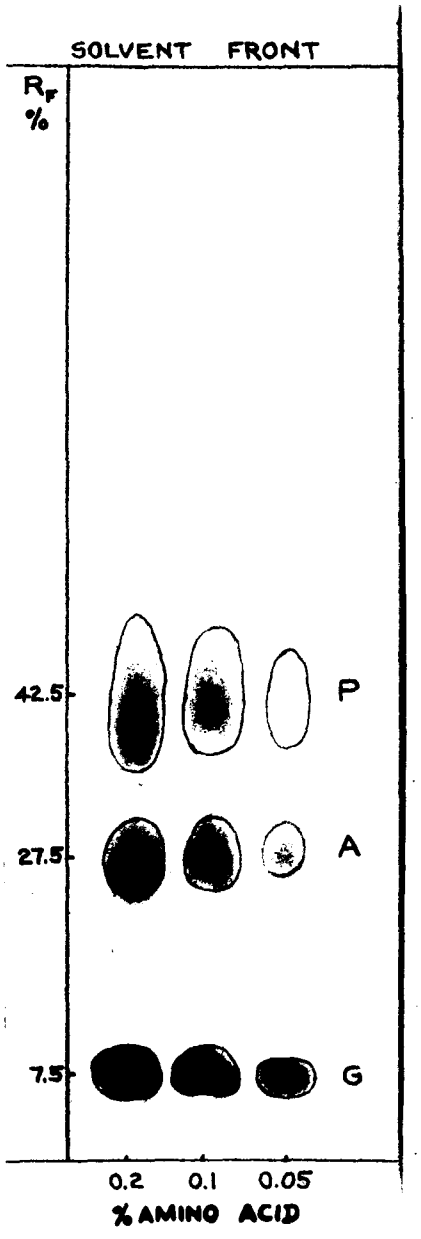

Fig. 3 Paper Chromatography of Phenylserine, Allophenylserine and Glycine.

lower with 0.1% ninhydrin solution than with 0.2%. Spot colors developed too slowly (60-90 minutes) when final drying was carried out at 60°, whereas undesirable pink flushing of the whole sheet frequently occurred at 100°.

With the butanol-water-acetone-ammonia mixture, the developed spots were initially orange-brown in color, darkening to violet in the course of a day or more, then gradually fading in several weeks. Phenylserine spots were generally elongated ovals with slightly less color intensity than the circular allophenylserine spots. Border definition was sufficiently sharp to permit pencil delineation.

Mixed solutions containing both phenylserine and allophenylserine were tested over a wide range of concentrations. A barely visible spot was obtained with either hydroxyamino acid at as low as 0.01% concentration in solution. The spot was still discrete when as much as 2.5% of the other isomer was present in the same solution.

2. Threonine and allothreonine

By use of the technique described in the preceding section, three spots each of 0.1% (W/V) solutions of threonine and allothreonine were placed on paper sheets. The solvent mixture which successfully separated phenylserine and allophenylserine was employed, with a five and one-half hour ascent period. Ninhydrin development was effected as before.

Threonine gave 100Rp values of 18, 18, 19 and allo-threonine 13, 14, 14 respectively. The spots were a vivid blue-violet in color, with well defined borders. Their shape was circular and area smaller than with phenylserine diastereomers run at the same concentration.

F. Effect of Condensation Time on Phenylserine Diastereomer Content and Yield

1. Observations on the condensation reaction

Several batches of phenylserine were synthesized according to the German patent procedure (1). The quality and quantity of each reactant used were the same as in the earlier described run. The electrically driven Hershberg stirrer was operated at the mxaimum speed commensurate with avoiding serious loss of the reaction mixture by splashing.

New phenomena were brought to light when the reaction course was observed closely under these conditions. As in earlier runs, benzaldehyde was added all at once*. One minute later, the reaction mixture was turbid, somewhat viscous and uniformly emulsified. Temperature had risen from 15° to 17°. After three minutes, with temperature at

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* Time periods subsequently noted are counted from this addition.

(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

20-22°, the emulsion suddenly set to a curdy thick white paste, and the agitator was brought to an abrupt halt. The material gradually redissolved upon mixing with the aid of a glass rod and hand-turning of the stirrer shaft. After 5-6 minutes, the stirring motor was again turned on at high speed. At this time, temperature reached the maximum of 25° and declined gradually thereafter at a rate varying with the water turnover in the external cooling bath. After 12-13 minutes, the reaction mixture was a completely transparent viscous light syrup. No further change was apparent up to 25-28 minutes, when precipitation commenced anew. In 28-32 minutes, the mixture rapidly attained solidity and temperature rose 1°. The agitator slowed gradually in this instance before finally stopping entirely.

2. Observations on acid cleavage of insoluble intermediates

The insoluble material finally formed from condensation of benzaldehyde and glycine was allowed to stand at room temperature for different periods of time with various batches. Concentrated hydrochloric acid (50.0 ml., ca. 0.6 mole) was then added dropwise from a burette during 30 minutes. The condensation cake was fragmented with a spatula and mixing was effected with a heavy glass rod to minimize local concentration of the acid. An external water bath at 15° was used for cooling throughout the operation. The Hershberg stirrer was turned on at maximum speed as soon as reduction of lump size permitted.

With two batches which were allowed to stand only one hour from the time of benzaldehyde addition, a thin white slurry, with few lumps, was present at the close of acidification. Thickening to a smooth paste occurred during further agitation for 30 minutes. Then, new lumps gradually developed, attaining a diameter greater than one cm. in another 30 minutes, and leaving a clear pale yellow supernatant. The mixture was chilled to 5° and stored at this temperature for two hours. Suction-filtration (very slow) was then effected, the cake being pressed as dry as possible with the aid of a rubber dam. A faintly yellow transparent aqueous filtrate, at pH 3.1 and with only a few ml. of supernatant benzaldehyde, was thus obtained.

Three batches run with four hours elapsing between benzaldehyde addition and acidification showed a different behavior. During 60 minutes of high speed agitation after acidification, the few lumps remaining of the insoluble condensation material totally disintegrated. As precipitation of fine particles occurred, the reaction mixture gradually thickened to a stiff paste. In this case, no tendency to agglomerate into new lumps was apparent. After cooling and storage at 5° for 1-24 hours, the paste was suction-filtered, a rubber dam being used as before. By contrast with the one hour run, filtration was rapid and yielded a pale yellow transparent filtrate at pH 4-4.7 completely devoid of supernatant benzaldehyde. Even the

white filter cake smelled only slightly of benzaldehyde. In one case, the filter cake was broken up, mixed again with the filtrate and acidified to pH 2.8 with concentrated hydrochloric acid. By dropwise addition of 50% sodium hydroxide, pH was then raised to 5.6. Upon filtration, the filtrate was again obtained free from all visible traces of benzaldehyde.

With two runs in which acidification was effected after a 24 hour condensation period, lumps of the initial solid were fragmented with more difficulty. During the 60 minute agitation period, the acid cleavage product precipitating gradually agglomerated into oily pellets, 1-2 mm. in diameter and smelling strongly of benzaldehyde. One batch was stored overnight at 5°; the other was treated with 100 ml. of ether which, upon further agitation for 30 minutes with external ice bath cooling, gave a thick smooth white paste, surrounded by free aqueous supernatant. In both runs, suction-filtration proceeded slowly and yielded a clear aqueous layer surmounted by a substantial quantity of benzaldehyde.

A strong benzaldehyde smell prevailed from commencement of acidification with a batch which had stood for 60 hours after addition of benzaldehyde. In this case, lumps of the initial condensation material were quite hard. Their dissolution was facilitated by addition of 150 ml. of ether during acidification and the subsequent one hour agitation period. Further processing and behavior were in every way similar to

the ether-treated 24 hour batch.

The cake remaining after acidification, cooling and filtration was fragmented and washed by mixing well with three 200-250 ml. volumes of boiling 95% ethanol, with filtration of the resultant thin slurry and suction of the crude phenylserine cake as dry as possible each time with the aid of a rubber dam. In the case of one four hour condensation run, the fragmented cake was initially hand-stirred with only 100 ml. of boiling 95% ethanol. The thin slurry which first formed quickly stiffened to a paste sufficiently thick that the containing beaker could be inverted without loss. Addition of a further 150 ml. of boiling 95% ethanol was necessary to give a filtrable slurry again. When the fragmented cake from a 24 hour run was treated with boiling 95% alcohol in the same portionwise fashion, mere slurrying occurred and no sudden stiffening to a paste. Subsequent ethanolic washing on these two batches was carried out as with the others. With all batches, alcohol-washed crude phenylserine was dried to constant weight over anhydrone at 50-60° and 10-15 mm. Yields in typical runs are summarized in Table 18.

Ethanol washings were distilled <u>in vacuo</u> to concentrates in which the proportion of benzaldehyde present varied in accord with the quantity split off during hydrochloric acid treatment. Thus, with four hour runs, all cleaved benzaldehyde appeared at this stage. With other runs, the amount

Table 18

Yield and Diastereomer Content of Crude Ethanol-Washed Phenylserine

Condensation time, hrs.	Crude product		Isomer fraction (a)	
	Wt., g.	% Yield	Phenylserine	Allophenylserine
1	33.4	46	0.55-0.60	0.40-0.45
4	44.3	61	0.80-0.85	0.15-0.20
24	52.7	73	1.00	0
60	49.0	68	1.00	0

(a) Procedure given in next section

corresponded to that mechanically retained within the filter cake prior to alcoholic washing. By treatment of the ethanol-benzaldehyde concentrates with ether in the manner described earlier, slightly colored amorphous materials containing varying amounts of the phenylserines were obtained. These were checked by paper chromatography, but usually not processed further.

Some aqueous filtrates from the acidification step were distilled in vacuo to a damp crystalline mass, which was extracted three times with 100 ml. volumes of boiling methanol. The residual sodium chloride showed negligible traces of the phenylserines when subjected to paper chromatography. Amorphous powders obtained by concentration of the methanol

extracts contained varying small amounts of the two phenylserine isomers, as indicated by paper chromatography.

3. Diastereomer content and yield by paper chromatography

Paper chromatography by the technique already described was used to estimate the proportion of phenylserine and allophenylserine present in the products of the various condensation runs. For this purpose, three mixed standard control solutions were prepared. Each of these contained phenylserine, allophenylserine and glycine at levels of 0.2%, 0.1% and 0.05% (W/V) respectively. In early runs, only the 0.1% controls were used, in duplicate or triplicate. Subsequently, all three controls were employed, with one spot of each per sheet. To facilitate later comparison, control spots were spaced uniformly across the sheet, with non-adjacent duplicates of up to five unknown samples also distributed along the same starting base line. Several dilutions of a given unknown were used where uncertainty existed as to its content of phenylserines, otherwise dilutions were set to give developed spots of approximately the same area as the 0.1% control. Repeat runs at different dilutions were made when results were inconclusive.

The results of paper chromatography applied to ethanol-washed crude phenylserine from batches run with different condensation times were summarized in Table 18. The values presented in Table 19 are based upon similar examination not

Table 19

Approximate Total Yield and Diastereomer Content

Condensation time, hrs.	Total % yield (a)	Isomer fraction		% Glycine unreacted
		Phenylserine	Allophenylserine	
1	60	0.55-0.60	0.40-0.45	15-20
4	80	0.80-0.85	0.15-0.20	5-10
24	85	0.96-0.98	0.02-0.04	2-4
60	80	0.96-0.98	0.02-0.04	1-2

(a) ± 5%

only of the main crop of each batch, but also of amorphous fractions obtained by working up ethanol washings and aqueous filtrates.

