1951

Organic bases and other agents for amino acid resolution

Ralph Burton Fearing
Iowa State College

ORGANIC BASES AND OTHER AGENTS FOR AMINO ACID RESOLUTION

by

Ralph B. Fearing

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Bio-organic Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1951

TABLE OF CONTENTS

INTRODUCTION

In general, techniques of resolution depend on crystal structure or on adsorption phenomena. The former is utilized in three types of procedure: (1) a supersaturated solution of a racemate may be seeded with one pure crystalline antipode; (2) the mirror-image crystals may occasionally be separated by tweezers, as did Pasteur; or (3) more reliably, diastereomers having different equilibrium solubilities may be prepared, by covalent bonding or by salt linkage, with a foreign asymmetric molecule of one configuration only. Crystallinity may be of less importance for covalent diastereomers. Under adsorption phenomena could be included enzymic methods as well as chromatographic resolution.

The most common procedures for resolution of amino acids have involved preparation of N-acylated derivatives suitable for selective enzymic reactions or for diastereomeric salt formation. These derivatives must subsequently be split to recover the amino acid, making three steps in all. The purpose of this investigation was to evaluate new procedures which might eliminate one or two steps in the resolution of monoamino monocarboxylic acids.

There are six general lines of attack which have been, or could be adopted in an effort towards simplification.

(a) Seeding techniques have a chance of success if some form of, or solution of, the amino acid can be found not characterized by a less-soluble racemic compound.

(b) Successful chromatographic separation would afford a one-step method.

(c) An attractive possibility, from a theoretical point of view, would be the formation of carbamino salts in the presence of asymmetric bases. Although addition of carbon dioxide to nitrogen in an amino acid constitutes derivatization, the formation and subsequent decomposition of this intermediate might be made spontaneous enough that the scheme could be classed as simplified resolution.

A discussion of possibilities (d), (e), and (f) requires consideration of the problem of forcing an amino acid dipolar ion to acquire a net electric charge. The monoaminomonocarboxylic variety, whose lack of basic amino or acidic carboxyl groups offers the greatest challenge, possesses a pK_A value in the region of 9.5, due to the RNH_3^+ portion (1). Since common aliphatic amines have pK_B's in the region 3.5 to 4.5 simple calculation shows that zwitter ions might be

---

(1) Cohn and Edsall. Proteins, Amino Acids, and Peptides. p. 84. New York, Reinhold Publishing Corp. 1943.

expected to surrender about half of their protons to an amine to form a negative ion, but any salt so formed would be metastable at best.

Another pK_A commonly given for amino acids, but actually referring to hydrochlorides, is in the range 2-2.5, due to the COOH group. From the point of view of the pure zwitter ion, this gives the COO^- group a pK_B of 14 - 2.5 = 11.5, making it even weaker than aniline. Even a relatively strong carboxylic acid such as d(+)tartaric acid cannot be expected to force protons on a zwitter ion completely enough for salt-formation.

(d) One way to deal with the zwitter ion problem might be to avoid it altogether. The amino acid itself might be synthesized by such a procedure that a resolution might be performed on an intermediate; the final step in manufacture would yield an optically active product.

Finally, a more direct approach for laboratory purposes is the use of optically active acids or bases strong enough to react completely with the COO^- or RNH_3^+ groups, respectively, of a zwitter ion.

(e) Various sulfonic acids have been used, with or without success, for resolution.

(f) A large part of the present work is devoted to

an investigation of a number of optically active strong bases in an attempt to produce crystalline salts of the types illustrated.

$$\left[RCH(NH_2)COO^-\right]\ \left[{}^+NRR'(CH_3)_2\right]$$

$$\left[RCH(NH_2)COO^-\right]\ \left[{}^+NH_2{=}C(NH_2)NHR\right]$$

Some of the alkaloids are easily converted to quaternary hydroxides. Consideration was also given to the problem of preparing strong bases from carbohydrates, amino acids, terpenes, sterols, and miscellaneous products of fermentation or chemical synthesis. Of these, only derivatives of (-)2-aminobutan-1-ol, d(+)2-dimethylaminobutan-1-ol, and d-camphor were prepared.

HISTORICAL

Ordinary Salt-Forming Procedures

The basic principles of resolution were laid down in 1860 in a classic series of lectures by Louis Pasteur (2). They are also classified and summarized in a review by Eichwald (3).

The most generally applied chemical resolution methods for amino acids, originated by Emil Fischer (4, 5, 6) near the turn of the century, have required destruction or moderation of basic character in the amino group. The free carboxyl group can then react with bases as weak as ordinary amines or alkaloids. Moderation of amino group basicity has been brought about by introduction of such acyl groups as formyl

---

(2) Pasteur. Researches on Molecular Asymmetry. Alembic Club Reprints No. 14. London, Simpkin, Marshall, Hamilton, Kent, & Co., Ltc. 1897.
(3) Eichwald, in Abderhalden's Handbuch der Biochemischen Arbeitsmethoden. Vol. 9, 645. Berlin, Urban and Schwarzenberg. 1919.
(4) Fischer, Ber., 32, 2451 (1899).
(5) Fischer, ibid., 33, 2370 (1900).
(6) Fischer and Mouneyrat, ibid., 33, 2383 (1900).

(7, 8, 9, 10, 11, 12, 13), benzoyl (4, 5, 6, 14, 15), m- or p-nitrobenzoyl (16, 17, 18, 19, 20), acetyl (21, 22), chloroacetyl (23), phenylcarbamido (24), nitroso (25), and benzenesulfonyl (26, 27). Sugasawa (28) dehydrated DL-Glutamic acid to pyrrolidonecarboxylic acid, through a sort of self-acylation, for resolution with quinine. Although formaldehyde effectively decreases amino group basicity, it does not appear to have been applied to resolution.

---

(7) Fischer and Schoeller, Ann., 357, 1 (1907).
(8) West and Carter, J. Biol. Chem., 122, 611 (1938).
(9) Windus and Marvel, J. Am. Chem. Soc., 53, 3490 (1931).
(10) Duffin and Wilkinson, British Patent 585,413 (1947). Abstracted in C. A., 41, 4175 (1947).
(11) Abderhalden and Zeisset, Z. physiol. Chem., 195, 121 (1931).
(12) Wood and du Vigneaud, J. Biol. Chem., 130, 109 (1939).
(13) Yaginuma, Hayakawa, and Arai, J. Agr. Chem. Soc. Japan, 8, 699 (1932). Original not seen. Abstracted in C. A., 26, 5073 (1932).
(14) Pacsu and Mullen, J. Biol. Chem., 136, 335 (1940).
(15) Dunn, Stoddard, Rubin, and Bovie, J. Biol. Chem., 151, 241 (1943).
(16) Colles and Gibson, J. Chem. Soc., 279 (1931).
(17) Winter, J. Am. Chem. Soc., 62, 3266 (1940).
(18) Arnstein, Nature, 164, 361 (1949).
(19) Zambito, Peretz, and Howe, J. Am. Chem. Soc., 71, 2541 (1949).
(20) Fischer and Jacobs, Ber., 39, 2942 (1906).
(21) du Vigneaud and Sealock, J. Biol. Chem., 96, 511 (1932).
(22) Shabica and Tishler, J. Am. Chem. Soc., 71, 3251 (1949).
(23) Abderhalden and Schmitz, Biochem. Z., 214, 158 (1929).
(24) Leuchs and Bormann, Ber., 52, 2086 (1919).
(25) Kenner and Mackay, Nature, 158, 909 (1946).
(26) Gibson and Levin, J. Chem. Soc., 2754 (1929).
(27) Berlingozzi and Naldi, Atti accad. Lincei, Classe sci fis., mat. e nat., 23, 874 (1936).
(28) Sugasawa, J. Pharm. Soc. Japan, No. 537, 934 (1926). Abstracted in C. A., 21, 2664 (1927).

Incorporation of an extra acidic group has been used by Levene and Schormüller (29) who resolved serine by esterification of the hydroxyl with phosphoric acid, and preparation of the brucine salt.

Fischer's original resolutions made use of most of the alkaloids, including morphine, quinidine, cinchonine, and brucine. Other bases used in salt formation have included strychnine (14, 15, 17), α-phenylethylamine (9, 21, 23), ephedrine (10), quinine (16, 18, 20, 30), and nor-pseudoephedrin (26). No method has been uniformly applied to all amino acids, but brucine salts of formylated amino acids have seen wide application*. Howe and Sletzinger (31) have used nitrobenzoyl-L-glutamic acid to resolve isomethadon.

Conversely, esterification of the carboxyl group frees the amino group to react with common acids such as tartaric. This principle has not been exploited significantly, although Linnell and Smith (32) used it for resolution of a racemic alanine derivative.

In the case of amino acids with extra acidic or basic groups, the zwitter ion portion can be ignored, and the

---

* See all references given on formylated amino acids.

(29) Levene and Schormüller, J. Biol. Chem., 106, 595 (1934).
(30) Gibson, Johnson, and Levin, J. Chem. Soc., 479 (1929).
(31) Howe and Sletzinger, J. Am. Chem. Soc., 71, 2935 (1949).
(32) Linnell and Smith, Nature, 162, 735 (1948).

amino acid resolved without substitution. This has been applied by Berg (33) to the resolution of lysine by camphoric acid, and by Pyman (34) to the resolution of histidine by tartaric acid. Arginine, with a zwitter ion involving guanidine and carboxyl groups, is left with a free amino group which accounts for the use of arginine itself as a resolving agent for biotin (35, 36). The diastereomeric amino acid salts were reviewed by Dunn and Rockland (37).

Enzymic Resolutions

The literature on enzymic, or biological, resolutions is very extensive, but they may be classified into three types. Type one involves simple destruction or other metabolism of one antipode. Many of these applications are discussed in an early review by Ehrlich (38). For example DL-alanine, according to Behrens (39) and Duschinsky and Jeannerat (40), yields only L-alanine upon treatment with D-amino acid oxidase. Stumpf and Green (41) found in

---

(33) Berg, J. Biol. Chem., 115, 9 (1936).
(34) Pyman, J. Chem. Soc., 99, 1386 (1911).
(35) Wolf, Mozingo, Harris, Anderson, and Folkers, J. Am. Chem. Soc., 67, 2100 (1945).
(36) Baker, Querry, Safin, and Bernstein, J. Org. Chem., 12, 138 (1947).
(37) Dunn and Rockland, in Anson and Edsall, Advances in Protein Chemistry, Vol. III, New York, Academic Press. 1947.
(38) Ehrlich, in Abderhalden's Handbuck der Biochemischen Arbeitsmethoden. Vol. 2, 559. Berlin, Urgan and Schwarzenberg. 1910.
(39) Behrens, J. Biol. Chem., 141, 465 (1941).
(40) Duschinsky and Jeannerat, Compt. rend., 208, 1359 (1939).
(41) Stumpf and Green, J. Biol. Chem., 153, 387 (1944).

P. vulgaris an L-amino acid oxidase which allowed separation of the D-forms.

The successful use of yeast in a sugar-containing medium was established early in this field by Abderhalden (42) and by Ehrlich (43), who reported production of D-glutamic acid, D-histidine, and D-isoleucine, but unsatisfactory results with aspartic acid, tyrosine and proline (44). Later, tyrosine was satisfactorily resolved (45). More recently, Kocher and Vogler (46) obtained D-methionine by the use of yeast. Among other organisms used in resolution are Oidium lactis for production of D-tyrosine (47), E coli to yield D-tryptophan (48), and certain fungi (49, 50). Edlbacher has obtained D-histidine (51) and D-aspartic acid (52) from urine of rabbits injected with the racemic form, although such a method would not seem practical on a large scale.

---

(42) Abderhalden, Z. physiol. Chem., 130, 205 (1923).
(43) Ehrlich, Biochem. Z., 8, 438 (1908).
(44) Ehrlich and Zamkow, ibid., 63, 379 (1914).
(45) Ehrlich, ibid., 182, 245 (1927).
(46) Kocher and Vogler, Helv. Chim. Acta, 31, 352 (1948).
(47) Chikano, Z. physiol. Chem., 180, 149 (1929).
(48) Majima, ibid., 243, 250 (1936).
(49) Ulpiani and Condelli, Gazz. chim. ital., 30, 382 (1900).
(50) Pringsheim, Z. physiol. Chem., 65, 96 (1910).
(51) Edlbacher and Baur, Verhandl. Ver. schweiz. Physiol., 18, 21 (1941). Abstracted in C. A., 38, 5578 (1944).
(52) Edlbacher and Schmid, Helv. Chim. Acta, 28, 1079 (1945).