G. Separation of Phenylserine and Allophenylserine via Solvates

1. Crude sodium salt

A sample (8.72 g., 0.048 mole) of anhydrous ethanol-washed crude phenylserine, which had been prepared with a one hour condensation period (i.e. 40-45% allophenylserine), was

fed into 25.0 ml. of 1.925 N (0.048 mole) sodium ethoxide in absolute ethanol. When rapid caking occurred, a further 25 ml. of absolute ethanol was added and the mixture was gently simmered for five minutes. By standing overnight at room temperature, filtering, twice washing with ethanol and drying, 9.40 g. (96% yield) of fine white powder was obtained.

Paper chromatography showed that the product was a mixture of phenylserine and allophenylserine sodium salts, the ratio of the two isomers being scarcely changed with respect to the starting material. In the ethanol filtrate were detected roughly 0.150 g. of allophenylserine, 0.005-0.01 g. of phenylserine and 0.025-0.05 g. of glycine.

2. Recrystallization tests

A series of 1.000 g. samples of the same crude phenylserine noted in the preceding section were dissolved in 10.0 ml. volumes of water by warming in a water bath. The solutions were treated with 0.1-1.0 volume of concentrated ammonium hydroxide and then with 0-3 volumes of acetone. These tests were discarded when no crystallization was observed after 48 hours at 5°.

Other test solutions, prepared in the same way, were treated with equal volumes of various organic solvents and stored overnight at 5°. Crystal crops were subsequently

filtered off, washed with cold solvent and dried over anhydrone at 50-60° and 10-15 mm. Recoveries, and observations on isomer content based on paper chromatography, are presented in Table 20.

Table 20

Recrystallization of Crude Phenylserine (a) from One Hour Condensation Run

Solvent	Crystalline product		Filtrate isomer content
	% Recovery	Isomer content	
Water (b)	28	ca. 95% P	slightly more A than P
50% Acetone	63	slightly more A than P	slightly more P than A
50% Methanol	54	slightly more P than in crude	slightly more A than P
50% Ethanol	75	slightly more P than in crude	slightly more A than P
50% Dioxane	67	ca. 65% A	predominantly P; ca. 0.02 g. A

A = allophenylserine, P = phenylserine

(a) ca. 55% P; 45% A
(b) 1.000 g. crude in 10.0 ml. of water: no organic solvent added.

3. Phenylserine monohydrate

A 2.500 g. sample of phenylserine, shown by paper chromatography to be isomerically pure, was dissolved in 30 ml. of boiling water. The hot colorless solution was suction-filtered to remove traces of lint, raised again almost to the boiling

point, and then, with steady swirling, 30 ml. of boiling ethanol was slowly added. The mixture was seeded when cooling to 5° during four hours failed to initiate crystallization. Rapid deposition of iridescent flakes followed. Under the microscope, these were seen to consist of thin well-formed hexagonal plates, layered like mica. Overnight storage at 5°, filtration, washing with 10 ml. of cold 50% ethanol and drying over anhydrone at 50-60° and 10-15 mm. during 18 hours gave 2.175 g. (87% recovery) of anhydrous phenylserine.

Anal. Calcd. for $C_9H_{11}O_3N$: N, 7.73; neut. equiv., 181.2

Found: N, 7.72, 7.85; neut. equiv. (formol), 181, 182, 183

Other samples of phenylserine, similarly recrystallized, were allowed to dry in air to constant weight after filtration and solvent washing. The monohydrate thus obtained was then desiccated over anhydrone at 50-60° and ca. 0.1 mm. during 16 hours.

Anal. Calcd. for $C_9H_{11}O_3N \cdot H_2O$: H_2O, 9.05

Found: H_2O, 9.01, 9.10, 9.05

4. Allophenylserine hemidioxanate*

A 2.500 g. sample of fibrous allophenylserine, prepared by cold alkaline hydrolysis of the corresponding ethyl ester

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* The prefix "hemi" is used to indicate a combining ratio of one molecule of dioxane to two molecules of allophenylserine.

hydrochloride in the manner already described, and shown by paper chromatography to be isomerically pure, was dissolved in 35 ml. of water by gentle simmering during ten minutes. The hot colorless solution was filtered to remove lint, raised again almost to the boiling point, and then, with steady swirling, 35 ml. of boiling dioxane was added. Steady deposition commenced of glittering needles, which, under the microscope, were found to be fairly long blunt-ended individual rods. Overnight storage at 5°, filtration, washing with 10 ml. of cold 50% dioxane and drying in air to constant weight gave 3.001 g. of allophenylserine hemidioxanate. Desiccation over anhydrone at 10-15 mm. and 50-60° during 18 hours decreased the weight to 2.998 g. (97% yield). No further weight loss occurred when the material was heated at 77° under ca. 0.1 mm. over phosphorus pentoxide in an Abderhalden dryer for two hours.

Anal. Calcd. for $C_9H_{11}O_3N \cdot 1/2C_2H_4O_2$: N, 6.22; neut. equiv., 225.2

Found: N, 6.05, 6.21; neut. equiv. (formol), 229, 231

The formation of this interesting compound was also observed under the microscope. A few milligrams of allophenylserine were dissolved in a drop of water on a slide by puddling with a fine glass rod. A cover slip was placed over the preparation to curtail evaporation. A drop of dioxane was then introduced under the cover slip from a capillary pipette.

Within a few seconds, long rods commenced rapid growth from the periphery of the clear solution inward, until, individually and in clusters, they pervaded the whole droplet. No such phenomenon was observed when acetone, methanol or ethanol were substituted for dioxane, nor when allophenylserine was replaced by phenylserine, threonine or allothreonine.

5. Allophenylserine hemihydrate

A solution of 11.124 g. of allophenylserine hemidioxanate in 100 ml. of boiling water was gently simmered for five minutes after the odor of dioxane was no longer detectable in the emerging steam (ten minutes in all) and then cooled rapidly under the cold water tap, a glass rod being used to rub the flask walls briskly at the same time. Rapid precipitation occurred of hard glittering tablets, which the microscope showed were thick perpendicular-sided hexagonal prisms. Overnight storage at 5°, filtration, washing with small volumes of ethanol, then ether and air drying gave 5.972 g. (63% yield) of allophenylserine hemihydrate. The material was recrystallized for analysis from boiling water with fast ice bath cooling and only ice water washing prior to air drying at room temperature. Water content was determined by desiccating a sample over phosphorus pentoxide at 77° and ca. 0.1 mm. during ten hours. Nitrogen determinations were effected both on the hemihydrate and the dried material.

Anal. Calcd. for $C_9H_{11}O_3N \cdot 1/2H_2O$: N, 7.37; H_2O, 4.74
Found: N, 7.34; H_2O, 4.85
Calcd. for $C_9H_{11}O_3N$: N, 7.73
Found: N, 7.70

The aqueous filtrate from which the hemihydrate was first isolated was raised to the boiling point and then treated with an equal volume of boiling dioxane. After overnight storage at 5°, filtration, washing and drying, 2.856 g. (26% recovery) of allophenylserine hemidioxanate was obtained.

6. Recrystallization of allophenylserine from various solvents: possible existence of other solvates

A solution of 5.496 g. of allophenylserine in 55 ml. of boiling water was allowed to cool slowly to room temperature with frequent swirling. A white semi-gelatinous mass composed of long thin fibrous needles, frequently bundled, slowly formed. Overnight storage at 5°, filtration, washing twice with 10 ml. volumes of ice water and drying in air at room temperature to constant weight led to 3.299 g. (60% recovery) of anhydrous allophenylserine. This material suffered no further weight loss on desiccation over anhydrone at ca. 0.1 mm. and 50-60° during five hours.

The aqueous filtrate was concentrated to roughly 25 ml., cooled to room temperature and then treated with an equal volume of ethanol. After storage at 5° for four hours, there

was obtained a thick white slurry which, under the microscope, was observed to consist of bundles and star-clusters of short sharp-pointed needles. Filtration, washing with cold 50% ethanol and drying in air to constant weight gave 1.315 g. (24% recovery) of anhydrous allophenylserine. This weight was unchanged by desiccation over phosphorus pentoxide at ca. 0.1 mm. and 77° during ten hours.

Another recrystallization filtrate containing pure allophenylserine was seeded with a few crystals of the hemihydrate, treated with an equal volume of ethanol and stored at 5° overnight. A crystal crop was recovered in which no form other than the thick hexagonal prisms of the hemihydrate were observed microscopically.

The aqueous filtrate from alkaline hydrolysis of allophenylserine ethyl ester hydrochloride (described on p. 128) was treated with an equal volume of ethanol (65 ml.) and stored overnight at 5°. A small iridescent mat, which was filtered off (very slow), was found under the microscope to consist of jagged-ended parallel-sided thin grooved plates, some of which were partly cleaved to individual needle forms. Air drying to constant weight at room temperature gave 0.230 g. of a solvated allophenylserine (identity established by paper chromatography). The material was dried at 77° and ca. 0.1 mm. over phosphorus pentoxide. A weight decline of 8.5% had been observed when unfortunate mechanical loss of the entire sample

occurred (calculated weight loss for monohydrate, 9.05%).

A solution of 2.500 g. of allophenylserine in 50 ml. of boiling water was treated with 50 ml. of boiling acetone and allowed to cool slowly to room temperature with frequent swirling. A heavy slurry of long thin fibrous thread-like needles formed. Overnight storage at 5°, filtration, washing twice each with 10 ml. volumes of 50% acetone, acetone, then ether and drying over anhydrone for twelve hours at 50-60° and 10-15 mm., yielded 1.818 g. (73% recovery) of anhydrous allophenylserine. Similar desiccation of air-dried samples obtained by the same route was observed to cause no appreciable weight loss. A further recrystallization from water-acetone was carried out for analysis.

Anal. Calcd. for $C_9H_{11}O_3N$: N, 7.73; neut. equiv., 181.2
Found: N, 7.54, 7.62; neut. equiv. (formol), 181, 181

Other samples of allophenylserine were recrystallized from water-ethanol and water-methanol in the manner just described for water-acetone. The materials recovered were desiccated directly to the anhydrous state in the usual manner, without intermediate air drying at room temperature to check for solvate formation. These solvent mixtures were not tested further when recoveries were noted to be substantially less than that via water-acetone.

7. Isolation of pure diastereomers from crude phenylserine

Fractionation of 89.2 g. of crude phenylserine from a one hour condensation run was effected in the light of observations made on behavior of the individual hydroxyamino acid diastereomers and mixtures during recrystallization from different solvents. The material, shown by paper chromatography to contain 40-45% allophenylserine, was first recrystallized from 890 ml. of boiling water to give a 26.7 g. (30% recovery) phenylserine fraction containing less than 5% allophenylserine. The aqueous filtrate, heated to the boiling point, was treated with an equal volume of boiling dioxane. From this treatment resulted a 43.0 g. (39% recovery) fraction of allophenylserine hemidioxanate containing roughly 5% phenylserine. The 50% dioxane filtrate was concentrated in vacuo to roughly 170 ml., heated to the boiling point, and treated with an equal volume of boiling ethanol. This led to 18.9 g. (21% recovery) of phenylserine wherein bare traces of allophenylserine were detectable. The 50% ethanol filtrate was shown to contain about 6.5 g. (7% of original starting material) of hydroxyamino acids by paper chromatography, with only slightly more phenylserine than allophenylserine present.

The two high phenylserine fractions were combined and twice recrystallized from 50% ethanol in the usual manner. The crude allophenylserine dioxanate middle fraction was

recrystallized once from 50% dioxane and then converted to the anhydrous form by recrystallization from 50% acetone. All recrystallization filtrates were combined, concentrated *in vacuo* to 200 ml. and treated hot with an equal volume of ethanol. After removal of the mixed tail crop thus obtained, the filtrate was again concentrated (40 ml.) and treated with ethanol to obtain a second mixed tail crop, the filtrate from this being discarded. Final estimated recoveries of phenylserine and allophenylserine, shown to be isomerically pure by paper chromatography, and of mixed tail fractions are summarized in Table 21.

Table 21

Fractionation of Pure Diastereomers from Crude Phenylserine

Fraction	Wt., g.	Phenylserine		Allophenylserine	
		Wt., g.	% Recovery	Wt., g.	% Recovery
Phenylserine	36.5	36.5	41	-	-
Allophenylserine	24.0	-	-	24.0	27
Tail crops	15.2	9.1	10	6.1	7
	5.4	3.2	4	2.2	3
Tail filtrate	2.4	1.2	1	1.2	1
Totals	83.5	50.0	56	33.5	38

8. Decomposition temperatures of phenylserine and allophenylserine

The effect of initial bath temperature and heating time on the decomposition temperatures of the two hydroxyamino acids and their solvates was studied. Samples of each material were tested with two or three different initial bath temperatures, and with a sample of anhydrous phenylserine run simultaneously each time to provide a basis of comparison. The approximate times elapsing between insertion of the sample into the bath and attainment of the decomposition temperature were measured with a stopwatch. Results are collected in Table 22.

H. Derivatives of Phenylserine and Allophenylserine

1. Hydrochlorides

Each of the pure anhydrous amino acids (1.812 g., 0.0100 mole) suspended in 18 ml. of dioxane was treated under anhydrous conditions with a brisk stream of dry hydrogen chloride for fifteen minutes. Solution, accompanied by evolution of heat, was complete within five minutes, and temperature declined thereafter. The clear solutions were diluted with equal volumes of dioxane and ether was added until cloudiness developed. After seven hours at 5°, the products were suction-filtered, washed with ether and dried in air at 50°. Each was recrystallized by dissolving in 10 ml. of methanol at room temperature,

Table 22

Decomposition Temperatures of Phenylserine and Allophenylserine

Run No. (a)	Compound	Temperature, °C. initial (b)	yellow	soft	gas	Heating time, min.
1a	P anhydrous	172	178	182	183	5.5
	P monohydrate	172	181	185	186	8.0
1b	P anhydrous	180	185	188	189	3.5
	P monohydrate	180	188	190	191	4.5
1c	P anhydrous	188	190	191	192	2.5
	P monohydrate	188	190	192	193	3.3
2a	P anhydrous	176	180	183	183	4.8
	A hemidioxanate	176	178	181	(c)	3.8
	A anhydrous	176	183	184	184	6.0
2b	P anhydrous	187	189	190	190	2.5
	A hemidioxanate	187	188	189	(c)	2.0
	A anhydrous	187	190	191	191	3.3
3a	P anhydrous	179	186	190	190	3.0
	A hemihydrate	179	181	191	192	3.7
	A hemihydrate, dehydrated	179	181	186	190	3.0
3b	P anhydrous	185	186	187	187	3.0
	A hemihydrate	185	186	187	187	3.2
	A hemihydrate, dehydrated	185	186	187	187	2.3
4a	P anhydrous	183	188	190	190	2.5
	A anhydrous	183	190	192	192	3.3
	P + A anhydrous	183	185	189	190	2.3
4b	P anhydrous	192	194	196	196	1.7
	A anhydrous	192	196	197	197	2.0
	P + A anhydrous	192	193	195	196	1.5

P = phenylserine, A = allophenylserine

(a) Run no. indicates the sets of samples tested simultaneously.
(b) Initial bath temperature
(c) Shrivels quickly after softening.

adding ether until cloudy and refrigerating for four hours, with filtration and drying as before. Further recrystallization from ether-acetic acid did not change the decomposition points appreciably. Both compounds had the form of blunt needles or blades.

Table 23

Diastereomeric Phenylserine Hydrochlorides

Series	Yield		M.p., °C.(a)		% Cl (b)		% N	
	Wt.,g.	%	Crude	Rxtzd.	Calcd.	Found	Calcd.	Found
Phenylserine	1.918	88	163	160	16.29	16.15 16.22	6.44	6.31 6.39
Allophenyl-serine	2.030	93	163	159	16.29	15.99 16.10	6.44	6.43
Mixture	-	-	159	-	-	-	-	-

(a) All with decomposition: initial bath temperature 150-155°, 4-5 minutes heating.
(b) Mercurimetric

2. Ester Hydrochlorides

Isomerically pure anhydrous phenylserine (3.624 g., 0.0200 mole), suspended in the appropriate alcohol (36 ml.), was treated under anhydrous conditions with a vigorous stream of dry hydrogen chloride for 2.5-3 hours, with sufficient heating to maintain gentle reflux. Complete solution of the amino acid occurred within five minutes, accompanied by evolution of heat. Except in the case of the isopropyl derivative, optimum recovery was achieved by concentrating the alcoholic

solution *in vacuo* almost to dryness, with care to avoid excessive heating, taking up the residual oil or paste in a small volume of the hot parent alcohol, and rapidly adding ether to incipient crystallization. The isopropyl compound started to precipitate after 1.5 hours of hydrogen chloride treatment, at the close of which it was redissolved by addition of an equal volume of boiling isopropanol (no ether added). The resultant ester hydrochlorides were isolated following overnight refrigeration, suction-filtration, washing with ether and drying in air at 50°. Small second crops were obtained from the filtrates by repeating the concentration procedure outlined above.

Table 24

Phenylserine Ester Hydrochlorides

Ester hydrochloride	Yield (a) Wt.,g.	%	M.p. °C.(b)	% Cl(c) Calcd.	Found	% N Calcd.	Found
Methyl	4.360	94	160(dec.)	15.30	14.99 15.03	6.05	6.00 6.01
Ethyl	4.796	98	140	14.43	14.61 14.71	5.70	5.61 5.66
n-Propyl	4.679	90	131	13.65	13.30 13.62	5.39	5.18 5.21
i-Propyl	4.947	95	164(d)	13.65	13.34 13.51	5.39	5.31 5.35

(a) Total yield of crude product, first and second crops
(b) Values cited are for the first crop of recrystallized material.
(c) Mercurimetric
(d) M.p. 165° (dec.) noted with another larger batch

For analysis, the first and second crops of each crude product were combined and recrystallized from a minimum of hot parent alcohol, with ether added to the point of incipient crystallization. The ester hydrochlorides were recovered in the range 92-97%. The melting point of the isopropyl compound rose only 1° (comparing first crops), and those of the other ester hydrochlorides remained unchanged. Microscopic examination showed the four substances to be of the thin hexagonal plate form, the methyl and isopropyl derivatives favoring a diamond-shaped modification and the ethyl and propyl compounds being frequently elongated to blades.

Samples of isomerically pure anhydrous allophenylserine (3.624 g., 0.0200 mole) were treated in the manner just described for phenylserine. Variations encountered in times

Table 25

Alcohol-Hydrogen Chloride Treatment of Allophenylserine

Alcohol	Time (min.) for solution	Time (min.) for crystallization	Time (min.) for reaction	Final vol. ml.
Methyl	5	15	150	90
Ethyl	5	15	150	126
n-Propyl	45	60	240	72
i-Propyl (a)	150	180	300	90

(a) After 120 minutes, 24 ml. more of i-propanol was added to promote solution.

observed for solution of the amino acid, for the start of precipitation of the ester hydrochloride from the hot alcoholic solution and for total reaction, as well as the volumes of boiling appropriate alcohol finally added to dissolve the precipitated product are summarized in Table 25. No ether was added prior to refrigeration and isolation of the first crops of crude products. Small second crops were obtained in the way described for phenylserine ethyl ester hydrochlorides. All four compounds were in the form of hexagonal or parallelogram plates.

Table 26

Allophenylserine Ethyl Ester Hydrochlorides

Ester hydrochloride	Yield (a) Wt.,g.	%	M.p. °C.(b)	% Cl (c) Calcd.	Found	% N Calcd.	Found
Methyl	4.485	97	180 (dec.)	15.30	14.98 15.00	6.05	5.98 6.05
Ethyl	4.733	96	178 (dec.)	14.43	14.54 14.71	5.70	5.63 5.64
n-Propyl	5.031	97	160	13.65	13.32 13.45	5.39	5.21 5.27
i-Propyl (d)	5.069	98	159 (dec.)	13.65	13.60	5.39	5.24 5.28

(a) Total yield of crude product, first and second crops
(b) Values cited are for the first crop of recrystallized material.
(c) Mercurimetric
(d) M.p. 165° (dec.) noted with another larger batch, esterified during 400 minutes, after recrystallization from isopropanol alone

Recrystallization was again effected from the parent alcohol-mixture, with 82-94% recovery. The melting points of the ethyl and isopropyl derivatives rose by 1° and 2° respectively, the others undergoing no change (comparing first crops).

3. Esters

Portions (0.0100 mole) of the various phenylserine and allophenylserine ester hydrochlorides, noted in the preceding section, were converted to the corresponding free esters by treatment with ammonia gas in ether, by using the procedure described earlier for preparation of phenylserine methyl ester. After removal of ammonium chloride, the ether solutions

Table 27

Reactants for Ester Synthesis

Ester hydrochloride	Wt., g.	Ether, ml.
Methyl	2.317	115
Ethyl	2.457	123
n-Propyl	2.597	130
i-Propyl	2.597	130

were evaporated to dryness in a dry air stream and the crude crystalline products were recrystallized from ether-Skelly D directly. Second crops were obtained by further addition of Skelly D to first crop filtrates, without concentration.

Table 28

Phenylserine and Allophenylserine Esters

Ester	Yield (a) Wt.,g.	Yield (a) %	M.p. °C. (b)	% N Calcd.	% N Found	Crystal form
Phenylserine						
Methyl	1.470	75	62	7.17	7.09 7.15	needles
Ethyl	1.602	77	84	6.69	6.54 6.58	mica plates
n-Propyl	1.900	85	59	6.27	6.18 6.22	needles, blades
i-Propyl	1.976	89	75	6.27	6.21 6.25	needles, blades
Allophenylserine						
Methyl	1.747	89	110	7.17	7.15 7.15	mica plates
Ethyl	1.937	92	86	6.69	6.50 6.57	needles
n-Propyl	1.996	89	63	6.27	6.23 6.24	needles
i-Propyl	1.896	85	75	6.27	6.27 6.30	needles

(a) Total yield of once recrystallized product, first and second crops

(b) Values cited are for the first crop of twice recrystallized material.

For analysis, first and second crops were combined and recrystallized from ether-Skelly B. Single crop recoveries were in the range 71-91%. The melting points of phenylserine isopropyl, and of allophenylserine methyl and isopropyl esters were unchanged; those of the other esters rose only 1° (comparing first crops).

4. Alkaline hydrolysis of ester hydrochlorides: absence of epimerization

Preparation of allophenylserine by hydrolysis of its ethyl ester hydrochloride with 1.95 N sodium hydroxide at room temperature has already been described. Another batch of the same starting material (9.828 g., 0.0400 mole) was treated by the same procedure, with an extra feature. Prior to acidification, 45 ml. of dioxane was added to the alkaline solution, which remained clear. As the pH was adjusted to 5 with concentrated hydrochloric acid, rapid precipitation of the hemidioxanate occurred. This adduct was subsequently recovered in virtually quantitative yield (8.966 g.) in a single crystal crop.

Isomerically pure phenylserine ethyl ester hydrochloride (9.828 g.) was hydrolyzed by the same technique, 43 ml. of ethanol being added prior to acidification. Thus, 6.480 g. (81% yield) of phenylserine monohydrate was obtained in one crop.

Paper chromatography was used to check isomer purity of the above crystal crops in 2.5% (W/V) aqueous solution, and of the undiluted filtrates from which they had been obtained. Only the expected isomers were detected in each case.

Several batches of impure phenylserine ethyl ester hydrochloride, from which most of the allo isomer had been removed by passage through acetone, were combined and recrystallized from ethanol-ether. A sample (9.828 g.) of the recovered material was subjected to alkaline hydrolysis in the same way as the pure isomer. By use of paper chromatography the crystalline phenylserine monohydrate obtained (6.304 g.) was found to contain ca.1% (0.06 g.) of allophenylserine, and the filtrate ca. 0.4 g. of this isomer. Thus, the starting material was contaminated with roughly 7% of the allo isomer.