A second type of enzymic reaction is the asymmetric hydrolysis of a peptide or other bond to yield a free L-amino acid and an unchanged D-derivative. Some of the pioneer work of this type was done by Abderhalden's group. By means of yeast, natural alanine and leucine bearing free amino groups were split from racemic peptides (53). Enzymic hydrolyses were not limited to peptides; Abderhalden and Schweitzer used other types of N-substitution requiring carboxypeptidase action; their trypsin-kinase hydrolyzed a chloracyl-L-phenylalanine but not the D-isomer (54). The enzymic specificity also extended to an asymmetric acyl group, in the case of *l*-α-bromocaproyl-L-tyrosine which was hydrolyzed faster than d-α-bromocaproyl-L-tyrosine (55).

Operating on the carboxyl end, a pancreatic extract hydrolyzed L-tyrosine ethyl ester preferentially (56). Brenner and co-workers (57) have also applied chymotrypsin to tryptophan methyl ester. An elegant method of this type, using the isopropyl ester of phenylalanine, has recently

---

(53) Abderhalden and Singer, Fermentforschung, 8, 187 (1925).
(54) Abderhalden and Schweitzer, Fermentforschung, 11, 224 (1930).
(55) Abderhalden and Bahn, Fermentforschung, 11, 399 (1930).
(56) Abderhalden, Sickel, and Ueda, Fermentforschung, 7, 91 (1923).
(57) Brenner, Sailer, and Kocher, Helv. Chim. Acta, 31, 1908 (1948).

been developed by Wretlind (58). Intestinal enzymes were early applied to leucinamide and alaninamide by Bergell and co-workers (59, 60).

Asymmetric removal of N-acyl groups, originated by Abderhalden, has been most thoroughly developed in recent years by Greenstein's group. At first they used hog kidney on acetyl-DL-alanine at pH 7.9 (61); their procedure was later improved by the use of chloroacetyl derivatives of several amino acids (62), and by use of pure pancreatic carboxypeptidase (63) in place of hog kidney. Separation of the unhydrolyzed chloroacetyl-D-amino acid was based on its solubility in ethyl acetate. A patent was recently granted for a very high-yielding variation of this process (64). In the case of histidine, cystine, and proline, however, it was necessary to start with the DL-amides and use either mushroom extract, or manganese-activated hog liver or kidney (65).

Other enzymes used in removal of acyl groups are

---

(58) Wretlind, J. Biol. Chem., 186, 221 (1950).
(59) Bergell and Brugsch, Z. physiol. Chem., 67, 97 (1910).
(60) Bergell and von Wulfing, ibid., 64, 348 (1910).
(61) Fodor, Price, and Greenstein, J. Biol. Chem., 178, 503 (1949).
(62) Price, Gilbert, and Greenstein, ibid., 179, 1169 (1949).
(63) Gilbert, Price, and Greenstein, ibid., 180, 473 (1949).
(64) Neuberg and Mandl. United States Patent 2,511,867 (1950). Abstracted in C. A., 44, 8365 (1950).
(65) Levintow, Price, and Greenstein, J. Biol. Chem., 184, 55 (1950).

Taka diastase (66, 67) and histozyme (68).

The third general type of enzymic resolution originated with Bergmann and Fraenkel-Conrat in 1937 (69) when they observed the precipitation of carbobenzoxy-L-amino acid anilides by the action of HCN- or sulfhydryl-activated papain on mixtures of carbobenzoxy-DL-amino acids and aniline. The acylated D-amino acid was recovered from the filtrate. The antipodal specificity is not always satisfactory (70, 71); yields over 50% of product from N-carboallyloxy-D-leucine (72) and as high as 83% from acetyl-D-phenylalanine (73) have been obtained using phenylhydrazine in place of aniline. This effect was believed to be due to the acyl group rather than the phenylhydrazine. Hydrolysis of the anilides occasionally leads to racemization (74) or decomposition (75).

When benzoyl derivatives are used, the unnatural

---

(66) Neuberg and Linhardt, Biochem. Z., 147, 372 (1924).
(67) Hoppert, ibid., 149, 510 (1924).
(68) Smorodintzev, J. Russ. Phys. Chem. Soc., 51, 156 (1920). Abstracted in C. A., 18, 1302 (1924).
(69) Bergmann and Fraenkel-Conrat, J. Biol. Chem., 119, 707 (1937).
(70) Fruton, Irving, and Bergmann, ibid., 133, 703 (1940).
(71) Bennett and Niemann, J. Am. Chem. Soc., 70, 2610 (1948).
(72) Milne and Stevens, ibid., 72, 1742 (1950).
(73) Bennett and Niemann, ibid., 72, 1798 (1950).
(74) Doherty and Popenoe, J. Biol. Chem., 189, 447 (1951).
(75) Hanson and Smith, ibid., 179, 815 (1949).

acylated D-amino acid is very difficult to obtain pure, due to smaller solubility of the racemic compound formed with any of the natural L-isomer (76). A survey of acylated amino acids during the present research indicated that, in general, benzoylated amino acids tend to form racemic compounds, with higher melting points, while acetyl- and formyl-amino acids most often do not. Thus both forms of S-benzylhomocysteine have been successfully obtained by this method starting with the acetyl derivative (77).

The influence of various acyl groups on anilide yield has been reported by Doherty and Popenoe (74). The effects of variations in the aromatic amine on yield also have been studied (78, 79).

Resolution by Adsorption

If adsorptive resolution is to be used as a preparative tool, an adsorbent of high capacity is desirable. Ratios of the weight of adsorbed material to that of adsorbent varied from 10^{-5} to 10^{-1} in a great variety of papers reviewed in Zechmeister's book (80). With good

---

(76) Dekker and Fruton, J. Biol. Chem., 173, 471 (1948).
(77) Reed, Kidwai, and du Vigneaud, Ibid., 180, 571 (1949).
(78) Huang and Niemann, J. Am. Chem. Soc., 73, 475 (1951).
(79) Waldschmidt-Leitz and Kuhn, Z. physiol. Chem., 285, 23 (1950).
(80) Zechmeister and Cholnoky. Principles and Practice of Chromatography. New York, Wiley and Sons. 1938.

inorganic adsorbents, a ratio of one part adsorbate to 100 parts adsorbent is common. Activated charcoal possesses exceptionally high absorptive power for amino acids (81) as well as for other compounds (82, 83). Ghosh and Khan (84) reported 96% adsorption of quinine by only twice its weight of charcoal.

However, the use of high-powered adsorbents such as Fuller's earth or charcoal for resolution would still necessitate prior formation of diastereomers. A few attempts at separation of diastereomers on such adsorbents have been made (85, 86); the process was successful in the case of the alkaloid mandelates on charcoal (87, 88), and N-methylvaline, as the L-α-bromoisovaleryl derivative on alumina (89). Paper partition chromatography was used recently (90) to separate an amine tartrate diastereomer and, more important, the diastereomers of β-phenylserine (91). Partial separation of diastereomers has been effected on

---

(81) Schaaf and Reinhard, Ber., 76B, 1171 (1944).
(82) Koschara, Z. physiol. Chem., 280, 55 (1944).
(83) Sahun, United States Patent 2,416,956 (1947). Abstracted in C. A., 41, 3491 (1947).
(84) Ghosh and Khan, J. Indian Chem. Soc., 23, 344 (1946).
(85) Jamison and Turner, J. Chem. Soc., 611 (1942).
(86) Hass, DeVries, and Jaffe, J. Am. Chem. Soc., 65, 1486 (1943).
(87) Ammon and Fischgold, Biochem. Z., 234, 39 (1931).
(88) Erlenmeyer and Hoffmann, Helv. Chim. Acta, 15, 1140 (1932).
(89) Cook, Cox, and Farmer, Nature, 162, 61 (1948).
(90) Bonino and Carassiti, Nature, 167, 569 (1951).
(91) Shaw and Fox, unpublished work in this laboratory.

activated carbon from the vapor phase (92).

Carbohydrates, such as powdered cellulose (93), have found some application as adsorbents. Although paper can be considered an optically active adsorbent, no record of a resolution prior to 1951 by this agent alone was found, in spite of the wide use of paper chromatography in amino acid chemistry. An attempt by Flood et al. (94) to resolve DL-arabinose by paper partition chromatography, using optically active organic phases, was unsuccessful.

It has been stated that no difference exists between the behavior of D- and L-amino acids on a starch column (95) or on paper (96). Although Dent observed two spots with glutamic acid, he did not interpret this as resolution*, although glutamic acid, incapable of DL-compound formation, would be the most likely possibility for chromatographic resolution. Very recently a small difference between the R_f values for D- and L-glutamic acid has actually been observed (97). These same workers also accomplished the first actual chromatographic separation of amino acid antipodes by resolving DL-tyrosine-3-sulfonic acid on a

---

(92) Isom and Hunt, J. Phys. Chem., 50, 28 (1946).
(93) Peterson and Reinecke, J. Biol. Chem., 181, 95 (1949).
(94) Flood, Hirst, and Jones, J. Chem. Soc., 1679 (1948).
(95) Stein and Moore, J. Biol. Chem., 176, 337 (1948).
(96) Dent, Biochem. J., 43, 169 (1949).
(97) Kotake, Sakan, Nakamura, and Senoh, J. Am. Chem. Soc., 73, 2973 (1951).

* This paper did not state whether active or racemic glutamic acid was used.

chromatopile.

Henderson and Rule (98) made extensive investigations of asymmetric adsorbents such as calcium lactate, emulsin, glucose, and lactose. They obtained resolution only with lactose. As adsorbate they chose p-phenylenebis-[3-iminocamphor] for its extremely high specific rotation of 1500°, and resolved it completely. However, the separation of milligram quantities of the antipodes required eight liters of solvent and 10 kilograms of adsorbent, an adsorbate-adsorbent ratio of 10^{-7}. Prelog and Wieland (99) and Havinga (100) reported resolutions with 400 parts lactose to one of adsorbate.

Carbohydrates have low absorptive capacity. Presumably this could be increased by substitution for hydroxyl hydrogens by acidic or basic radicals, to yield ion-exchange types of adsorbents. A considerable number of such derivatives are known, such as cellulose trisulfate (101), cellulose phosphate (102), sulfoethylcellulose (103), carboxymethylcellulose (104, 105), aminocellulose (106),

---

(98) Henderson and Rule, J. Chem. Soc., 1568 (1939).
(99) Prelog and Wieland, Helv. Chim. Acta, 27, 1127 (1944).
(100) Havinga, Chem. Weekblad, 43, 556 (1947).
(101) Traube, Blaser, and Lindemann, Ber., 65B, 603 (1932).
(102) Reid and Mazzeno, Ind. Eng. Chem., 41, 2828 (1949).
(103) Timell, Svensk Papperstidn., 51, 254 (1948). Abstracted in C. A., 43, 396 (1949).
(104) Klug and Tinsley, British Patent 623,276 (1949). Abstracted in C. A., 43, 7685 (1949).
(105) Oel-u. Chemie-Werk A.-G., Swiss Patent 247,440 (1947). Abstracted in C. A., 43, 4854 (1949).
(106) Scherer and Feidl, Rayon Textile Monthly, 22, 607 (1941). Abstracted in C. A., 36, 261 (1942).

aminoalkylcellulose (107), and p-aminobenzylcellulose (108). They have not been investigated for chromatographic resolution purposes however. In some cases their solubility might prove objectionable.

Fractions with very small rotations were produced by Tsuchido, Kobayashi, and Nakamura (109) by adsorption of asymmetric octahedral cobalt complexes on optically active quartz powder. Karagunis and Coumoulos (110), using D- or L-quartz, also obtained small rotations with a cobalt complex having specific rotation of 3000°.