IV. DISCUSSION AND CONCLUSIONS

A. Phenylserine from Condensation of Benzaldehyde and Glycine

In early phases of the investigation, a method was sought by which phenylserine could be obtained in reasonable quantity with a minimum of manipulation, and by which analogues might be prepared. First, the Forster and Rao modification (1) of the original Erlenmeyer procedure (2) was tested. Cold ethanol washing of the condensation intermediate was found to be poorly effective in removing excess base, as well as time-consuming. Yield of the final product was only moderate.

Reaction of benzaldehyde with glycine in presence of aqueous potassium hydroxide occurred more slowly, possibly due to greater solubility of intermediate potassium salts involved. Phenylserine was obtained in lower yield and purity. Recrystallization of the crude product was impeded by the presence of by-products, probably diphenylhydroxyethylamines.

The German patent procedure (3) proved to be eminently satisfactory. The condensation proceeded smoothly and intermediate processing offered no difficulties. Phenylserine,

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(1) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).
(2) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).
(3) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

uncontaminated by aminol bases, was produced in good yield. Anomalous recovery of 103% upon recrystallization was noted, but was considered at the time to be due to incomplete dehydration.

Seemingly minor points of processing are of concern. Banzaldehyde which has been exposed to atmospheric oxidation or which is otherwise impure frequently fails to condense with glycine. The material may be redistilled or drawn from a freshly opened bottle, the remainder of whose contents are adequately protected by sealing the screw top well with plastic tape. Aqueous filtrates from the crude cake retain phenylserine in amounts varying with pH. Loss at this point is reduced by use of an equivalent quantity of hydrochloric acid, which is predetermined by titration of an aliquot against the sodium hydroxide stock solution used to dissolve the glycine initially. Elution of phenylserine on hot alcoholic washing varies with the amount of aqueous phase left in the crude cake, as well as with the volume of alcohol used.

Decomposition temperatures of the various phenylserine fractions fell in the range 189-197°. Degree of hydration, mode of preparation or number of the crystal crop had little apparent effect. Reproducibility was poor, variation occurring with both temperature and duration of heating. Thus, no indication of more than a single isomer was provided.

A lone attempt to extend the German procedure to synthesis of β-2-furylserine was unsuccessful. Other workers (1) have recently reported similar inability to condense furfural with glycine in presence of aqueous sodium hydroxide.

B. Transformation of Phenylserine to Chloramphenicol

Phenylserine was successfully converted to the racemic form of the antibiotic. The sequence of reactions found most satisfactory for the purpose is summarized in Fig. 4, with the yield for each step indicated. The overall yield of the process approximated 8%. Only cursory attention was given to improving the efficiency of the last three steps, procedures for which were first set forth by the Parke, Davis and Co. group in their classic synthesis of chloramphenicol (2).

The main crop of phenylserine prepared by the German patent procedure consists of a single pure diastereomer, a fact for which conclusive evidence is presented later in this discussion. The transformation to chloramphenicol, to which a threo configuration has been assigned (3), clearly establishes phenylserine as a structural analogue of the natural amino acid, threonine. This feature is of advantage for synthesis

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(1) K. Hayes and G. Gever, J. Org. Chem., 16, 269 (1951).
(2) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).
(3) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, ibid., 71, 2458 (1949).

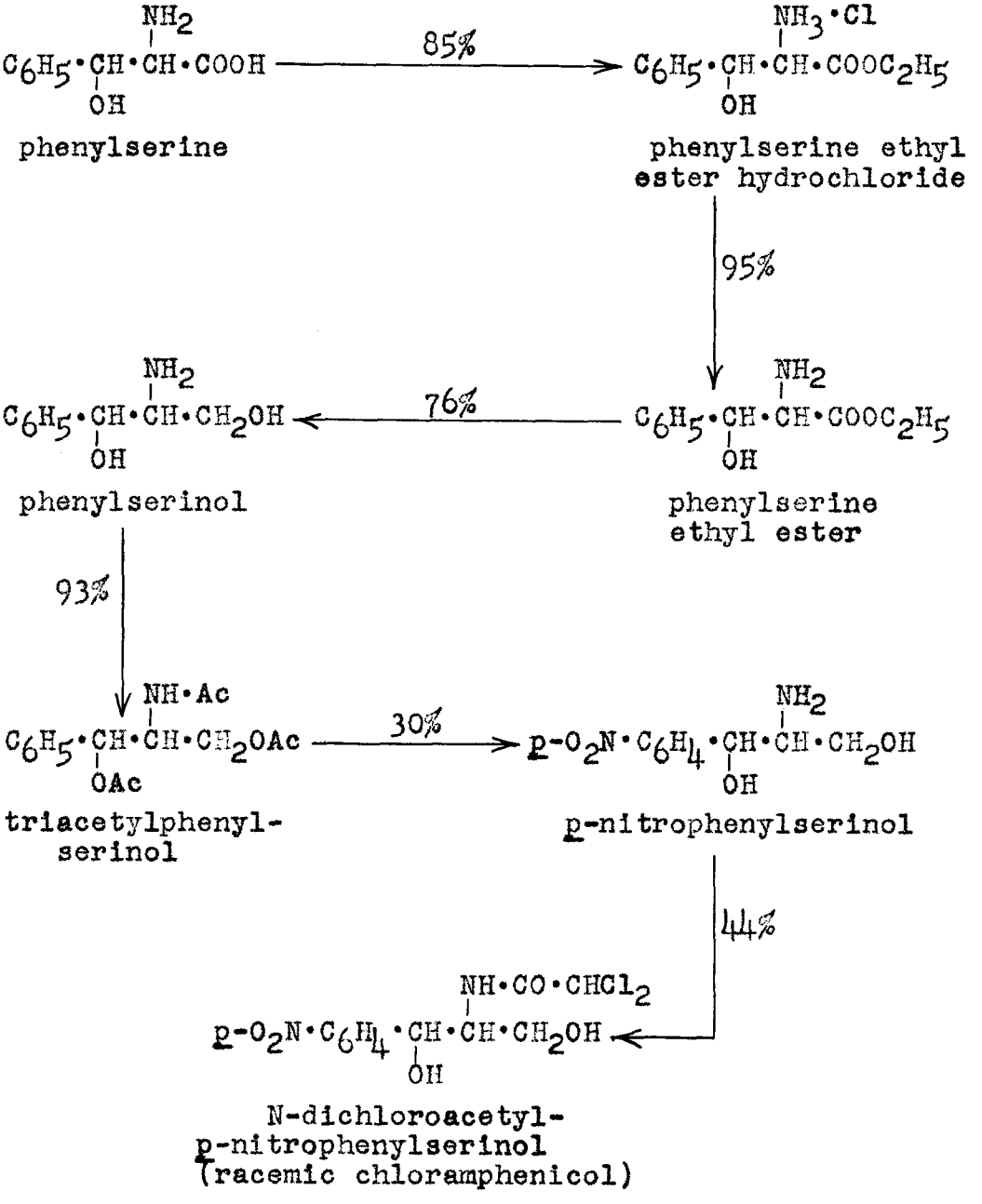

Fig. 4 Transformation of Phenylserine to Chloramphenicol

of the antibiotic, since it obviates troublesome separation of isomers at the aminediol stage.

Phenylserine ester hydrochlorides were obtained readily in excellent yield, nicely crystalline form and high purity. These compounds are not appreciably hygroscopic and can be stored indefinitely at room temperature. The Hillmann procedure (1) for conversion to the free esters, by treatment with 2% ammonia in cold chloroform, was found poorly applicable, due to formation of ammonium chloride in troublesome colloidal form. Transformation is effected without such manipulative difficulty by bubbling ammonia gas through an ether suspension of the ester hydrochlorides. The free bases are easily isolated in high yield subsequently. The recrystallized esters may be kept in a desiccator over anhydrone for some weeks, although, even then, declining melting point suggests deterioration. Impure products, or those open to the atmosphere, degenerate more rapidly to oily gums smelling of ammonia and benzaldehyde. In view of this instability, isolation and storage of the intermediate ester hydrochlorides is deemed more practical than direct conversion of phenylserine in large quantity to the free esters, even though the latter course gives higher yields and involves less manipulation.

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(1) G. Hillmann, Z. Naturforsch., 1, 682 (1946).

Reduction of phenylserine esters with lithium aluminum hydride was accomplished via modification of procedures used by the Karrer group with other amino acid esters (1). Introduction of the ester by way of Soxhlet extraction is a convenient way of controlling reaction vigor. On one occasion when water was added through a dropping funnel to the reaction mixture to destroy excess lithium aluminum hydride, small flames and sparks flickered through the ether slurry, with carbonized areas appearing on the flask wall. Thus, the danger of violent explosion at this point is noteworthy. By contrast, decomposition proceeded quietly when ether saturated with water was used. Yields of phenylserinol were only moderate on most occasions. Inefficient extraction of ths aminediol from the lithium-aluminum residues is considered to be the cause, rather than incomplete ester reduction. This contention is supported by the recovery pattern observed in the case where twelve ether extractions gave a 76% yield. Hot ethanol or chloroform are poorly effective as extracting solvents. An attempt to proceed, without isolation of phenylserinol, directly to its triacetyl derivative, although fairly successful, was time-consuming and somewhat impractical from

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(1) P. Karrer, P. Portmann and M. Suter, Helv. Chim. Acta, 31, 1617 (1948); 32, 1156 (1949).
P. Karrer and P. Portmann, ibid., 31, 2088 (1948); 32, 1034 (1949).
P. Karrer, M. Suter and P. Waser, ibid., 32, 1936 (1949).

the standpoint of cost. To achieve better yield, other solvents should be tested, as well as the possibility of solubilizing the interfering aluminum hydroxide by use of citrate, tartrate or other complexing agents.

Elimination of *erythro* compounds was achieved at the N,O-diacetylphenylserinol stage by the Parke, Davis and Co. group (1). This derivative is no longer important in the phenylserine route to chloramphenicol, in which only *threo* compounds are encountered. Good yields of the N-acetyl and N,O-diacetyl derivatives are not always attained, due to formation of mixtures upon attempted partial acetylation. By contrast, triacetylation proceeds almost quantitatively.

As already remarked by the Parke, Davis and Co. group, triacetyl-*p*-nitrophenylserinol is not easily purified without loss, and acid hydrolysis of the covering groups without isolation, is the more practical approach. Both this step and the final dichloroacetylation operation need further study to improve recoveries.

Shortly before the findings of this study were reported (2), announcement was made of work along the same lines by two

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).
(2) K. N. F. Shaw and S. W. Fox, Abstracts of Papers, 118th Am. Chem. Soc. Meeting, p. 28N (1950).

groups in Italy (1,2). Subsequently, additional papers in the same vein appeared in the literature (3,4,5,6). It had originally been planned to use knowledge acquired from the phenylserine-chloramphenicol sequence in attempting the synthesis of heterologues and other derivatives of possible chemotherapeutic value. However, in the face of clearly indicated interest in problems of this sort displayed by various pharmaceutical groups, attention was returned to phenylserine itself, with which promising new leads had already been obtained.

C. Characterization of Allophenylserine

As already discussed under REVIEW OF LITERATURE, Erlenmeyer contended that benzaldehyde-glycine condensation produced not only phenylserine, but also minor amounts of its expected diastereomer. Later investigators neglected or failed to verify this contention.

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(1) C. G. Alberti, B. Asero, B. Camerino, R. Sannicolò and A. Vercellone, Chimica e industria (Milan), 31, 357 (1949).
(2) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).
(3) K. Vogler, Helv. Chim. Acta, 33, 2111 (1950).
(4) K. Hayes and G. Gever, J. Org. Chem., 16, 269 (1950).
(5) C. F. Huebner and C. R. Scholz, J. Am. Chem. Soc., 73, 2089 (1951).
(6) G. W. Moersch (to Parke, Davis and Co.). U. S. Patent 2,538,792. January 23, 1951.

On one occasion, tail crops of phenylserine from potassium hydroxide-catalyzed condensation, which showed the same melting range but much finer crystalline form than first crop material, were treated with hydrogen chloride in ethanol. It was of particular interest to observe the unexpected precipitationof an ethyl ester hydrochloride which was in appearance quite different from that of phenylserine. The same phenomenon was encountered when tail crops from the German patent procedure were used. The new compound was readily converted to its parent ester by treatment with ammonia in chloroform. The probability that the two substances were isomers of the corresponding phenylserine derivatives was first suggested by analytical data. Confirmatory evidence was provided by lithium aluminum hydride reduction of the new ester to dl-erythro-1-phenyl-2-amino-1,3-propanediol, which was further characterized as its N-acetyl, N,O-diacetyl and triacetyl derivatives by use of Parke, Davis and Co. procedures (1).

The free hydroxyamino acid, named allophenylserine because of its obvious structural relation to allothreonine, is easily prepared by cold alkaline hydrolysis of the ester hydrochloride. Although its decomposition temperature, alone or mixed with phenylserine, falls in the same range as that of the latter, allophenylserine is individually distinct in

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(1) J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., J. Am. Chem. Soc., 71, 2463 (1949).

its crystal form.

Findings of this investigation partially justify Erlenmeyer's early claim, insofar as it is reasonable to suppose that he obtained allophenylserine considerably contaminated with the less soluble phenylserine, from the mother liquors of benzaldehyde-glycine condensation. However, crystallization studies, discussed later, point to the improbability of his having prepared isomerically pure allophenylserine by mere recrystallization from water-ethanol.

D. Partial Separation of Phenylserine and Allophenylserine via Ethyl Ester Hydrochlorides

After characterization of allophenylserine, it was of obvious interest to ascertain just how conditions of benzaldehyde-glycine condensation could be modified to produce this diastereomer in quantity. Before such investigation could be pursued, the problems of distinguishing and separating the compound from phenylserine required attention.

Crude phenylserine was prepared by the German patent procedure (1) with only four hours standing allowed before acidification of the condensation intermediate, instead of the prescribed twenty-four. To determine whether recrystallization of the product from various media brought about any

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(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

separation of the hydroxyamino acid diastereomers, conversion to the ethyl ester hydrochlorides was used. Allophenylserine ethyl ester hydrochloride precipitating out was weighed. That remaining in solution was estimated on the basis of rough solubility data, and thus a tentative indication of isomer content in the original crude product and the recrystallized fractions was provided. Recrystallization from water lowered allophenylserine content from no greater than 30% in the crude material to less than 10%. Likewise, successive recrystallization of crude product from 50% methanol and 50% ethanol gave crystals apparently quite devoid of allophenylserine. Dioxane showed promise of utility in cleaning up tail fractions containing allophenylserine from mother liquors, although this solvent again gave anomalously high recoveries.

At this point, the means were available for securing a pure phenylserine head fraction and for recovering a tail fraction of increased allophenylserine content. By treating the latter with hydrogen chloride in ethanol, varying amounts of pure allophenylserine ethyl ester hydrochloride were obtainable. With an eye to further fractionation of the mixed ethyl ester hydrochlorides then recovered from ethanol mother liquors, the solubilities of the individual ethyl ester hydrochlorides in various organic solvents were compared. Although the allo compound alone proved to be poorly soluble

in dioxane, it dissolved readily in the presence of its diastereomer. Manipulative difficulties arising from high freezing point further disqualified dioxane as a separating solvent. The possibility that acetone might prove suitable was suggested by a report that the ethyl ester hydrochlorides of threonine and allothreonine differed greatly in their solubilities in this solvent (1). The two ethyl ester hydrochlorides in the phenylserine series also showed wide solubility differences in acetone, but, curiously, order was reversed, the allo compound being the less soluble. Again, solubility of allophenylserine ethyl ester hydrochloride was found to rise in presence ofphenylserine ethyl ester hydrochloride, but not to as great a degree as in dioxane. While of use for augmenting stocks of allophenylserine ethyl ester hydrochloride, acetone passage was not entirely satisfactory, since a substantial fraction of phenylserine derivative with an estimated 5-10% allo content remained.

The scheme of isomer separation devised on the basis of the foregoing observations is summarized in Fig. 5. Several disadvantageous features are visible. Recovery of each isomer in pure form is moderate, but incomplete. Too many operational steps are involved to permit more than a rough estimate of isomer content in the original crude condensation

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(1) D. F. Elliott, J. Chem. Soc., 589 (1949).

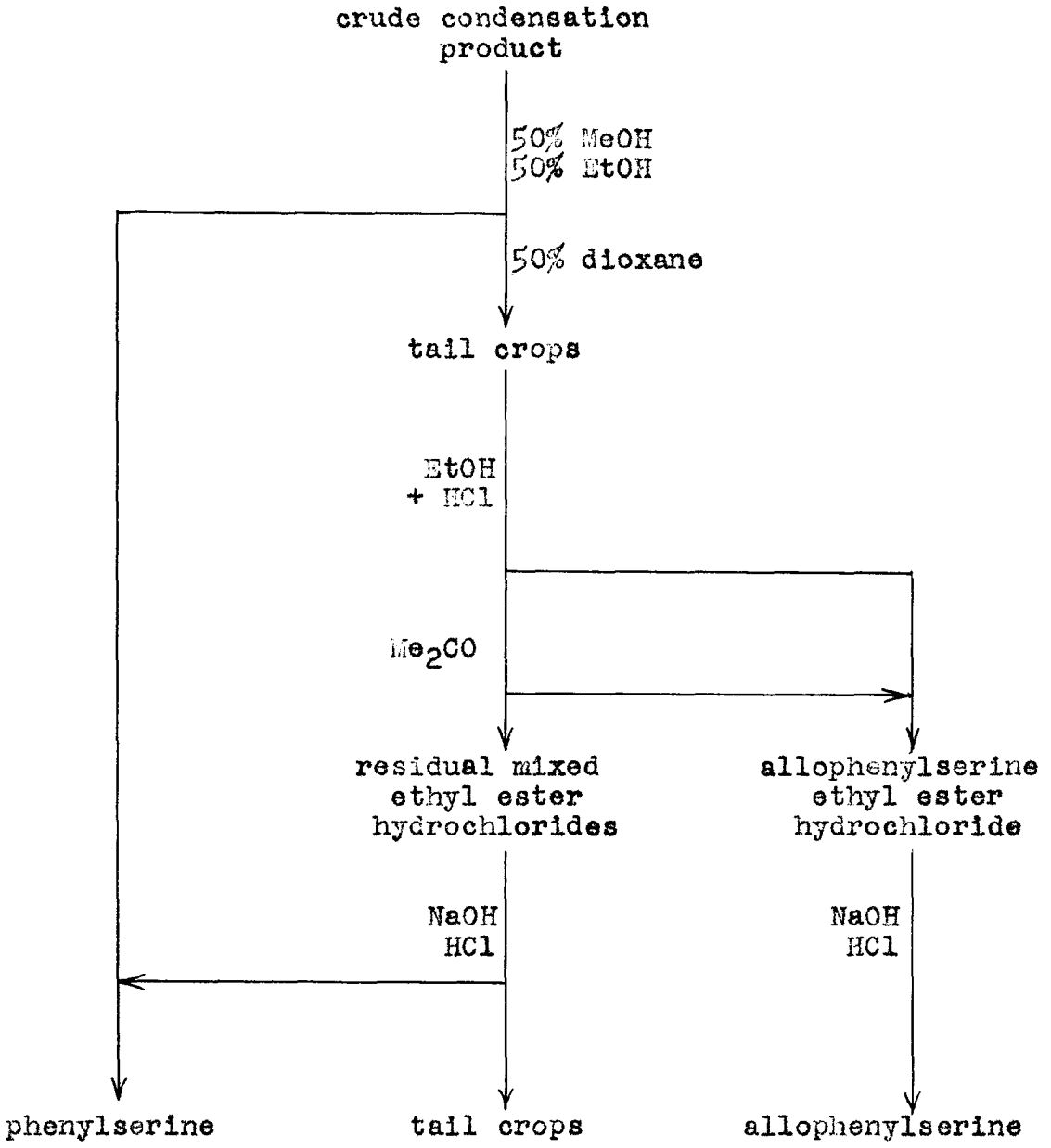

Fig. 5 Partial Separation of Phenylserine and Allophenylserine via Ethyl Ester Hydrochlorides

product. Thus, a detailed study of the condensation reaction itself is not facilitated.

E. Paper Chromatography

The problem of isomer estimation similarly arose in studies on synthesis of threonine and allothreonine. In these cases, a solution was found in the form of a microbiological assay method specific for L-threonine (1). Whereas the likelihood of finding an organism requiring phenylserine or allophenylserine for growth appeared to be small, the prospect of developing an assay method based upon competitive inhibition of phenylalanine or threonine utilization seemed more reasonable. However, paper chromatography, given first attention in view of its simple technique, speed and low cost, was found to satisfy all needs of this study.

Phenylserine and allophenylserine are separated on paper chromatograms by use of the upper water-poor layer from a mixture containing by volume 50% butanol, 6.25-7.5% acetone, 6.25-7.5% concentrated ammonium hydroxide and the rest water. For optimum results, acetone level is critical over a small range, whereas the amount of ammonia has much less effect, except at relatively high or low levels. Outside the specified limits for these components, separation of the two isomers,

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(1) K. Pfister, E. E. Howe, C. A. Robinson, A. C. Shabica, E. W. Pietrusza and M. Tishler, J. Am. Chem. Soc., 71, 1096 (1949).

reflected in ΔR_F values, is less extensive. The migration of one isomer is not influenced by that of the other, regardless of the relative concentration of either. As is now well known, R_F values vary somewhat with such factors as different solvent batches, grade and grain of paper and external temperature. Any difficulties arising in this regard are readily avoided by running suitable controls along with unknowns. The more serious effect of sample pH is of no concern here, since the solvent mixture employed is effectively buffered.

The paper chromatographic technique is qualitatively useful over the concentration range 0.01-2.5% (W/V) with either diastereomer; e.g. one part of allophenylserine in 250 of phenylserine is detectable when a 2.5% solution is chromatographed. For approximate quantitative work and for comparison purposes, solutions containing 0.05-0.2% of the given hydroxyamino acid are satisfactory. Greater analytical accuracy and precision, not sought in this study, could be achieved by use of standard volumetric micropipettes for dispensing solutions on the paper sheets, and by estimating areas of the ninhydrin-developed spots photometrically. The sensitivity, 25 mg. or less, is an obvious advantage.

The paper chromatographic method proved to be a powerful tool in this investigation. The effect of changing reaction conditions in benzaldehyde-glycine condensation on ratio of

diastereomers in the crude product was readily followed. Processing losses became controllable. The efficiency of schemes devised to separate the isomers and the isomeric purity of various fractions thereby obtained were easily checked. The possible applicability of paper chromatography in similar fashion to other organic reaction studies is thus worthy of consideration.

The solvent mixture used with the phenylserines was found also to be effective in separating the structural analogues, threonine and allothreonine. No attempt was made in this case to vary the proportion of solvent constituents in order to attain maximum ΔR_F. With only slight improvement in this direction, the method would be of value for following isomer content in threonine synthesis, especially with production on an industrial scale. Similar investigation of other amino acids with more than a single asymmetric centre, e.g. isoleucine, hydroxyproline, would be of interest. Paper chromatographic separation of peptide diastereomers has already been achieved (1).