Although Willstatter's original attempts to resolve racemic alkaloids on wool were unsuccessful (111), Porter and Ihrig (112) reported about one per cent resolution of a dye on only two and a half parts wool by weight. Martin and Kuhn (113) obtained rotations as high as 1° by "multiplied" partition of DL-mandelic acid on a wool belt. Kögel et al. observed no difference in tissue-staining by dyes

---

(107) Hardy, United States Patent 2,136,296 (1939). Abstracted in C. A., 33, 1495 (1939).
(108) Panciralli, Boll. reparto fibre tessili vegetali staz. sper. ind. carta e fibre tessili vegetali, 32, 314 (1937). Abstracted in C. A., 31, 7247 (1937).
(109) Tsuchido, Kobayashi, and Nakamura, Bull. Chem. Soc. Japan, 11, 38 (1936).
(110) Karagunis and Coumoulos, Nature, 142, 162 (1938).
(111) Willstatter, Ber., 37, 3758 (1904).
(112) Porter and Ihrig, J. Am. Chem. Soc., 45, 1990 (1923).
(113) Martin and Kuhn, Z. Elektrochem., 47, 216 (1941).

derived from D- or L-camphorquinone (114). Ingersoll concluded that crystalline adsorbents are better than amorphous ones, but that chromatographic adsorption requires unreasonable quantities of adsorbent (115). The subject has also been reviewed by Zechmeister (116, 117) and by Lederer (118).

Types of Diastereomers Other Than Salts

The idea of using optically active moieties in covalent connection has occurred to a number of investigators. This might eliminate salt formation or enzyme reactions entirely, although it does not constitute a very great over-all simplification. On the amino nitrogen, the introduction of a menthoxyacetyl group enabled Holmes and Adams to resolve alanine, valine, and phenylglycine (119). Berlingozzi and Lenoci tried unsuccessfully to use the α-bromoisovaleryl radical (120).

Although Ågren (121) found no difference between natural and unnatural cysteine in its reaction with sugar,

---

(114) Kögel, Faber and deBoer, Rec. trav. chim., 69, 482 (1950).
(115) Ingersoll. Organic Reactions. Vol. II, p. 389. New York, Wiley and Sons. 1944.
(116) Zechmeister, Ann. N. Y. Acad. Sci., 49, 220 (1948).
(117) Zechmeister. Progress in Chromatography 1938-1947. New York, Wiley and Sons. 1950.
(118) Lederer. Progres recents de la Chromatographie Paris, Hermann. 1949.
(119) Holmes and Adams, J. Am. Chem. Soc., 56, 2093 (1934).
(120) Berlingozzi and Lenoci, Gazz. chim. ital., 68, 721 (1938).
(121) Ågren, Acta Physiol. Scand., 1, 105 (1940).

Maurer and Schiedt were able to prepare ether-soluble N-glucosides by reaction with acetobromoglucose (122) and later resolved phenylglycine in this manner. In other cases no crystalline products could be obtained (123). Kynurenine, an aromatic amino acid, has been resolved as a molecular complex with sucrose (124).

Apparently no attempts have been made to esterify the carboxyl of amino acids with optically active alcohols; attempts to use menthol in this laboratory were unsuccessful. Bergmann et al. resolved phenylalanine by chemical preparation of acetylphenylalanyl-L-glutamic acid (125).

Other Unusual Methods of Separating Diastereomers

S. W. Bergmann (126) made a study of solubility theory applied to diastereomers. Assuming ion affinity and the energy of ion solvation to be the same for two dissolved diastereomers, he concluded that the only difference between them is in solid lattice energy, and therefore separation of ionic diastereomers necessitates crystal

---

(122) Maurer and Schiedt, Z. physiol. Chem., 206, 125 (1932).
(123) Maurer and Schiedt, ibid., 213, 110 (1932).
(124) Butenandt and Weichert, ibid., 281, 122 (1944).
(125) Bergmann, Stern, and Witte, Ann., 449, 277 (1926).
(126) Bergmann, Arkiv. Kem. Mineral Geol., 9, No. 42 (1926).

production. He devised a simple equation for the difference in solubility between two diastereomers involving chiefly the difference in specific gravities of the solid salts. Experiments in which the dense diastereomer crystal was always the more difficulty soluble, substantiated this formula. An apparent exception to Bergmann's proposition was noted by Hollander and du Vigneaud (127); when the diacetylcystine salt of brucine was crystallized from water or methanol, the dextro anion appeared in the precipitate, but with less polar alcohols, the first fractions contained levo-diacetylcystine. However, this might have been due to two different crystal habits of one diastereomer, different solvates, or simply to the rate of crystal formation, which is often a more determining factor than solubility. Stewart and Allen (128) studied such a phenomenon at -70°, where rates of crystallization would be slow.

A more significant exception to the assumption of equal affinity of ions in a diastereomeric pair is the finding of Shapiro and Newton that brucine salts could be partially separated by counter current partition between water and chloroform (129). The ion affinity assumption does not apply to covalent diastereomers, which have been

---

(127) Hollander and du Vigneaud, J. Biol. Chem., 94, 243 (1931).
(128) Stewart and Allen, J. Am. Chem. Soc., 54, 4027 (1932).
(129) Shapiro and Newton, ibid., 65, 777 (1943).

partially separated by fractional distillation (130).

Resolution of Intermediates in Synthesis

As stated in the introduction, the resolution of intermediates in the preparation of amino acids could avoid the necessity for derivatizing the amino acids. The resolution of amino alcohols by tartaric acid is easy; oxidation of the alcohol should yield an optically active amino acid. Although this has not been applied to optically active amino acids, it has been possible by careful cooling to produce glycine, in 50% yield, from unsubstituted ethanolamine sulfate by the use of $KMnO_4$ (131). Or, aminonitriles could be resolved, then hydrolyzed.

A process in which formylaminomalonic ester is used as starting material for a series of amino acids has been patented by Roche Products, Ltd. The side chains are introduced as halides in the usual fashion, and after decarboxylation of the α-formylaminodicarboxylic acid, an ordinary brucine resolution yields the active formylamino acid. A whole series of resolved amino acids can thus be prepared, without having to formylate each one individually (132).

---

(130) Bailey and Hass, J. Am. Chem. Soc., 63, 1969 (1941).
(131) Billman, Parker, and Smith, J. Biol. Chem., 180, 29 (1949).
(132) Cohen, Hughes, and Silk, British Patent 621,706 (1949). Abstracted in C. A., 44, 2017 (1950).

The α-bromo acids are a logical choice for another type of resolution of intermediates. Levorotary norleucine has been prepared from resolved α-bromocaproic acid (133). According to Fischer and Carl (134), amination of the bromo acid results in nine per cent racemization. However, a Walden inversion has been used in preparation of O-methyl-D-tyrosine from the bromo acid, derived from the L-antipode by treatment with nitrosyl bromide (135). Some of these methods are perhaps more suited to manufacturing processes than to laboratory resolution.

Some Simplified Resolutions Not Involving Derivatives

The seeding of optically active amino acids from supersaturated solutions is made difficult by the fact that DL-compounds of most amino acids are less soluble than pure isomers, in a few cases by a factor of over two (136). Dalton and Schmidt found only glutamic acid lacking the property of racemic compound formation (137). Thus, as might be expected, pure L- and D-glutamic acid hydrochlorides

---

(133) Levene and Mardashew, J. Biol. Chem., 117, 707 (1937).
(134) Fischer and Carl, Ber., 39, 3996 (1906).
(135) Rivers and Lerman, J. Endocrinol., 5, 223 (1948).
(136) Cohn and Edsall. Proteins, Amino Acids, and Peptides. p. 190. New York, Reinhold Publishing Corp. 1943.
(137) Dalton and Schmidt, J. Biol. Chem., 103, 549 (1933).

have been seeded from 20% HCl (138). Similarly, taking advantage of the fact that DL-histidine hydrochloride is not stable as a racemic compound above 40°, Duschinsky started with a mixture of L- and DL-histidine HCl; by working rapidly he was able to filter off somewhat more L-isomer than the original excess. After removal of much of the DL compound, he then obtained some of the D-isomer from the filtrate (139).

DL-Compound formation means also that an amino acid of less than a certain optical purity cannot be recrystallized to a 100% L- or D-antipode, barring some unpredictable supersaturation. Wood and Gutmann, in resolving radioactive benzyl-DL-cysteine, used about 65 parts of unlabelled L-antipode for coprecipitation, resulting of course in dilution of the radioactivity (140).

Purification of optical isomers from partially resolved mixtures can also be accomplished in a "reverse" manner, by taking advantage of the exaggerated difference in solubility between active and racemic forms of amino acid salts of Vogler's acid. In the case of the phenylalanine salt of his 2-nitro-4'-chlorodiphenylamine-4-

---

(138) Kögl, Halberstadt, and Barendregt, Rec. trav. chim., 68, 387 (1949).
(139) Duschinsky, Chemistry & Industry, 53, 10 (1934).
(140) Wood and Gutmann, J. Biol. Chem., 179, 535 (1949).

sulfonic acid (not an asymmetric acid), the racemic form was only 1/250 as soluble as the L-isomer (141). Removal of the DL form from a partially racemic mixture allowed eventual purification of L-phenylalanine.

The question of solubility of antipodes in optically active solvents has received some limited study. Ebert and Kortum, as well as many others, concluded that there was no difference in solubility (142). Slight separations of mandelic acid antipodes have been noted however, on partition between ether and a water solution of levulose (143) and between water and carvone (144). Attempts to adsorb histidine and leucine from suspension in optically active solvents have indicated slight differences between antipodes at liquid interfaces (145). Use of optically active solvents in a chromatographic experiment referred to earlier (97, p. 14), was completely without effect on R_f values.

Compounds lacking sufficiently strong acidic or basic functions sometimes form crystalline addition products with relatively complex molecules. Brown and Hammick (146)

---

(141) Vogler and Koenig, Helv. Chim. Acta, 31, 183 (1948).
(142) Ebert and Kortüm, Ber., 64B, 342 (1931).
(143) Tolloczko, Ztschr. physikal. Chem., 20, 412 (1896).
(144) Schroer, Ber., 65, 966 (1932).
(145) Karagunis and Nikolaidis, Kolloid-Z., 106, 112 (1944).
(146) Brown and Hammick, J. Chem. Soc., 1395 (1948).

attempted without success to use a tetranitrodiphenic acid derivative (asymmetric due to hindrance) to resolve hydrocarbons. Sobotka (147) has had some success with desoxycholic acid complexes for resolution. None of the common amino acid complexing agents are optically active, although an addition product with dioxane has recently improved separation of diastereomeric β-phenylserines (91).

Use of the Acidic or Basic Properties of Unsubstituted Amino Acids

Przylecki et al. have studied the stability of salts of unsubstituted monoaminomonocarboxylic acids with amines or carboxylic acids. Precipitates from solutions of amino acids with volatile amines were analyzed for nitrogen content to determine to what extent the amine was retained by amino acid. Although in most cases analysis indicated the residue was mostly free amino acid, that from leucine and dimethylamine bordered on a true salt, or at least a 1:1 complex (148).

---

(147) Sobotka, Naturwissenschaften, 19, 595 (1931).
(148) Przylecki, Cichocka, Hofer, and Rafalowska, Biochem. Z., 299, 230 (1938).

The most difficult problem is with monoaminomonocarboxylic acids. The use of strong sulfonic acids would be indicated here. As early as 1913, Colombano et al., attempted the preparation of camphorsulfonates and bromocamphorsulfonates of alanine, leucine, and tyrosine, but were not able to resolve any of these (149). Berlingozzi (150) obtained only soluble compounds with camphorsulfonic acid. Although Triem also reported excessively soluble bromocamphorsulfonic acid derivatives, he was able completely to resolve leucine with cholestenonesulfonic acid by crystallization from absolute alcohol, in 40% yield (151). Ingersoll and his group (152) have been successful in resolving α-amino-phenylacetic acid with L-camphorsulfonic acid.

The ionization constants of alkylamines are somewhat greater than that of acetic acid. However, when the two are "combined" in one amino acid molecule the resulting α-substitution has a weakening effect on the basicity of amino groups, while the acidity of the carboxyl group is

---

(149) Colombano and Sanna, Atti. accad. Lincei, 22, II, 234 (1913). Abstracted in C. A., 8, 339 (1914).
(150) Berlingozzi and DeCecco, Atti V. congr. nozl. chim. pura applicata Rome, Pt. I, 307 (1935). Abstracted in C. A., 31, 3875 (1937).
(151) Triem, Ber., 71B, 1522 (1938).
(152) Ingersoll, J. Am. Chem. Soc., 47, 1168 (1925).

greatly increased (153). From the zwitter ion point of view, the RNH_3^+ should then be somewhat more capable of losing a proton than is the COO^- of accepting one. Salt formation with dimethylamine has already been mentioned, and stronger bases react readily with the zwitter ion.