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(1) J. W. Hinman, E. L. Caron and H. N. Christensen, J. Am. Chem. Soc., 72, 1620 (1950).

F. Effect of Condensation Time on Phenylserine Diastereomer Content and Yield: Possible Reaction Mechanism

In some early condensation runs by the German patent procedure (1), only four hours, instead of the prescribed twenty-four, were allowed to elapse between congelation of the insoluble intermediates and acidification. When crude products were converted to ethyl ester hydrochlorides, more allo compound was obtained than expected. This was the first indication that condensation time was related to diastereomer content. Closer examination of the situation became possible after the paper chromatographic method had been devised.

Total yield of the phenylserines is only moderate with a short condensation time, due to incomplete reaction (indicated by presence of unreacted glycine). The high yield obtained with a 24 hour period is fully in accord with the claims of the German patent (1). Yield appears to fall slightly with a longer condensation time, possibly because of slow side reaction leading to the diphenylhydroxyethylamines.

The effect of condensation time on the allophenylserine content of crude products is striking. With a one hour period, allophenylserine is formed to an extent comparable to phenylserine. With increasing condensation time, the allophenylserine level falls rapidly, the compound no longer

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(1) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

being demonstrable in the crude product when a 24 hour interval is used. The smaller amounts of this diastereomer then produced remain dissolved in aqueous mother liquors or are eluted during hot ethanol washing.

This course of events partly explains why earlier investigators were unable to secure allophenylserine. Forster and Rao (1) specified 24 hours prior to acidification, whereas Erlenmeyer's procedure (2) vaguely called for a standing period of several hours. In the latter instance, with more procedural steps involved, the likelihood of losing allophenylserine by solution was correspondingly greater. In this light, it is open to question whether allophenylserine was present in preparations synthesized by other workers according to either of these methods. The proximity of decomposition temperatures of the two isomers, alone or mixed, and the lack of other suitable means of differentiation heretofore, are features which would have complicated recognition of allophenylserine, even had it been encountered as a major product.

The problem of easily synthesizing allophenylserine has been solved. Yield of this isomer, as well as of phenylserine, could be improved by slightly modifying the

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(1) M. O. Forster and K. A. N. Rao, J. Chem. Soc., 1943 (1926).
(2) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).

procedure to curtail losses to the aqueous filtrate or to the ethanol wash. If desired, refinements of paper chromatographic technique, suggested in the preceding section, could be employed to secure analytical data more accurate than the obvious approximations presented in Tables 18 and 19. Of greater potential interest would be the study of the condensation time-isomer ratio relation in other similar reaction systems. The recently reported synthesis of β-2-thienylserine (1) along lines of the German patent procedure (2) is worthy of reinvestigation. The possibility of preparing other heterocyclic serine diastereomers is also attractive, particularly from the chemotherapeutic standpoint.

Mechanisms offered in the past to explain formation of phenylserine from benzaldehyde and glycine have been discussed under REVIEW OF LITERATURE. N-Benzalglycine in anionic form and the sodium salt of N-benzalphenylserine were postulated as intermediates, with only the latter claimed to have been isolated. It is clear from observations made in the course of this investigation that the reaction is by no means as simple as supposed hitherto, and that probably still more intermediates are involved.

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(1) G. Weitnauer, Gazz. chim. ital., 81, 162 (1951).
(2) Ges. für Kohlentechnik m.b.H. German patent 632,424. July 8, 1936.

A tentative reaction path, which reasonably explains the findings of this study is presented in Fig. 6. It is to be emphasized that for conclusive verification, the scheme still requires the actual isolation and characterization of individual intermediate compounds.

When high speed agitation of the condensation reactants was effected by means of a Hershberg stirrer, precipitation of a new substance was observed after 2-3 minutes. This phenomenon, not reported by other investigators, did not occur with slow mixing of the reactants. If the initial reaction is heterogeneous, the increased surface area resulting from finer dispersion of the benzaldehyde droplets could be responsible. Thus, with slow stirring, concentration of an early intermediate could remain low, as a result of its undergoing further reaction. At higher speeds, momentary precipitation of this intermediate would follow from the fact that its solubility value had been exceeded. The more rapid final congelation of the reaction mixture occurring at higher stirring rates (30 instead of 45 minutes) shows the influence of this factor.

It is reasonable to suppose that reaction commences with nucleophilic attack on the benzaldehyde carbonyl group by the glycine nitrogen, followed by loss of a proton from the latter. Acquisition of a proton by the oxygen of the former benzaldehyde part of the adduct, elimination of the resultant

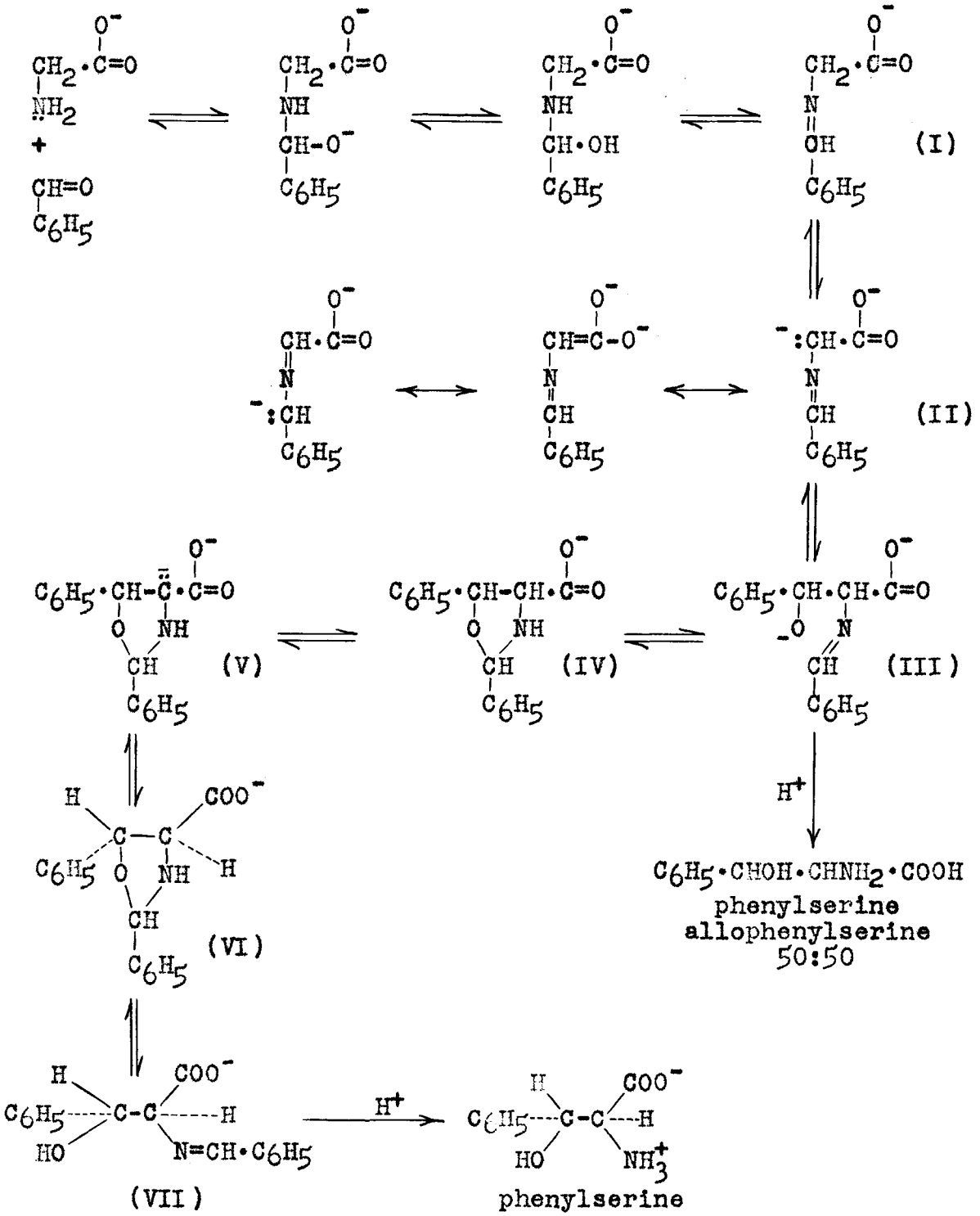

Fig. 6 Tentative Reaction Mechanism for Synthesis of the Phenylserines

hydroxyl group, with a concurrent electron shift from the nitrogen, and, finally, loss of another proton from the nitrogen would lead to the Schiff base anion (I) of benzalglycine. At high concentration, this moiety would precipitate from solution as its sodium salt.

To arrest reaction at this point, and thereby to facilitate isolation of the first solid intermediate, several approaches merit consideration. Dilution of the reaction mixture with a less polar aprotic solvent, e.g. dioxane, or adjustment of pH toward neutrality (dry ice) might be of value. Alternatively, the purpose might be better served by initial use of lower pH, of a weaker basic catalyst or by operating at a lower temperature to decrease reaction rates. Once isolated, the compound could be readily checked for identity with sodium benzalglycinate.

The rapid redissolving of the first intermediate could be related to base-catalyzed formation of an α-carbanion (II), of which several contributing forms are depicted in Fig. 6. Nucleophilic attack by II on another molecule of benzaldehyde would proceed in a non-stereospecific fashion to give III (two geoisomeric pairs of diastereomers). Extensive precipitation of sodium salts from III with rising concentration would correspond to solidification of the condensation reaction mixture after 30 minutes. Some dissolved III could undergo cyclization to give the oxazolidinecarboxylate

structure (IV), for which four diastereomeric forms are equally possible and whose sodium salts may also be poorly soluble. A certain amount of unreacted I could also be present. Such a combination of features would aptly explain why acidification after a one hour condensation period leads to free benzaldehyde in fair quantity, roughly equal amounts of phenylserine and allophenylserine (but not in high total yield), and some free glycine.

If the insoluble condensation intermediates are allowed to stand for four hours, acidification is no longer accompanied by appearance of free benzaldehyde in the medium, even at pH 2.8. As lumps of the condensation material slowly disintegrate, another new substance is thrown down. Its precipitation occurs to a greater extent, and the yield of phenylserine finally obtained is greater, when sufficient acid is added to give pH 4-5 than when pH is adjusted to ca. 5.7, the approximate isoelectric point of phenylserine. When this acidification product is washed with hot ethanol, benzaldehyde is split out and a crude phenylserine remains in which the proportion of the allo isomer is relatively low.

A reasonable explanation for the processing behavior just described can be offered with the aid of the sequence in Fig. 6. With the passage of time, increased conversion of III to IV could occur. Under the influence of the hydroxyl ion present, there could arise a small amount of

the doubly charged anion V, in which coplanarity of the carboxylate group with the oxazolidine ring is worthy of note. Under conditions of equilibrium, return of the proton to the 4-carbon of V would proceed with due regard for steric considerations. Thus, formation of VI, in which the β-phenyl and α-carboxylate groups are trans to one another, would be favored, rather than the corresponding cis-oxazolidine structure. If the sodium salts of IV and VI were less soluble than those from III, the former would slowly increase in quantity at the expense of the latter to a point where III would be a minor constituent of the condensation cake. Under such circumstances, acidification would give the free 2,5-diphenyloxazolidinecarboxylic acids, with the 4,5-trans form predominating. Poor solubility of this compound in the aqueous reaction medium at room temperature would impede a further ring-opening reaction. In the course of ethanol washing at higher temperatures, hydrolytic cleavage would lead to a crude phenylserine of low allo content.

Isolation of the acidification product in a four hour condensation run should not prove too difficult, since it displays moderate stability. Its observed solubility in 50% methanol or 50% ethanol may prove applicable to its purification. Whether the compound is an oxazolidinecarboxylic acid or whether it contains a C=N link could possibly be checked by examination of infrared absorption spectra.

Behavior in the case of a 24 hour or longer condensation period could be correlated with a further transformation of VI to the sodium salt of N-benzalphenylserine (VII). Acid cleavage of VII, proceeding in the same fashion as with III, would lead exclusively to phenylserine. Only small amounts of allophenylserine would arise simultaneously, from hydrolysis of residual quantities of earlier intermediates.

Some consideration was given to other possible reaction mechanisms for the synthesis of phenylserine and allophenylserine. One involved 2-phenyloxazolidone; another included 2-phenyl-3-aziridinecarboxylic acid. Neither route proved as satisfactory as the one just described for closely correlating experimental observations.

Reference has been made under REVIEW OF LITERATURE to Erlenmeyer's studies on the diphenylhydroxyethylamines, which form as by-products in the condensation of benzaldehyde and glycine, and which can constitute the main products if reaction conditions are suitably changed. A threo configuration was recently shown (1) for isodiphenylhydroxyethylamine, which is the predominating diastereomer obtained by this route. The preferential formation of this compound is reasonably explained by extension of the reaction sequence postulated earlier for synthesis of the phenylserines.

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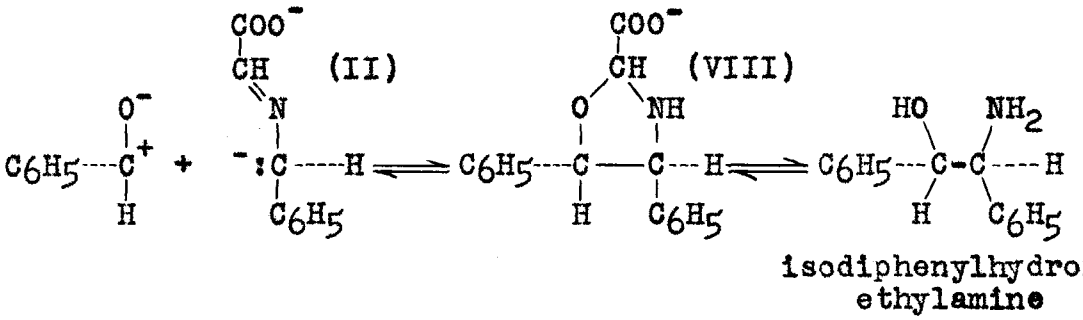
(1) J. Weijlard, K. Pfister, E. F. Swanezy, C. A. Robinson and M. Tishler, J. Am. Chem. Soc., 73, 1216 (1951).

Steric considerations may again be invoked in dealing with the reaction between II and benzaldehyde, which would give



4,5-<u>trans</u>-diphenyl-2-oxazolidinecarboxylic acid (VIII) rather than the corresponding <u>cis</u> compound. Isodiphenylhydroxyethylamine would result from acid cleavage of VIII.

Several purposes could be served by further investigation of the glycine-benzaldehyde condensation. It may prove possible to arrest the reaction sequence at some intermediate in such a way that high yield could be attained without epimerization of the allophenylserine precursor. In this regard, use of bases other than sodium hydroxide to catalyze condensation might lead to desirable shifting of equilibria between intermediates, as a result of solubility changes. Epimerization may possibly be avoided by employing a weaker base in the system. Experiments should also be run to determine the influence of reaction temperature. A better understanding of the reaction mechanism, to be gained in such a study, may permit extension of the reaction scope in a beneficial manner.

Recent work (1,2) on interconversion of the individual stereoisomers of threonine and allothreonine via oxazoline intermediates points the way to solution of the same problem in the phenylserine series. However, an easier approach may lie through the reaction sequence presented for synthesis of the phenylserines. If this scheme be valid, then overnight standing of a well-agitated mixture of allophenylserine and benzaldehyde in aqueous sodium hydroxide should result in epimerization via the oxazolidine intermediates V and VI. If such be the case, phenylserine would be obtained upon acidification of the reaction mixture. It would be of some commercial importance if epimerization of allothreonine could be effected in the same manner.

G. Separation of Phenylserine and Allophenylserine via Solvates

The problem of separating phenylserine and allophenylserine at the hydroxyamino acid level became simplified with paper chromatography available to follow the progress of fractionation. It was recently reported (2) that the sodium salt of threonine is much less soluble in ethanol than that of allothreonine, a finding which proved well-suited to

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(1) D. F. Elliott, J. Chem. Soc., 589 (1949); 62 (1950).
(2) K. Pfister, C. A. Robinson, A. C. Shabica and M. Tishler, J. Am. Chem. Soc., 71, 1101 (1949).

separation of these isomers. In this investigation, a limited parallel was observed with the phenylserine diastereomers. Although the sodium salt of phenylserine dissolved in ethanol to a lesser extent than that of allophenylserine, both are so poorly soluble that they show little promise for separation purposes. Further, slight scission of the molecule occurs under the basic conditions involved.

Portions of crude phenylserine (40-45% allophenylserine) from a one hour condensation run were recrystallized from different aqueous-organic media. Paper chromatography of the various fractions thus obtained revealed a useful solubility pattern. Although phenylserine is less soluble than allophenylserine in water or aqueous alcohols, the order of isomer solubility is reversed to a moderate extent in 50% aqueous acetone, and very sharply in 50% aqueous dioxane. Better recovery is achieved via 50% ethanol, but the allophenylserine content of the crystal crop is only a little less than that of the starting material, whereas with water alone, although recovery is lower, the product contains much less allophenylserine. With 50% acetone, the level of allophenylserine in the crystal crop improves somewhat; in 50% dioxane, this isomer is precipitated almost quantitatively. Total recovery is satisfactory in both cases. An explanation for this solubility behavior resulted from a study of the individual hydroxyamino acids, with samples whose isomeric purity had

been established by paper chromatography.

Phenylserine was well characterized by Erlenmeyer (1). In this study, the substance was rechecked, both in the anhydrous state and as the monohydrate, in the course of preparing control samples for analytical work, and while examining behavior of the compound on recrystallization. Treatment of hot aqueous solutions of the compound with an equal volume of hot ethanol has been found highly satisfactory for achieving final purification, excellent recovery and good crystalline form.

In the early part of this investigation, dioxane was considered merely as a satisfactory agent for recovering phenylserine tailings from aqueous mother liquors. However, in two instances discussed earlier, recovery was observed to exceed 100%. The reason for this anomaly became clear with the discovery that allophenylserine and dioxane combine in a 2:1 ratio to form a poorly soluble compound. The adduct precipitates rapidly in nicely crystalline form when a boiling aqueous solution of allophenylserine is treated with an equal volume of boiling dioxane. Mixture at lower temperatures sometimes leads to troublesome gelation.

The fact that evacuation at moderate temperatures does not cause removal of dioxane from the addition compound

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(1) E. Erlenmeyer, Jr., and E. Frühstück, Ann., 284, 36 (1894).

indicates strong binding of the solvent molecule. However, when an aqueous solution is simmered gently, the odor of dioxane is readily apparent in the vapors. If boiling is continued for a few minutes, the dioxane is completely expelled and the free amino acid can be recovered in anhydrous form from the solution.

The infrared absorption spectra of anhydrous dioxane, and of Nujol mulls of phenylserine, allophenylserine and allophenylserine hemidioxanate were examined. Phenylserine and allophenylserine in the anhydrous state show general similarity, with only minor changes apparent in allophenylserine hemidioxanate. In the dioxane adduct, it is noteworthy that the many broad absorption bands characteristic of dioxane are no longer readily apparent. Observation of such drastic alteration makes untenable an early view that allophenylserine hemidioxanate might be a clathrate compound. The possibility that the substance may be an oxonium type salt is open to consideration (1).

The ability of allophenylserine to unite with dioxane is of considerable practical value to phenylserine chemistry. It provides the means by which this otherwise difficultly accessible hydroxyamino acid can be readily separated from its isomer, and by which recrystallization can be effected with

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(1) P. Karrer, "Organic Chemistry", Elsevier Publishing Co., New York, 1950, 4th English ed., p. 246.

high recovery.

By recrystallization from hot 50% acetone, allophenylserine is obtained with good recovery in the anhydrous state as a bulky fluff of fine thread-like needles. The same form, with slightly lower recovery, results from slow cooling of hot aqueous solutions. By contrast, if a hot water solution of allophenylserine is suddenly chilled, or if clear solutions at room temperature are permitted to evaporate, dense hexagonal prisms of the hemihydrate are produced.

The form in which allophenylserine is obtained from 50% ethanol varies according to other processing factors. Short needles of anhydrous material resulted in one case where a clear aqueous solution of the hydroxyamino acid was treated with ethanol at room temperature. On the other hand, only the hemihydrate was produced under similar circumstances when a seed of this crystalline form was initially added. On one occasion, addition of ethanol to an aqueous allophenylserine solution containing sodium chloride caused precipitation of still another solvate, in the form of rafted needles, which may have been a monohydrate. This material, unfortunately destroyed, was of some historical interest, since Erlenmeyer had claimed isolation of an allophenylserine monohydrate under comparable conditions (1). Reasons for the

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(1) E. Erlenmeyer, Jr., Ann., 307, 70 (1899).

probable isomeric impurity of his material have already been discussed.

A few minor points concerning recrystallization of allophenylserine merit brief note. Although more soluble in water than its isomer, allophenylserine in the anhydrous fluffy form is poorly wettable and hence dissolves slowly. Considerable simmering is required to prepare concentrated solutions. In a recent report (1) on preparation of "erythrophenylserine", this substance, as well as phenylserine itself, was stated to decompose on boiling, even in neutral media, with liberation of benzaldehyde. On some occasions in this investigation, aqueous solutions of the pure phenylserine diastereomers have been concentrated at atmospheric pressure with no sign of appreciable decomposition. The technique which has been described for recrystallization of allophenylserine from 50% acetone (mixing while hot, intermittent swirling) is designed to avoid gel formation and to furnish the compound in readily filtrable form. Ether washing is advised to eliminate traces of mesityl oxide, the odor of which is sometimes faintly apparent.

By the use of integrated data from recrystallization experiments, the crude products of one hour condensation runs were effectively separated into isomerically pure fractions of

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(1) I. Elphimoff-Felkin and H. Felkin, Compt. rend., 232, 241 (1951).

phenylserine and allophenylserine. A typical fractionation is summarized in Fig. 7. Processing by the route outlined offers no particular difficulty and requires little actual working time. Recoveries of each isomer are excellent (values in Fig. 7 are related to the initial crude product, with no allowance made for the fact that tail crops can be reworked with subsequent fractionation runs).

A brief study of the decomposition temperatures of phenylserine and allophenylserine, as well as of their various solvates, was made in order to reemphasize the relative insignificance of this physical property as a criterion of purity, isomeric or otherwise, of the phenylserine diastereomers. It is to be noted that time and duration of heating are factors affecting the decomposition temperature considerably more than degree of solvation or isomeric purity, and that the testing of mixtures gives scant information of value with these compounds.

H. Derivatives of Phenylserine and Allophenylserine

Phenylserine hydrochloride, m.p. 157°, has been mentioned as an intermediate in conversion of phenylserine to its ester hydrochlorides (1). More recently, "erythrophenylserine"

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(1) G. Carrara and G. Weitnauer, Gazz. chim. ital., 79, 856 (1949).