Although heavy metal salts of amino acids are well known, investigation of the literature yields varying statements on the difficulty of preparation of alkali metal salts of amino acids. Voss and Guttmann (154) attempted the preparation of sodium glycinate by use of sodium ethylate in alcohol, but found it difficult, as did Curtius (155), to obtain the pure anhydrous salt, either because of decarboxylation or anhydride formation. However, when the preparation was carried out in liquid ammonia with sodium metal, the product was a crystalline powder. On the other hand, sodium salts of leucine and alanine were prepared from sodium ethoxide. Spies and Chambers (156) stated that pure tryptophan is stable in 5N alkali at 185° in the absence of oxygen, and required two hours even for racemization under such conditions. Evidently the alkali metal salts are

---

(153) Orthner and Hein, Biochem. Z., 262, 461 (1933).
(154) Voss and Guttmann, Ber., 63B, 1726 (1930).
(155) Curtius, J. prakt. Chem., 2 26, 160 (1882).
(156) Spies and Chambers, Anal. Chem., 21, 1249 (1949).

reasonably stable, and soluble in ethanol as well as water (157, 158). The salts of glycine, cystine (157), leucine (159), alanine, phenylalanine (154), and histidine (160) have been characterized. A patent has been granted for a preparation of sodium salts of amino acids by the action of sodium cyanide and an amine on aldehydes (161). Sodium salts have been used to separate isoleucine from alloisoleucine (162). Strong base resins such as Dowex 2 have been used to separate leucine, methionine and glutamic acid from each other (163).

For resolution purposes it would be necessary to induce a stable, non-acidic, asymmetric cation to precipitate with the anion of amino acid. The two most common types of cation are the quaternary ammonium and guanidinium ions. Prior to this work no amino acids had ever been resolved by this means, although quinine methohydroxide and cinchonine methohydroxide have been used in resolutions of

---

(157) Toennies and Lavine, J. Biol. Chem., 90, 203 (1931).
(158) Greenberg. Amino Acids and Proteins, p. 28. Springfield, Charles C. Thomas. 1951.
(159) Fodor and Weitzmann, Z. physiol. Chem., 154, 290 (1926).
(160) Galat, J. Am. Chem. Soc., 69, 707 (1947).
(161) Bersworth, United States Patent 2,387,735 (1945). Abstracted in C. A., 40, 1171 (1946).
(162) Shabica, United States Patent 2,456,742 (1948). Abstracted in C. A., 43, 3029 (1949).
(163) Davies, Hughes, and Partridge, J. Chem. Soc., 2285 (1950).

stronger carboxylic acids (164, 165), in particular pantothenic acid, which can be considered anacylated amino acid (166).

Formation of Carbamino Acids

Another approach to resolution which has apparently not been tried is the formation of carbamino acid salts by simple introduction of CO_2 into an alkaline amino acid solution. Although esters with free amino groups easily yield the carbamino anion in cold, dry ether (167), the reaction with an unsubstituted amino acid occurs only at very high pH; however, the rate of the reaction in a cold solution is greater than that of carbonate formation (168). Thus of the two competing reactions below (in which M^{++} represents a bivalent cation), (a) is favored. This has

(a) $$M^{++} + RCH(NH_2)COO^- + CO_2 \longrightarrow RCH(NHCOO^-)COO^- + M^{++} + H^+$$

(b) $$M^{++} + CO_2 + OH^- \longrightarrow MCO_3 + H^+$$

---

(164) Wolf, Mozingo, Harris, Anderson, and Folkers, J. Am. Chem. Soc., 67, 2100 (1945).
(165) Major and Finkelstein, J. Am. Chem. Soc., 63, 1368 (1941).
(166) Stiller and Wiley, ibid., 63, 1237 (1941).
(167) Frankel and Kotchalski, ibid., 65, 1670 (1943).
(168) Stadie and O'Brien, J. Biol. Chem., 112, 723 (1936).

been made the basis for some amino acid separations (169, 170). The above reactions would suggest that absence of hydroxyl ion should favor reaction (a).

The attempted application of this idea to resolution is treated in the experimental section.

Use of Asymmetric Cations

Both of the latter methods (with or without carbamino anion formation) require the preparation of asymmetric quaternary ammonium, or guanidinium cations. One type of asymmetric quaternary cation, extensively investigated by Wedekind, contains four different radicals attached to nitrogen. Some of these have been resolved by tedious fractional crystallization (171, 172, 173), but their ready autoracemization makes them unsuitable for use (172, 174). More stable are those derived from alkaloids, and others discussed in the experimental section.

Although all quaternary ions are perfectly inert from an acid-base point of view, some of the guanidinium

---

(169) Siegfried and Schutt, Z. physiol. Chem., 81, 260 (1912).
(170) Neuberg and Kreb, Biochem. Z., 40, 498 (1912).
(171) Frohlich and Wedekind, Ber., 40, 1646 (1907).
(172) Wedekind and Frohlich, ibid., 39, 4437 (1906).
(173) Jones, Proc. Camb. Phil. Soc., 14, 376.
(174) Wedekind, Trans. Far. Soc., 10, 126 (1914).

ions are unsuitable for the purpose. According to Davis and Elderfield (175), phenylguanidine base has an ionization constant of only 5.9×10^{-4}. Thus aromatic guanidine bases would be too weak to produce a stable salt consisting of guanidinium cation and amino acid anion. The same is true of any asymmetrically disubstituted guanidine. The symmetrical trialkyl guanidines are again strong bases.

---

(175) Davis and Elderfield, J. Am. Chem. Soc., 54, 1499 (1932).

EXPERIMENTAL*

The following approaches to simplified resolution were undertaken:

1. Seeding and use of optically active solvents.
2. Asymmetric adsorption.
3. Use of strong organic bases combined with amino acids or carbamino acids.

All of these ideas have their counterparts in experiments reported in the literature in other connections, or in fields other than amino acid chemistry. The third has apparently never been applied, outside of this laboratory, to the resolution of any unsubstituted amino acid, as far as the literature search could reveal. The nature of the problem thus called for an exploratory approach, in which any suggestion of success would be followed by development of improved conditions. Another objective was constantly kept in mind; the method should be simple and rapid, once the proper reagents had been secured.

---

* All melting points reported herein were corrected. Nitrogen analyses were run by the macro Kjeldahl method, except for some micro Kjeldahl figures followed by (miK). Halogen analyses were run by the Volhard titration and sulfate analyses in the usual gravimetric manner, calculated as per cent sulfur. Per cent water in hydrates was based on loss in weight from heating in vacuo below the melting point. The neutral equivalents of bioxalates and bitartrates were determined by titration with NaOH to the brom thymol blue end point; amines were titrated with HCl to the methyl red end point.

Seeding and Use of Optically Active Solvents

There are very few optically active solvents which are readily available. Leucine hydrochloride was found to be 3.2% soluble in secondary butanol, an inexpensive solvent. However, the resolution of secondary butanol by the procedure of Pickard and Kenyon (176) proved too cumbersome to merit systematic investigation. Commercial 85% L(+)lactic acid (syrupy) of at least 94% optical purity was therefore used.

Acidic solvents

Attempted resolution of DL-leucine hydrochloride from L(+)lactic acid. Pure D- and L-leucine hydrochlorides were made from recrystallized leucines for seeding purposes. A solution of 33 g. of racemic leucine hydrochloride and four drops of concentrated HCl in 26 ml. of 85% L(+)lactic acid was prepared and filtered while warm. The clear filtrate was divided into two parts. To one was added a few seeds of D-leucine hydrochloride and to the other L-leucine hydrochloride. As soon as the precipitates were appreciable, they were quickly filtered off, in the hope that the opposite form of the hydrochloride would not have

---

(176) Pickard and Kenyon, J. Chem. Soc., 103, 1923 (1913).

crystallized as rapidly. The precipitated samples were dissolved in a small amount of water, treated with ammonia to a pH of about five or six, filtered, and washed free of lactate. The residues proved to be racemic in both cases.

Attempted resolution of DL-leucine hydrochloride from HCl. Hydrochloric acid was used by Kögl et al. (138) in a resolution of glutamic acid hydrochloride by seeding. DL-Leucine (14 g., .11 mole) was dissolved in 60 ml. of 7.5M HCl with warming. Cooling of the supersaturated solution and seeding with a small crystal of the pure L-isomer hydrochloride, gave DL-leucine hydrochloride only.

Attempts to crystallize active phenylalanine, methionine, and valine from L(+)lactic acid. DL-Phenylalanine (10 g.) was dissolved with heating in 22 ml. of syrupy 85% L(+)lactic acid. After two days the precipitate which gradually formed was filtered through a coarse sintered-glass crucible and washed with alcohol. The precipitate had an observed optical rotation of +.05°, which was not significant considering the reading error.

The above was repeated with 9.5 g. of DL-methionine in 40 ml. of L(+)lactic acid, and with seven g. of DL-valine in 30 ml. of L(+)lactic acid, with similar results.

Some optically active amine solvents

A search for conveniently available amines which might be produced in optically active form led to investigation of the following:

sec-Butylamine

Fenchylamine (from fenchone)

3-Aminoheptane (from 3-heptanone)

2-Aminobutan-1-ol

Some preliminary experiments on salt-forming tendency. A few preliminary experiments, similar to those of Przylecki et al. (148), were performed to learn whether the affinity of amino acids for an asymmetric amine such as sec-butylamine might be sufficient to produce a precipitate consisting at least partially of amino acid-amine salt. Although it might seem desirable to use pure amines, or amine-alcohol mixtures, small proportions ofwater were absolutely essential for solvent action. The dl-sec-butylamine was used at first.

DL-Phenylalanine (1.8 g.) was dissolved in a mixture of eight ml. of pure dl-sec-butylamine and one ml. of water, then cooled in dry ice. DL-Valine (1.1 g.) was similarly dissolved in 20 ml. of 85% sec-butylamine. In no case was there any precipitate on overnight cooling to -80°, although the solutions were close to saturation before cooling.

To each solution was then added about 25 to 30% of its volume of pure acetone. Small precipitates were obtained. The one from the phenylalanine experiment was filtered and washed with ether. It was then dissolved in warm water and titrated with .009N H_2SO_4 to the methyl orange end point. Assuming that the ether washing removed all physically adhering amine, this indicated the chemically bound amine (.216 millimole) amounted to about 60% of theoretical.

The valine experiment indicated similarly that 70% of the theoretical amine was held by valine in the precipitate. This is essentially in agreement with Przylecki et al. (148).

Because of the volatility of sec-butylamine, it seemed preferable first to investigate the active antipodes of the other amines listed. Ketones served as starting materials for two of these.

Products from ketones. Eastman fenchone was found to have a low specific rotation, even when fractionated. The oxime was prepared from this product by the procedure of Wallach (177) in the hope that recrystallization of it would increase the optical purity so that reduction would

---

(177) Wallach, Ann., 315, 278 (1901).

yield a satisfactory fenchylamine. However, recrystallization lessened the rotation, suggesting DL-compound formation.

3-Aminoheptane was prepared in 67% yield from Eastman practical butyl ethyl ketone by the usual Leuckart reaction, except that a Dean-Stark trap was used to separate water formed by the reaction. An attempt to resolve this amine with (+)tartaric acid in alcohol gave a partially solid salt in the refrigerator, which melted to an oil at room temperature.

Commercial Solvents Co. 2-aminobutan-1-ol was found to be very conveniently resolved by (+)tartaric acid, and so was used in this work.

Resolution of 2-aminobutan-1-ol. Baker and Adamson (+)tartaric acid (930 g., 6.2 moles) was dissolved in 1500 ml. of distilled water. To this was added, with cooling, Commercial Solvents technical 2-aminobutan-1-ol (553 g., 6.2 moles). The warm solution was cooled in the refrigerator to yield 400 g. of crystals, washed with alcohol, in which the crystals were only 1.5%soluble. A red-brown color was removed by the alcohol. The solid was recrystallized from 220 ml. of water to give 378 g. of pure (-)2-aminobutan-1-ol (+)bitartrate monohydrate, m.p. 102-3°.

$$[\alpha]_D^{26.5} = +10.5^\circ \pm 0.2^\circ \text{ (5\% in water)}$$

Anal. Calcd. for $C_8H_{17}O_7N \cdot H_2O$: N, 5.4; neut. equiv., 257; H_2O, 7.0.

Found: N, 5.3; neut. equiv., 259; H_2O, 7.0.

The filtrate was used to recrystallize another crop obtained by evaporation of the original filtrate. Another 212 g. were thus obtained. The total yield was 590 g. (2.3 moles). Since the technical grade amine was only about 75% pure, this represented about 85 to 90% yield. (-)2-Aminobutan-1-ol (+)bitartrate appears to be a new compound, previous resolutions of 2-aminobutan-1-ol having been accomplished indirectly by way of the N-benzyl derivative (178).

Liberation of (-)2-aminobutan-1-ol from bitartrate. The (-)2-aminobutan-1-ol (+)bitartrate (590 g., 2.3 moles for a monohydrate) was almost all dissolved in 1500 ml. of water. It was then treated with successive small portions of calcium hydroxide powder, with stirring, until about 230 g. had been added. The calcium tartrate was filtered and washed with 100 ml. of water. The pH was noted with alizarin yellow indicator; 20 more g. of calcium hydroxide was added and the solid filtered. The filtrate gave the same alizarin yellow color, and had a pH of 10.6 according to the pH-meter. The aqueous amine was fractionated from

---

(178) Stoll, Peyer, and Hofmann, Helv. Chim. Acta, 26, 929 (1943).

water and gave 138 g. (1.55 mole, 68% of theory) of pure (-)2-aminobutan-1-ol; b.p. 80°/13 mm., or 54°/1.5 mm., $d_4^{26.1}$ = .0.9390, and $[\alpha]_D^{26}$ = -9.92° ± .01° for the pure compound. The literature value is +9.8° for the other antipode (178). The product forms an azeotrope with water (when distilled at water-pump pressure), which contains about 2% amine. Therefore higher yield could probably be had by use of less water, or by use of alcohols, in which case the solid calcium hydroxide and amine bitartrate would have to be very thoroughly stirred.

Attempted asymmetric crystallization of active phenylalanine from (-)2-aminobutan-1-ol. Since pure amines do not dissolve most amino acids, a few preliminary experiments were run to determine the maximum proportion of 2-aminobutan-1-ol to water for dissolving phenylalanine. Then DL-phenylalanine (5.9 g., 0.035 mole) was dissolved with heating in 15 ml. of (-)2-aminobutan-1-ol and five ml. of water. After overnight cooling, a precipitate was obtained and treated with 16% acetic acid in alcohol to produce free phenylalanine. Subsequent washing with alcohol removed the amine acetate. The remaining amino acid was completely inactive.

Attempted asymmetric crystallization of active valine from (-)2-aminobutan-1-ol. A mixture of 21 ml. of (-)2-aminobutan-1-ol and 11 ml. of water was heated to

dissolve 5.7 g. of DL-valine. After overnight cooling, the precipitate which was obtained was acidified as before with alcoholic acetic acid, and washed with alcohol to yield three g. of valine. When this latter was dissolved in 25 ml. of dilute hydrochloric acid, it showed no rotation of light, although in this case full resolution would have given an observed rotation of 5°, so that even a small degree of resolution could have been detected.

Asymmetric Adsorption

Use of cholic acid

A small column was made from an ordinary test tube, with a narrow extension on the bottom. A glass wool plug was inserted as a support, then a suspension of ten g. of cholic acid in water was slowly poured on top of the plug. When 125 ml. of water had been poured through, the column was considered ready for use (at no time did solvent drop below the top of the cholic acid). Another glass wool plug on top prevented disturbance of the surface. Five ml. of D-phenylalanine solution (1%) was placed on top of the column, then the passage of water through the column was resumed for development of a liquid chromatogram. A dropping funnel on top of the column provided a reservoir as well as a hydrostatic pressure. Successive seven to eight ml. fractions were taken and analyzed for amino acid by

the ninhydrin test. The second fraction gave a very strong test, and the third a weaker test. No amino acid was detected in the following fractions.

The experiment was duplicated with L-phenylalanine, with the identical results. The rapid appearance of amino acid at the bottom of the column proved that no significant adsorption took place in either case from a water solution.

Use of nitrocellulose

A new column was made from a 30 cm. length of tube joined tightly to a microfilter funnel with a short length of rubber. This was gradually filled with a suspension of somewhat fibrous nitrocellulose (Hercules Powder Co.) in a solvent composed of 6.5 parts of n-butanol and one part water. When the solvent level had dropped almost to the top of the adsorbent in the usual washing procedure, one ml. of 0.1% D-phenylalanine was placed on top and a liquid chromatogram developed with the same butanol-water mixture used in making up the column and the amino acid solution.

Successive five ml. portions of filtrate were taken, with the aid of slight vacuum and the hydrostatic head of a dropping funnel of solvent on top of the column in the usual fashion. A ninhydrin test showed the presence of amino acid mostly in fraction five, and some in six. Again

the identical result was noted using L-phenylalanine. Thus there was no difference in adsorption tendency between antipodes, with this particular solvent-adsorbent combination.

Use of casein

Saturated aqueous solutions of DL-proline, phenylalanine, leucine, and methionine were shaken five hours with glass beads and with ten times the solute weight of Pfanstiehl Co. pure casein, then allowed to stand for 72 hours (except for 12 hours in the case of leucine). They were then filtered, charcoaled, and refiltered. As a blank, a 1% solution of glycine was treated in the same manner. All solutions, when compared with that of glycine, showed a differential rotation. However, the "blank" rotation in the glycine solution was significantly different from the instrument blank, suggesting that casein was being dissolved to an extent which might cast doubt upon the supposed resolutions of other amino acids. Positive biuret tests confirmed the presence of casein in all solutions. In view of an excessive rotation in the methionine solution, samples were evaporated to dryness both before and after the experiment. It was found that the solute content had increased, rather than decreased. Obviously this indicated that the solubilization of casein accounted for much of the reading, if not all.

Use of phosphorylated cotton

Phosphorylated cotton was prepared by the method of Jurgens et al. (179). According to those authors, the ion-exchange capacity of the product is one equivalent per kg. Therefore saturated solutions of DL-methionine, DL-phenylalanine, DL-tryptophan, and DL-valine, and a 5% solution of DL-proline were shaken with half the theoretical weight of cellulose phosphate on that basis and allowed to stand 24 to 56 hours. They were then drained from adsorbent. Examination of the filtrates after charcoal treatment (to improve reading accuracy) gave no rotation reading at all.

Repetition of the above with DL-phenylalanine by chromatographic technique in a column likewise gave no significant observed rotation in a series of fractions.

Organic Salts of Unsubstituted Amino Acids, or of N-Carboxyamino Acids

Method of attack

This section of the investigation was organized into three phases:

1. The search for convenient asymmetric bases not

---

(179) Jurgens, Reid, and Guthrie, Textile Rsch. Journal, 18, 42 (1948).

requiring elaborate fractionation for resolution.

2. Production* of D- and L-antipodes of some amino acid to be used as a test substance.

3. Exploratory work directed toward production of crystalline salts with the amino acid, or N-carboxyamino acid.

The alkaloids are a logical starting point from which quaternary salts are usually easy to obtain. Both the steroid and terpene groups contain alcohols and ketones. Preparation of amines from the ketones through reduction of oximes produces an additional asymmetric carbon atom, with consequent lowering of yields due to tedious fractional crystallization in obtaining pure diastereomers. The alcohols might be utilized without this difficulty by substitution, for alcoholic hydrogen, of a convenient radical having on its other end a bromine atom or dimethylamino group, either of which could then be converted to quaternary salts.

Some consideration was given to the advisability of making racemic guanidines or quaternary bases from synthetic products such as 3-aminoheptane or 2-iodooctane. However, the liberation, resolution, and re-liberation of

---

* The method actually used for this was itself a new resolution.

these compounds did not seem worth pursuing, until more easily prepared resolving agents had been tried first. The resolution of 2-aminobutan-1-ol was described earlier. The Williamson reactionpermitted easy preparation of oxygen-substituted derivatives of this resolved amine, from which a series of optically active quaternary ions was readily obtained. Thus most of the preparations are derivatives of 2-aminobutan-1-ol, or of alkaloids.

Derivatives of (+) and (-)2-aminobutan-1-ol and of ethanolamine

(+)2-Guanidinobutan-1-ol sulfate. This was made by a variation of the procedure of Paden and McLean (180) from (-)2-aminobutan-1-ol by using sulfuric acid (8 g., 0.16 equivalent) in 50 ml. of 95% alcohol, (-)2-aminobutan-1-ol (16 g., 0.18 mole), and Eastman reagent grade cyanamide (10 g., almost 0.25 mole). The slight excess of (-)2-aminobutan-1-ol produced a desirable alkaline pH. Drying of the resulting syrup by azeotropic distillation of moisture with 25 ml. of isoamyl alcohol at 70 mm. pressure gave crystals weighing (after absolute alcohol-ether wash) 19 g., or about two-thirds of the theoretical yield; m.p. 138-141°. The product was not hygroscopic. Recrystallization from water-alcohol gave 14.5 g. (50% over-all yield); m.p. 183-4°,

---

(180) Paden and McLean, United States Patent 2,425,341 (1947). Abstracted in C. A., 41, 7414 (1947).

$[\alpha]_D^{28.5} = +33.1 \pm .2^\circ$ (2% in water)

Anal. Calcd. for $C_{10}H_{28}O_6N_6S$: S, 8.93; N, 23.5.
Found: S, 8.90; N, 23.5.

(-)α-Ethylcholine iodide. Literature methods for the preparation of quaternary salts directly from primary amines involve the use of alkali to remove hydrogen iodide as it is formed according to the following reaction:

$$RNH_2 + 3CH_3I + 2KOH \longrightarrow RN^+(CH_3)_3I^- + 2KI + 2H_2O.$$

(-)α-Ethylcholine iodide was prepared from (-)2-aminobutan-1-ol according to the above equation, by the procedure of Trier (181). The product was obtained in potassium-free crystalline form only with some difficulty. It is believed that water formed in the reaction, as well as potassium iodide impurity, made crystallization of this compound somewhat more tedious than it ought to be. Some consideration was given to the possibility of eliminating the alkali metal ion from this preparation.

In efforts to find bases to remove hydrogen iodide by production of more insoluble iodides, (-)2-aminobutan-1-ol, at least a two to one excess of methyl iodide, and excess lead monoxide (or lead carbonate) were shaken in

---

(181) Trier, Z. physiol. Chem., 80, 409 (1912).

alcohol at room temperature for three days. The lead compound seemed to adsorb most of the product, since filtration and complete evaporation of the solvent yielded less than 25% of the expected weight.

The reaction was repeated with purified silver oxide, leading to the quaternary base directly, in 85% yield, based on titration. However, there was evidence that the preparation still contained complexed silver ion. Finally the hydroxide was made in good yield and free of metal ions by starting with the following reaction:

$$EtCH(NH_2)CH_2OH + 3CH_3I + Ca(OH)_2 \longrightarrow EtCH(CH_2OH)\overset{+}{N}(CH_3)_3I^- + CaI_2 \cdot 2H_2O$$

After overnight shaking of (-)2-aminobutan-1-ol (0.9 g., 0.01 mole) in methanol with about 100% excess of methyl iodide and 100% excess of calcium hydroxide, the filtered reaction mixture was treated with excess silver hydroxide, to yield (-)α-ethylcholine (hydroxide), the soluble calcium ion being removed as the hydroxide (with silver iodide). As proof of the yield of (-)α-ethylcholine, the iodide was then prepared by addition of hydriodic acid to the quaternary hydroxide and evaporation of solvent. Recrystallization of the residue from sec-butanol gave good crystals of the pure but very hygroscopic iodide, m.p. 226° (for physical constants and analysis, see the section on the antipode)

The drawback to all the foregoing methods is the necessity of using large quantities of somewhat expensive methyl iodide and silver oxide. It proved much simpler to dimethylate (-)2-aminobutan-1-ol by the Eschweiler-Clarke method (182). Thus all derivatives were tertiary amines, from which quaternary salts could be prepared by simple addition of only one mole of methyl iodide.