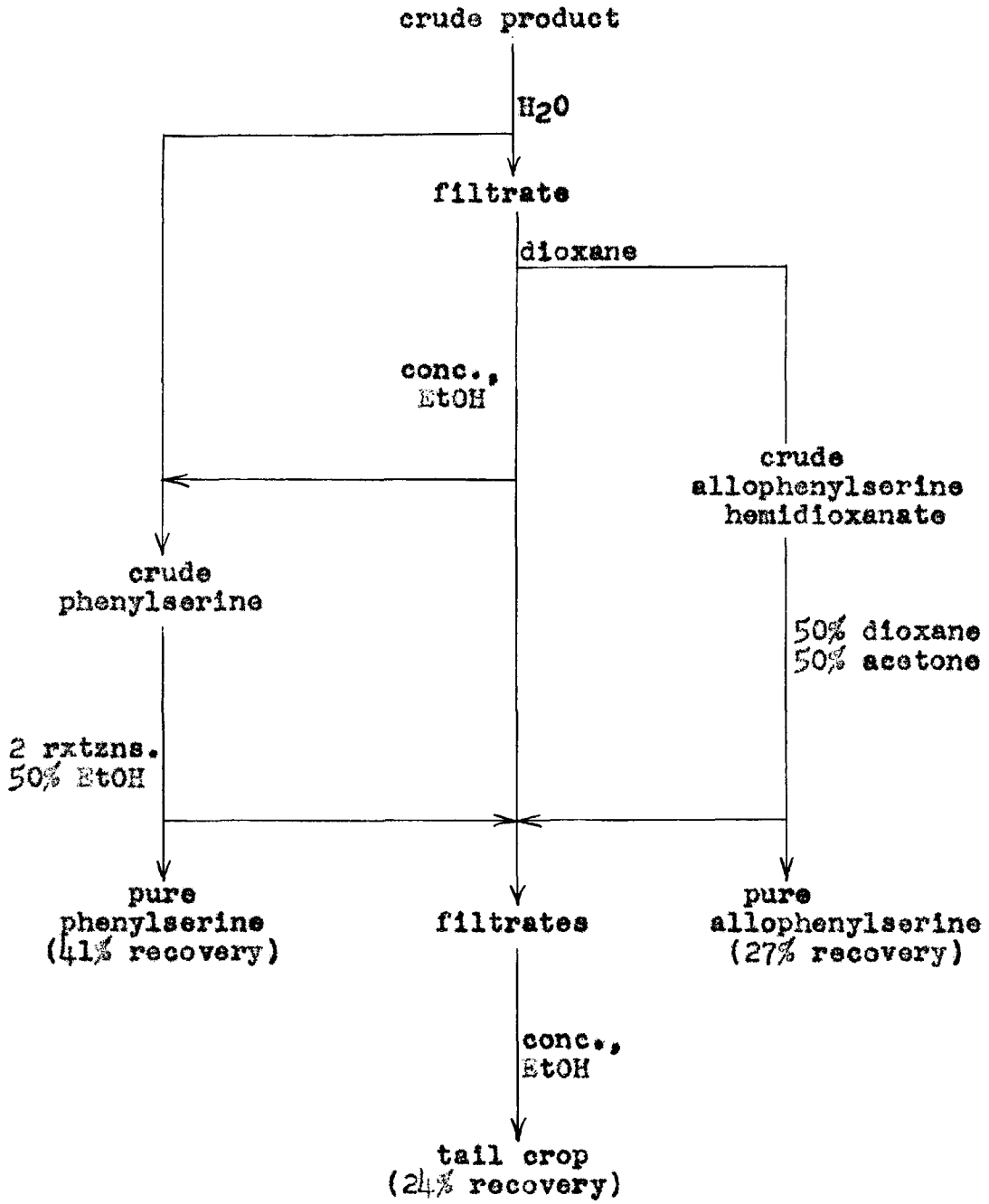

Fig. 7 Fractionation of Pure Diastereomers from Crude Phenylserine

hydrochloride, m.p. 212°, was briefly noted (1). Neither compound was characterized by analytical or other data.

In this study, the hydrochlorides were readily prepared by brief treatment of the isomerically pure individual hydroxyamino acids in anhydrous dioxane with dry hydrogen chloride. Addition of ether to the resultant clear solutions precipitated the products in high yield. Recrystallization could not be effected from fresh dioxane in which the compounds were poorly soluble and showed signs of decomposition on prolonged simmering. Purification was effected via methanol-ether, with short contact time and avoidance of elevated temperatures to minimize possible formation of methyl ester hydrochlorides. Each hydrochloride decomposed at ca. 160°.

In the early phases of this investigation, phenylserine methyl and ethyl ester hydrochlorides were prepared from phenylserine monohydrate. Allophenylserine ethyl ester hydrochloride was obtained as an unexpected by-product from processing phenylserine tail crops. When it became possible to check isomeric purity of the amino acids by paper chromatography, repetition of these syntheses was deemed in order. The fact that the various ester hydrochlorides were then obtained in almost quantitative yield is attributed in part to use of anhydrous starting materials, and higher temperatures

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(1) I. Elphimoff-Felkin and H. Felkin, Compt. rend., 232, 241 (1951).

during hydrogen chloride treatment. In the allo series, it may prove possible to curtail reaction time by initial use of larger volumes of the respective alcohols. The two series of ester hydrochlorides are quite stable, not hygroscopic, and crystallize well.

The melting points of phenylserine methyl, ethyl and propyl ester hydrochlorides were observed to be slightly higher than those recently reported by other workers (Table 3). The differences may reflect greater isomeric purity. The "phenylserine" methyl ester hydrochloride, m.p. 185° (Table 1), which was obtained via condensation of glycine methyl ester with benzaldehyde in methanol (1), may prove to be identical with allophenylserine methyl ester hydrochloride, m.p. 180° (dec.). Likewise, "erythrophenylserine" ethyl ester hydrochloride (2), m.p. 186°, seems to correspond with allophenylserine ethyl ester hydrochloride, m.p. 178° (dec.). However, in both cases, data which would permit certain correlation were not provided.

The various ester hydrochlorides in both series were converted to the corresponding free esters by treatment with ammonia gas in ether. Yields were good, but slightly lower than in earlier runs where second crops were recovered by

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(1) E. D. Bergmann, M. Genas and H. Bendas, Compt. rend., 231, 361 (1950).
(2) I. Elphimoff-Felkin and H. Felkin, Compt. rend., 232, 241 (1951).

concentration of filtrates. The melting points of phenylserine methyl, ethyl and propyl esters checked well with those recently cited by other workers (Table 3). The identity of "erythrophenylserine" ethyl ester (1), m.p. 86°, with allophenylserine ethyl ester, m.p. 86°, is uncertain, due to lack of information on the former substance.

The possibility of securing the respective optical isomers of phenylserine and allophenylserine most readily by selective enzymatic hydrolysis of the esters attaches particular importance to this group of derivatives. Highly effective resolution procedures involving action of pancreatic preparations on racemic isopropyl esters have recently been reported for phenylalanine (2) and for methionine (3). Once resolution is accomplished, it would be of interest to transform both D- and L-phenylserine to optically active intermediates in the chloramphenicol series of compounds, in order to conclusively correlate configuration of the antibiotic with that of natural amino acids.

Since enzymatic resolution of amino acids via their esters involves isolation of the unchanged D-ester, and then its hydrolysis to the D-amino acid, it was of interest to

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(1) I. Elphimoff-Felkin and H. Felkin, Compt. rend., 232, 241 (1951).
(2) K. A. J. Wretlind, J. Biol. Chem., 186, 221 (1950).
(3) M. Brenner. Swiss Patent 266,637. May 16, 1950.

ascertain whether cold alkaline hydrolysis of phenylserine and allophenylserine ester hydrochlorides was accompanied by appreciable epimerization. Paper chromatography, which under the conditions employed would have detected as little as 0.1-0.2% epimerization, showed no trace of unexpected isomer in the hydrolysate from the ethyl ester hydrochloride of either phenylserine or allophenylserine respectively.

Two minor features of interest were encountered in the course of alkaline hydrolysis experiments. Allophenylserine was recovered quantitatively as the hemidioxanate when dioxane was added to the alkaline solution of the ethyl ester hydrochloride just prior to final acidification. When phenylserine ethyl ester hydrochloride residues from the early acetone separation scheme were hydrolyzed, allophenylserine corresponding to ca. 7% of the starting material was demonstrated in the hydrolysate by means of paper chromatography. This value checked well with an earlier estimate (5-10%) based on approximate solubility data.

V. SUMMARY

1. The literature concerning the phenylserines was reviewed with respect to methods of synthesis, chemical reactions, and potential biochemical significance. The problems of steric configuration and heterocyclic analogues were considered. Apparent contradictory findings and possible areas for future research were indicated.

2. The most suitable route found for phenylserine synthesis was that described in German patent 632,424, by the directions of which benzaldehyde was condensed with glycine in aqueous sodium hydroxide. Advantages of the method are high yield, facile processing and less side reaction.

3. An attempt to extend the German patent procedure to synthesis of β-2-furylserine was unsuccessful.

4. Phenylserine was converted via lithium aluminum hydride reduction of its esters to racemic chloramphenicol. A threo configuration for the hydroxyamino acid was thus demonstrated. The advantage of this route to the antibiotic is the fact that the various intermediates belong to a single steric series.

5. It was found that, although condensation of benzaldehyde and glycine furnishes phenylserine as the main product, small amounts of a more soluble diastereomer also form. This

was demonstrated when Fischer esterification of condensation reaction tail crops yielded a new ethyl ester hydrochloride, much less soluble than that of phenylserine. Cold alkaline hydrolysis of this ethyl ester hydrochloride produced a new hydroxyamino acid, which was named allophenylserine after conversion of its ester to erythro intermediates in the chloramphenicol series had established its structural relation to the amino acid, allothreonine.

6. Phenylserine and allophenylserine were individually but incompletely recovered from crude condensation products by making use of the inversion of their solubility relation observed in proceeding from the free hydroxyamino acids to the corresponding ethyl ester hydrochlorides.

7. By use of a mixture containing n-butanol, aqueous ammonia and acetone, phenylserine and allophenylserine were effectively separated on paper chromatograms. Optimum conditions were established for employing paper chromatography as an analytical tool with these isomers.

8. Threonine and allothreonine were also separated on paper chromatograms by use of the same solvent mixture.

9. With the aid of paper chromatography, an investigation was made of the relation of phenylserine diastereomer ratio in the crude products to the time allowed for benzaldehyde-glycine condensation. With a one hour period, allophenylserine was formed to an extent comparable to phenylserine. With

increasing condensation time, the allophenylserine level fell rapidly. The compound was no longer demonstrable in the crude product when a 24 hour interval was used, under which conditions the yield of phenylserine approached a maximum. New intermediates in the condensation reaction were recognized, but not isolated.

10. A reaction mechanism was offered to explain why phenylserine and isodiphenylhydroxyethylamine, both possessing a threo configuration, are the preferred products of glycine-benzaldehyde condensation, rather than their respective diastereomers. Possible applications to further research were suggested.

11. Solvates of allophenylserine were prepared with water and with dioxane, in which the ratio of hydroxyamino acid to solvent is 2:1 for both compounds.

12. With paper chromatographic control, isomerically pure phenylserine and allophenylserine were fractionated with good recoveries from the crude product of one hour condensation runs. Effective separation was based upon the finding that although allophenylserine is more soluble than phenylserine in many aqueous solvent mixtures, its dioxane adduct is very poorly soluble.

13. The decomposition temperatures of phenylserine and allophenylserine, as well as those of their various solvates, and of their mixtures, were observed to fall in the same close

temperature range, so that this physical property is of no value as a criterion of isomeric purity.

14. The hydrochlorides of phenylserine and allophenylserine were prepared by treatment of the individual hydroxyamino acids with hydrogen chloride in dioxane.

15. The methyl, ethyl, n-propyl and i-propyl ester hydrochlorides of phenylserine and allophenylserine were synthesized according to a modified Fischer method in almost quantitative yield.

16. The various ester hydrochlorides were transformed to their respective esters by treatment with ammonia in ether. The prospect of securing the four optically active hydroxyamino acids by selective enzymic hydrolysis of the esters made these derivatives of interest.

17. Cold alkaline hydrolysis of phenylserine and allophenylserine ethyl ester hydrochlorides was shown, with the aid of paper chromatography, to proceed without epimerization.

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