(-)2-Dimethylaminobutan-1-ol. This was prepared by a variation of the Eschweiler-Clarke method (182). (-)2-Aminobutan-1-ol (+)bitartrate (394 g., 1.53 moles), prepared as described previously, was treated directly with 90% formic acid (340 ml., 7 moles) and U.S.P. formalin solution (270 ml., containing 3.3 to 3.5 moles of formaldehyde). (It was not necessary to isolate free (-)2-aminobutan-1-ol beforehand.) After overnight standing, the mixture was refluxed for six hours, and freed of formic acid with the aid of extra water and evaporation under reduced pressure. To the resulting syrup was added potassium hydroxide pellets (300 g., 5.3 moles) with cooling. The solid potassium tartrate was allowed to settle, so that the two liquid layers could be decanted and separated. The aqueous layer and the solid were extracted twice with ether, then all organic solutions were dried with potassium carbonate and

---

(182) Clarke, Gillespie, and Weisshaus, J. Am. Chem. Soc., 55, 4571 (1933).

fractionated through a 40 cm. Vigreux column to yield (-)2-dimethylaminobutan-1-ol, b.p. 61°/15 mm. Yield 163 g., 91% of theory. Further description of the product appears below under the preparation of the (+)antipode.

(+)2-Dimethylaminobutan-1-ol bioxalate. Filtrates from the (-)2-aminobutan-1-ol and (+)bitartrate preparation, containing mostly the (+) amine, were combined and partially evaporated to yield 740 g. of impure amine salt which was filtered from a dark colored mother liquor. The latter was easily miscible with ether, a proof that all amine salt had been removed. (It was calculated from this that the original Commercial Solvents technical 2-aminobutan-1-ol was only about 75% pure.)

The Eschweiler-Clarke dimethylation was run as before, with this salt (740 g., 2.8 moles), 90% formic acid (630 ml., 13 moles), and U.S.P. formalin solution (500 ml., containing about 6.3 moles of formaldehyde). In this preparation, after liberation of free amine, the ether extracts of the aqueous phase were individually evaporated to compare the amounts of amine therein. This indicated that the ether extraction had been reasonably complete, since the second extraction gave only 30 g. of amine compared with 90 g. for the first one. The combined amine samples were "flashed" away from inorganic residue by rapid non-fractionating vacuum distillation, then re-fractionated

through a 60 cm. glass helix column to give a 97 g. fraction with an equivalent weight of 119 (theory 117) and a 109 g. fraction with an equivalent weight of 121. Another 40 g. was somewhat less pure. The total crude yield of 246 g. was 2.1 moles, or 75% of theory; b.p. = 93°/73 mm.

This was combined with oxalic acid dihydrate (265 g., 2.1 moles) and crystallized from 420 ml. of 95% ethanol to yield 142 g. of pure (+)2-dimethylaminobutan-1-ol bioxalate. Further fractional crystallization brought the total yield to 174 g., 0.84 mole. The original dimethylated amine had consisted of 82% (+)antipode; thus the yield could be considered as about 49% on this basis. The melting point is 117°.

$$[\alpha]_D^{26} = +20.6^\circ \pm .5^\circ \text{ (1.6\% in water)}$$

Anal. Calcd. for $C_8H_{17}O_5N$: N, 6.75; neut. equiv., 207.

Found: N, 6.64(mlK); neut. equiv., 205.

All subsequent fractions, regardless of recrystallization, had rotations of +9° to +10° (corresponding to 56% racemic and 44% optically active salt). It was concluded that, with an optically inactive acid like oxalic, the amine forms a DL-compound.

(+)2-Dimethylaminobutan-1-ol. From the bioxalate

(155 g., 0.75 mole) in a slurry with 100 ml. of water, the amine was liberated in the usual fashion by potassium hydroxide with the aid of two ether extractions. After drying with potassium carbonate, the product was distilled at reduced pressure to give 85 g., 96% yield, of pure (+)2-dimethylaminobutan-1-ol; b.p. 92°/68 mm., d_4^{30} = .8805, $n_D^{17.5}$ = 1.4460.

$$[\alpha]_D^{30} = +5.2^\circ \pm .1^\circ \text{ (10\% in water)}$$

Anal. Calcd. for $C_6H_{15}ON$: neut. equiv., 117.2.
Found: neut. equiv., 119.

Methiodide. Hygroscopic, bipyramidal prisms (recrystallized from dry acetone); m.p. 228°.

$$[\alpha]_D^{28.5} = +4.4^\circ \pm .2^\circ \text{ (4\% in water)}$$

Anal. Calcd. for $C_7H_{18}ONI$: N, 5.4; I, 49.0.
Found: N, 5.3; I, 48.7.

Most of the following compounds could also be considered derivatives of (±)2-dimethylaminobutan-1-ol, which is thus adequately characterized. The above methiodide is also called (+)α-ethylcholine iodide.

(+)2-Dimethylamino-n-butyl benzyl ether. (+)2-Dimethylaminobutan-1-ol (23.4 g., 0.2 mole) and potassium metal (8 g., 0.2 mole) were added to one liter of dry

dioxane and refluxed for about four hours with stirring to speed the solution of metal. After solution of the metal, benzyl chloride (25.3 g., 0.2 mole) was added dropwise over a two-hour period, followed by four hours more reflux. The precipitated potassium chloride was filtered off and washed with a little ether. The filtrate was fractionated through a 60 cm. glass helix column to yield four g. of product, 10% of theory; b.p. 92-98°/<1 mm. A later preparation of the antipode was much more satisfactory however, and can be consulted for physical constants and analysis.

Methiodide. Recrystallized from butanol and acetone, m.p. 142.5-143°.

$$[\alpha]_D^{27} = +11.9^\circ \pm .5^\circ \text{ (1.5\% in water)}$$

Since the above rotation reading remained unchanged after ten hours, it was assumed that these quaternary salts would not racemize on standing in solution.

Anal. Calcd. for $C_{14}H_{24}ONI$: N, 4.0; I, 36.4.
Found: N, 3.9(miK); I, 36.3.

In order to study ways to improve the yield of ether, a few experiments were run with cheap inactive amino alcohols. Due to pharmacological interest in the ether derivatives of N-substituted ethanolamines, the patent literature on these compounds is too extensive to list here. Among

several other synthetic methods found, the Williamson reaction has been used often, with the amino group on either the alcohol or alkyl halide portion, and with a variety of conditions. Wright et al. (183) reported yields ranging 18-67% from N,N-dimethyl-β-chloroethylamine. Of those prepared in this laboratory, β-dimethylaminoethyl butyl ether and β-diethylaminoethyl benzyl ether have previously been reported, without characterization, in antihistamine research papers.

β-Dimethylaminoethyl butyl ether and derivatives. This compound was prepared in the same way as the previous example, except that a more concentrated solution of reactants was employed. The yield was 33% with b.p. = 63°/17 mm.

Bioxalate. Flakes from butanol and ether, m.p. 108-9°.

Anal. Calcd. for $C_{10}H_{21}O_5N$: neut. equiv., 235.5; N, 5.94.

Found: neut. equiv., 237; N, 5.95(miK).

Methiodide. Recrystallized from acetone-ether, m.p. 75°.

---

(183) Wright, Kolloff, and Hunter, J. Am. Chem. Soc., 70, 3098 (1948).
(184) Levy, Kohler, and Justin-Besancon, Compt. Rend., 200, 259 (1935).
(185) Binet and Kohler, Compt. rend. soc. biol., 135, 345 (1941).
(186) Illg and Smolinski, Roczniki Chem., 23, 418 (1949). Abstracted in C. A., 45, 2888 (1951).

Anal. Calcd. for $C_9H_{22}ONI$: I, 44.2; N, 4.88.
Found: I, 43.9; N, 4.91(miK).

Although high concentration of alkoxide is desirable in the Williamson reaction, there is a limit imposed by the solubility of this alkoxide. Lithium metal was found unsuitable here, and the slightly increased solubility of potassium derivatives was not enough to warrant use of this dangerous and expensive metal. Stirring was, of course, very essential to keep the metal dispersed with larger surface area in the initial phase of reaction.

The remaining reactions were run with sodium, in as little solvent as possible, for higher concentrations. As the following preparations indicate, the solubilities of sodium derivatives of amino alcohols depend greatly upon the individual compound, the solvent, and the temperature. The sodium salt of diethylaminoethanol was very soluble in trioxane, while that of (-)2-dimethylaminobutan-1-ol was quite insoluble in the same solvent. However, the latter dissolved readily in xylene.

For the best yields the most important consideration of all was found to be a long reaction time, at least 12 hours well above 100°, even though separation of sodium halide began almost immediately. Alkyl chlorides were too unreactive for convenient use; it was also profitable to

use some excess of the alkoxide to minimize addition of halide to the nitrogen atom toward the end of the reaction.

β-Diethylaminoethyl amyl ether. Some techniques of operating with molten trioxane were illustrated here, although this solvent is not particularly recommended. Redistilled β-diethylaminoethanol (88 g., 0.75 mole) and sodium metal (17.2 g., 0.75 mole) were mixed in 200 g. of molten trioxane, stirred and refluxed with air passing through the condenser, this cooling being sufficient to condense trioxane without freezing it. When a clear solution was obtained, n-amyl bromide (73 g., 0.48 mole) was added dropwise with more stirring and reflux for a total of four hours' heating.

About 300 ml. of Skelly D was added, and the mixture was stirred and cooled. Most of the trioxane crystallized, so that the petroleum could be decanted. The process was repeated with fresh Skelly D. A titration on each petroleum extract of the trioxane proved that two extractions were sufficient. Here the combined petroleum solutions were washed with water, removing a little trioxane and inorganic content. Fractionation then gave some unreacted β-diethylaminoethanol, and finally a pure fraction of β-diethylaminoethyl amyl ether. Yield 45 g., 50% based on the amyl bromide; b.p. 102°/16 mm., $d_4^{29} = .812$, $n_D^{26.5} = 1.4254$.

Anal. Calcd. for $C_{11}H_{25}ON$: neut. equiv., 187.1
Found: neut. equiv., 187.5.

Bioxalate. m.p. 85-86°.

Anal. Calcd. for $C_{13}H_{27}O_5N$: neut. equiv., 277; N, 5.06.
Found: neut. equiv., 275; N, 4.98.

Ethiodide. m.p. 108°.

Anal. Calcd. for $C_{13}H_{30}ONI$: I, 37.0; N, 4.09.
Found: I, 36.8; N, 4.16(micro K).

β-Diethylaminoethyl allyl ether. β-Diethylaminoethanol (46 g., 0.4 mole), sodium metal (9.2 g., 0.4 mole), and allyl bromide (42 g., 0.35 mole) were used in a preparation similar to the previous one. The crude, dried amine was treated with sodium to remove unreacted amino alcohol which would otherwise be difficult to fractionate from the product. Fractionation then gave 27 g. of amine, 50% based on the allyl bromide; b.p. 67°/15 mm, $n_D^{28} = 1.4337$.

Anal. Calcd. for $C_9H_{19}ON$: neut. equiv., 157.
Found: neut. equiv., 158.

Ethiodide. Recrystallized from acetone, m.p. 90-90.5°.

Anal. Calcd. for $C_{11}H_{24}ONI$: I, 40.6; N, 4.48.
Found: I, 40.8; N, 4.52.

β-Diethylaminoethyl benzyl ether. The previous type of experiment was repeated with sodium (17.2 g., 0.75 mole), β-diethylaminoethanol (88 g., 0.75 mole), and benzyl chloride (63 g., 0.5 mole). After the first four hours' reflux most of the trioxane was fractionated directly from the reaction flask to concentrate the reagents; the tempera- of the reaction mixture reached 173° during this distil- lation. The reaction must have been greatly favored by this treatment, as the large yield indicates. After re- moval of trioxane, water (200 ml.) was added to the cooled flask, and stirred to dissolve solid residue. The resulting two layers were separated and the top phase extracted again with water. All water solutions were extracted with ether. The combined and potassium carbonate-dried organic solution was fractionated with water-pump vacuum up to 134°, when the product began coming over steadily; then a Hyvac pump was used, and the amine distilled. Yield 92 g., 89%, based on the benzyl chloride; b.p. 97-100°/ 0.5 mm., $d_4^{29} = .930$.

Anal. Calcd. for $C_{13}H_{21}ON$: neut. equiv., 207.
Found: neut. equiv., 207.

Ethiodide. m.p. 103-103.5°.

Anal. Calcd. for $C_{15}H_{26}ONI$: I, 35.0; N, 3.86.
Found: I, 35.2; N, 3.98(miK).

Bioxalate. m.p. 102.5-103°.

Anal. Calcd. for $C_{15}H_{23}O_5N$: neut. equiv., 297; N, 4.72.

Found: neut. equiv., 296; N, 4.76.

(-)2-Dimethylamino-n-butyl benzyl ether. (-)2-Dimethylaminobutan-1-ol (41 g., 0.35 mole) and sodium metal (8 g., 0.35 mole) were allowed to react at reflux in 250 g. of trioxane, but here the sodium derivative was very insoluble. Addition of an equal volume of dioxane cleared up the mixture enough for a satisfactory reaction. After the sodium had dissolved, benzyl chloride (38 g., 0.3 mole) was added dropwise and the liquid was refluxed six hours. The solventswere then distilled off until the pot temperature reached 155°. The remainder was stirred with 100 ml. of water and separated in a separatory funnel. The top layer was extracted with 40 ml. of water containing a little potassium hydroxide. It was then dried with potassium hydroxide pellets and fractionated to yield 45.5 g., 0.22 mole, 73% of theory; b.p. 136°/17 mm. or 117°/4 mm., $n_D^{28} = 1.4936$, $d_4^{28} = 0.9279$.

$$[\alpha]_D^{28} = -4.85 \pm .05^\circ \text{ (3\% in absolute alcohol)}$$

Anal. Calcd. for $C_{13}H_{21}ON$: neut. equiv., 207.3.

Found: neut. equiv., 208.

Bioxalate. In order to check whether any racemization had occurred during this procedure, a sample of the amine (1.45 g., 6.98 millimoles) was combined with oxalic acid (880 mg., 6.98 millimoles) in 76% alcohol and read immediately in the polarimeter. The specific rotation was calculated as -14.1° for this bioxalate. When the pure bioxalate was prepared in isoamyl alcohol and precipitated with ether, then recrystallized from alcohol-ether, the crystals obtained melted sharply at 81.5-82°.

$$[\alpha]_D^{28} = -14.5^\circ \pm .05^\circ \text{ (in the same solvent)}$$

Anal. Calcd. for $C_{15}H_{23}O_5N$: neut. equiv., 297; N, 4.71.

Found: neut. equiv., 294; N, 4.77.

Since the purified bioxalate had practically the same rotation as that calculated from the unpurified mixture, it is believed that the distillate was reasonably pure even before bioxalate formation, and did not represent any extensive racemization.

Methiodide. Recrystallized from ethanol and acetone, m.p. 141-2°. See previous description of the (+)enantiomer.

Benzyl bromide addition product. Recrystallized from alcohol-ether, m.p. 124°.

$[\alpha]_D^{24} = +4.5^\circ \pm .1^\circ$ (5.5% in 95% ethanol)

Anal. Calcd. for $C_{20}H_{28}ONBr$: Br, 21.1; N, 3.7.
Found: Br, 20.9; N, 3.6.

(+)2-Dimethylamino-di-n-butyl ether. (-)2-Dimethylaminobutan-1-ol (41 g., 0.35 mole) and sodium (8 g., 0.35 mole) reacted together in about 350 ml. of a solvent consisting of 50% dioxane and 50% trioxane. After five hours' reflux the sodium dissolved, and n-butyl bromide (32.2 ml., 0.3 mole) was added dropwise. After refluxing for eight hours, most of the solvent was then fractionated from the product and sodium chloride; the latter was then extracted with water in two portions. The organic layer, after drying with potassium hydroxide pellets, was fractionated through a 60 cm. glass helix column to give 26.5 g. pure product, 52% of theory; b.p. 87.5°/17 mm, $d_4^{28} = .8155$, $n_D^{29} = 1.4218$.

$[\alpha]_D^{29} = +.47^\circ \pm .1^\circ$ (8% in absolute ethanol)

Anal. Calcd. for $C_{10}H_{23}ON$: neut. equiv., 173.
Found: neut. equiv., 172.4.

Bioxalate. In view of the low rotation, the bioxalate was again used to check for racemization as before; that from the distillate without recrystallization had $[\alpha]_D^{28} = -8.03^\circ \pm .1^\circ$ (9% in 76% ethanol).

The pure bioxalate was recrystallized from ethanol with ether, m.p. 77-78°.

$$[\alpha]_D^{28} = -7.9° \pm .1° \text{ (in the same solvent)}$$

Anal. Calcd. for $C_{12}H_{25}O_5N$: neut. equiv., 263; N, 5.3.
Found: neut. equiv., 262; N, 5.25.

Evidently no racemization had occurred in the preparation.

Methiodide. Extremely hygroscopic crystals from ethanol-ether, m.p. 110-112°.

$$[\alpha]_D^{28} = -10.4° \pm .2° \text{ (6.5\% in 95\% ethanol)}$$

Even after drying over phosphorus pentoxide in a vacuum desiccator, the material was still somewhat pasty and moist.

Anal. Calcd. for $C_{11}H_{26}ONI$: N, 4.4; I, 40.3.
Found: N, 4.3(miK); I, 40.9.

(-)2-Dimethylamino-n-butyl o-chlorobenzyl ether.

Sodium 8 g., 0.35 mole) was dissolved in a solution of (-)2-dimethylaminobutan-1-ol (41 g., 0.35 mole) in 700 ml. of a 1:1 mixture of dioxane and trioxane. After several hours of stirring and reflux, the sodium was all dissolved; the stirred mixture was treated dropwise with Eastman reagent grade o-chlorobenzyl chloride (48.3 g., 0.3 mole).

After six hours' reflux, the solvent was partially distilled off, thus furthering the reaction as discussed in previous sections. After five more hours of reflux, more solvent was distilled off, and 100 ml. of water was added and stirred to dissolve sodium chloride. Separation of layers gave a top organic layer, the density of which was near to that of water, so that a second extraction with water required addition of Skelly B to facilitate separation of layers. Fractionation through a 20 cm. Vigreux gave 55 g. of amine, 0.228 mole, 76% based on the o-chlorobenzyl chloride; b.p. 122-127°/<1 mm., n_D^{30} = 1.5050, d_4^{24} = 1.0199.

$$[\alpha]_D^{26} = -3.40^\circ \pm .05^\circ \text{ (10\% in absolute ethanol)}$$

Anal. Calcd. for $C_{13}H_{20}ONCl$: neut. equiv., 241.5.
Found: neut. equiv., 241.0.

Bioxalate. The possibility of racemization was again checked by preparation of the bioxalate, which had m.p. 103° and $[\alpha]_D^{25} = -10.8^\circ \pm .1^\circ$ (10% in 79% ethanol). That prepared directly in solution from the distillate had $[\alpha]_D^{26} = -10.4^\circ \pm .1^\circ$ (under the same conditions).

Anal. Calcd. for $C_{15}H_{22}O_5NCl$: neut. equiv., 331; N, 4.23.
Found: neut. equiv., 329; N, 4.15.

Thus very little, if any, racemization took place during

the reaction.

Methiodide. Recrystallized from alcohol, m.p. 167.5°.

$$[\alpha]_D^{25} = -8.8^\circ \pm .2^\circ \text{ (2.5\% in 80\% ethanol)}$$

Anal. Calcd. for $C_{14}H_{23}ONClI$: I, 33.2; N, 3.66.
Found: I, 33.5; N, 3.64(miK).

Benzyl bromide addition product. Recrystallized from alcohol-ether, m.p. 124°.

$$[\alpha]_D^{24} = +4.54^\circ \pm .1^\circ \text{ (5.5\% in 95\% ethanol)}$$

Anal. Calcd. for $C_{20}H_{27}ONClBr$: Br, 19.3; N, 3.4.
Found: Br, 19.2; N, 3.3.

(-)2-Dimethylamino-n-butyl p-chlorobenzyl ether.

(-)2-Dimethylaminobutan-1-ol (33 g., 0.28 mole) was dissolved in 500 ml. of xylene. Sodium metal (6.5 g., 0.28 mole) was added, and dissolved with stirring and reflux, to a clear solution, thus demonstrating the superiority of xylene as a solvent in this case. The crystals in a sample of Eastman practical p-chlorobenzyl were filtered from molten impurity and washed with a little Skelly A. Then the crystals (40.2 g., 0.25 mole) were dissolved in a small amount of xylene and added dropwise to the above stirred, refluxing solution. After six hours of reflux, most of the solvent was fractionated off, involving another

eight hours of increased heating. Titrations indicated only a negligible amount of amine co-distilled.

At the end of this period, about 90 ml. of water was added and stirred. The organic layer was washed once more with 60 ml. of water, during which addition of hexane was necessary for good separation. Fractionation yielded 50.5 g. of product, 0.207 mole, 83% of theory based on the p-chlorobenzyl chloride; b.p. 127°/1 mm., d_4^{25} = 1.0256, n_D^{23} = 1.5058.

$$[\alpha]_D^{28} = -4.5^\circ \pm .2^\circ \text{ (5\% in absolute ethanol)}$$

Anal. Calcd. for $C_{13}H_{20}ONCl$: neut. equiv., 242.
Found: neut. equiv., 244.

Methiodide. Crystallized from alcohol, m.p. 158°.

$$[\alpha]_D^{27} = -11.1^\circ \pm .2^\circ \text{ (7\% in 75\% ethanol)}$$

Anal. Calcd. for $C_{14}H_{23}ONClI$: I, 33.2; N, 3.66.
Found: I, 33.4; N, 3.65(miK).

Benzyl bromide addition product. Recrystallized from dry acetone, m.p. 122.5°.

$$[\alpha]_D^{26} = +6.65^\circ \pm .2^\circ \text{ (4\% in 95\% ethanol)}$$

Anal. Calcd. for $C_{20}H_{27}ONClBr$: Br, 19.4; N, 3.40.
Found: Br, 19.5; N, 3.33.

Syntheses directed toward production of guanidine derivatives

d-Camphoroxime. Eastman reagent grade d-camphor (500 g., 3.3 moles) was dissolved in about two liters of methanol. Hydroxylamine hydrochloride (500 g., 7.2 moles) was then added, with the aid of another liter of methanol. While this mixture was being stirred mechanically, sodium hydroxide pellets were gradually added, causing liberation of considerable heat. The flask was surrounded by cool water, and more sodium hydroxide was added, to total 750 g. (18.5 moles), by which time the mixture had heated almost to boiling. After standing overnight, most of the methanol was distilled off with stirring; then three liters of water was added to produce a clear solution. This technique differs somewhat from that of Angeli and Rimini (187). The free oxime was liberated by addition of glacial acetic acid (1200 g., 20 moles). The precipitate was washed free of chloride ion, then dried to weigh 545 g., 99% of theory, m.p. 118°. A melting point of 120° was reported by Angeli and Rimini (187). The apparent predominance of one geometrical isomer is remarkable here. No effort was made to recrystallize the product, which was satisfactory for the following step.

---

(187) Angeli and Rimini, Gazzetta chim. Ital., 26 II, 35 (1896).

d-Bornylamine hydrochloride. The following variation of Forster's procedure (188) was used: d-camphoroxime (300 g., 1.8 moles) was dissolved in 1.5 liters of n-butanol. While this was stirred and heated, small portions of sodium were added until precipitation of sodium butoxide made further addition inconvenient. A total of 190 g. (8.2 moles) had been added at this stage. (This was only a slight excess over the theoretical 7.2 moles.) The slurry was separated from a few small pellets of unreacted sodium by pouring through a large Buchner funnel without filter paper. To the filtrate was added one liter of water. After shaking, the aqueous layer was found to be free of camphoroxime (sodium salt). To the organic phase was added one-half liter of 6N hydrochloric acid. It is remarkable that the amino hydrochloride remained entirely in the organic phase at this point, even upon addition of xylene. The entire mixture was boiled to remove most of the solvent. Evaporation of added water helped to remove organic solvent. Systematic fractional crystallization of the residue from water gave 30 g. of product, 15% of theory based on a statement by Forster (188) that the reduction produces bornyl- and neobornyl amines in a 3:2 ratio; $[\alpha]_D^{26} = +22.7^\circ \pm .3^\circ$ (4% in absolute ethanol). A specific rotation of +22.7° (4% in absolute ethanol) was reported by

---

(188) Forster, J. Chem. Soc., 73, 386 (1898).

Forster (188). A further 21 g. crop was obtained with $[\alpha]_D^{27} = +21.0^\circ \pm .3^\circ$ (4% in absolute ethanol). Both fractions sublimed without decomposition at 360°. From this, as well as an amino-cholestene preparation made similarly but not reported, it was concluded that tedious fractionation is necessary to secure a good yield of optically pure amine diastereomer from oxime reduction.

Before the preparation of d-bornylguanidine sulfate was attempted, some general methods of guanidine synthesis were investigated.

n-Butylguanidine sulfate. Since cyanamide is an expensive reagent, some experiments were directed toward evaluating cheaper methods, using the synthesis of n-butylguanidine as a test reaction to conserve valuable asymmetric starting materials. Although dimethylcyanamide is much cheaper, the resulting guanidine, substituted on two of the nitrogens, is not a strong base.

In the course of attempts to use technical calcium cyanamide in guanidine synthesis, a rough analysis was made on this reagent:

Carbon (insoluble in HOAc)	16.7%
CaNCN (assuming no other Ca compounds)	77%
Slightest traces of other soluble cations, some Fe.	

Experiments (using an excess of this material) involved liberation of free cyanamide with oxalic acid, water, and carbon dioxide. In each case the aminium sulfate was present so that it could react simultaneously with cyanamide. Only the carbon dioxide method was successful, to give 29% of the n-butyl derivative, m.p. 215°.

Since the chief impurity in guanidine preparations is likely to be the aminium sulfate, a titration was devised to determine (approximately) RNH_3^+ in the presence of guanidine salts. The unknown was brought to the alizarin yellow end point (pH 12) with .2N sodium hydroxide. Only the aminium sulfate, if any, was titrated. An immediate end point was considered to be an indication of freedom from aminium sulfate.

In view of the unpleasant mercaptan odors from the use of S-methylisothiourea sulfate, and favorable reports of the efficiency of O-methylisourea (189), some effort was devoted to synthesis of this reagent. The sulfate was easily prepared by way of the bisulfate.

O-Methylisourea bisulfate. The reaction of methyl sulfate with urea was described by Werner (190) as being so highly exothermic that it could be performed conveniently

---

(189) Kapfhammer and Müller, Z. physiol. Chem., 225, 1 (1934).
(190) Werner, J. Chem. Soc., 105, 927 (1914).

only in small test-tube batches. However in one variation of this method, urea (60 g., one mole) was shaken gradually into dimethyl sulfate (126 g., one mole) maintained at 110-115°, with stirring. The monomethyl sulfate salt of methylisourea was produced as an oil. Liberation (with calcium hydroxide) of free methylisourea base was not convenient at this point because the highly soluble calcium methylsulfate interfered with distillation.

An amount of sulfuric acid calculated to displace methylsulfuric acid was dissolved in ether (slowly with cooling) and added to the oil from the reaction, along with about the same volume of acetone. When cooled and shaken so as to distribute the initial seeds, 58 g., or a 35% yield of methylisourea bisulfate was obtained, after washing with acetone; m.p. 115-118°. A melting point of 119-120° was reported by Hughes et al. (191). The acetone condensed to dark red compounds in the acid solution, but did not affect the yield, as shown by comparison with other methods.

In a preparation on a larger scale, urea (300 g., 5 moles) and dimethyl sulfate (630 g., 5 moles) were placed in a flask, covered with a layer of cyclohexane and heated cautiously. At 80°, the reagents under the cyclohexane

---

(191) Hughes, Saroff, and Carney, J. Am. Chem. Soc., 71, 2476 (1949).

were not completely miscible, but, when stirred, reacted satisfactorily. Previously 105° had been claimed as necessary for this reaction (192). The refluxing cyclohexane prevented the temperature from going much above 80°; no external heating was necessary. After less than an hour the three phases became two (cyclohexane was always immiscible), and soon the temperature dropped, no longer sustained by an exothermic reaction. The reaction was not self-sustaining below 75°, in spite of five varied "catalysts" tried. An attempt was made to crystallize the normal sulfate, by adding the proper amount of sulfuric acid without success.* However, an excess of sulfuric acid was added slowly, then the mixture was very cautiously extracted three times with ether (heat was produced when the first portion of ether was added). Finally, addition of an equal volume of n-butanol and cooling gave good crystals of the bisulfate, which were washed with butanol and finally with acetone. Drying gave 249 g., a 29% yield.

The typical 30% yield was not due to isolation difficulty since the product was highly insoluble in butanol and acetone and easily crystallized. All other reaction methods, with higher temperatures, and addition of reagents gradually in both possible orders, gave the same yield (30

---

* This could have been due to lack of seeds.

(192) Ongley, Trans. Proc. Roy. Soc. New Zealand, 77, 10 (1948).

to 35%). Methylisourea bisulfate (327 g.) from several preparations was recrystallized from one liter of methanol by addition of 2.5 liters of ether, to yield 274 g., m.p. 119°.

Methylisourea sulfate. Methylisourea bisulfate (192 g., 1.12 equivalents) was dissolved in water and cooled by an ice bath.

$$2CH_3OC(NH_2)_2HSO_4 + Ba(OH)_2 \longrightarrow [CH_3OC(NH_2)_2]_2SO_4 + BaSO_4 + 2H_2O$$

The bisulfate ion was neutralized by 572 ml. of 1.95N barium hydroxide solution.* Centrifugation gave a clear solution. Evaporation of water at reduced pressure was aided toward the end by addition of butanol. After addition of acetone the crystals were filtered and washed with acetone, yielding 119 g., with m.p. of 159-60°. Recrystallization from 200 ml. of water by addition of 800 ml. of acetone yielded 96 g. of the pure salt (70% yield), with m.p. 171-2°.

Anal. Calcd. for $C_4H_{14}O_6N_4S$: S, 13.0; N, 22.7.
Found: S, 12.9; N, 22.6(miK).

Although many other salts of methylisourea have been described, the normal sulfate seems not to have been previously reported.

n-Butylguanidine sulfate. Methylisourea sulfate (6.15 g., 50 milliequivalents) and n-butylamine (3.65 g., 50 milliequivalents) were combined in 30 ml. of water.

---

* This concentration was achieved at 60°.

$$CH_3OC(NH_2)_2(SO_4/2) + C_4H_9NH_2 \longrightarrow C_4H_9NHC(NH_2)_2(SO_4/2) + CH_3OH$$

As the equation indicates, complete reaction is accompanied by disappearance of titratable base. Titrations on small aliquots showed that after five minutes' reflux the reaction was 88% finished. After one-half hour the solution was evaporated to near-dryness in vacuo to yield a white solid. Addition of 25 ml. of absolute alcohol, then evaporation to about 20 ml. and addition of 20 ml. isoamyl alcohol gave four g. of product, which was washed with ether. This one crop was 50% of theoretical, m.p. 210-211°. A melting point of 206° was reported by Davis and Elderfield (175).

The melting point of recrystallized butyl guanidine sulfate of higher purity from a previous preparation was 215°. This large effect of impurities on the melting point was also noticed in the case of the derivative from 2-aminobutan-1-ol, the melting point of which was raised 45° in one recrystallization.

Morpholinecarboxamidine sulfate. Methylisourea sulfate (6.15 g., 50 milliequivalents) and morpholine (7.5 g., 86 milliequivalents) were mixed in 30 ml. of 50% methanol. After standing at 25-30° for 36 hours, 3.3 g. of prisms separated. The solution was evaporated

and alcohol added to yield three more grams. Total yield 6.3 g., 70% of theory. Decomposition point 306°.

Anal. Calcd. for $C_{10}H_{24}O_6N_6S$: S, 9.00; N, 23.6.
Found: S, 9.02; N, 23(mlK).

d-Bornylguanidine sulfate. When this compound was first prepared with stoichiometric quantities under conditions similar to the above, the reaction was about 75% complete in 12 hours, but the precipitate contained some d-bornylamine sulfate, as indicated by titration to the alizarin yellow end point, and confirmed by a low nitrogen content. It proved difficult to remove this impurity, so the reaction was repeated, this time with excess methylisourea as follows: d-bornylamine (3.5 g., 22.8 milliequivalents) in 30 ml. methanol was treated with methylisourea sulfate (4.07 g., 33 milliequivalents), and enough barium hydroxide solution to remove the 10 excess milliequivalents of sulfate ion. After a brief warming, the solution was filtered from barium sulfate and concentrated. Cooling yielded 1.5 g. of precipitate which, after recrystallization from 90% methanol, gave no titration to the alizarin yellow end point, m.p. 326-30° (dec.).

$$[\alpha]_D^{28} = +21.4^\circ \pm .6^\circ \text{ (3.5\% in 50\% ethanol)}$$

Anal. Calcd. for $C_{20}H_{44}O_4N_6S$: S, 6.5; N, 17.2.
Found: S, 6.4; N, 17.1.

Derivatives of alkaloids

Brucine methiodide. Recovered brucine (46 g., 0.10 mole) was dissolved in 200 ml. of warm 95% ethanol. To this was added, all at once, methyl iodide (27.5 g., .192 mole). The precipitation of brucine methiodide was quantitative, the yield being 61 g. (0.10 mole), m.p. 296° (dec.). A melting point of 295°(dec.) was reported by Gulland et al. (193).

Phenoxypropylbrucinium bromide. Brucine (9.6 g., 20 millimoles) and γ-bromopropyl phenyl ether (4.5 g., 21 millimoles) were combined in 120 ml. of 95% ethyl alcohol, heated two hours, and cooled. The yield, after washing with alcohol, was 9.0 g.; m.p. 201°. The product on heating to 120° lost weight corresponding to a monohydrate.

Anal. Calcd. for $C_{32}H_{37}O_5N_2Br \cdot H_2O$: Br, 12.7; N, 4.4.
Found: Br, 12.62; N, 4.2.

Phenylethylbrucinium bromide. Brucine (23.3 g., 50 millimoles) and β-phenylethyl bromide (9.5 g., 50 millimoles) were combined in 200 ml. of 95% alcohol, and heated

---

(193) Gulland, Perkin, and Robinson, J. Chem. Soc., 1627 (1927).

three hours. Cooling produced 24 g. of white crystals; m.p. 216-20°(dec.).

Anal. Calcd. for $C_{31}H_{35}O_4N_2Br$: Br, 13.8; N, 4.8.
Found: Br, 13.85; N, 4.65.

Cinchonine methiodide. This was prepared from Fisher Scientific Co. cinchonine base (26.5 g., 0.09 mole) and methyl iodide (6.3 ml., 0.1 mole) in two liters of 98% alcohol. After standing one day, the solution was concentrated in vacuo to yield 22 g. of cinchonine methiodide, m.p. 268-272°(dec.). Since literature melting points for the methiodide (269°) and free base (264°) are almost the same (194), an iodide analysis was run.

Anal. Calcd. for $C_{20}H_{25}ON_2I$: I, 29.2.
Found: I, 29.6.

Experiments on resolution with (-)2-aminobutan-1-ol

Of the various possible methods for evaluating the usefulness of the asymmetric cations prepared, the best seemed to be their direct combination with single antipodes of amino acids. This method would eliminate the necessity of starting with samples large enough to detect partial resolution in the polarimeter. A description of

---

(194) Claus and Muller, Ber., 13, 2290 (1880).

the simple technique used for evaluation will be found later.

Some effort was devoted to obtaining pure samples of D- and L-antipodes of some amino acid to be used as a test substance. Acylated amino acids were used mostly in this part of the work.

Benzoyl-DL-tryptophan. DL-Tryptophan (Merck, 12 g., 0.059 mole) was benzoylated by the usual Schotten-Baumann procedure to yield 17.4 g. (96%) with m.p. 196°. A melting point of 194° was reported by Elks et al. (195).

Formyl-DL-methionine. Racemic methionine was formylated according to the procedure of du Vigneaud et al. for the formylation of DL-cystine (196) with 70% yield, m.p. 103°. A melting point of 100° was reported by Kolb and Toennies (197).

Formyl-DL-phenylalanine. DL-Phenylalanine was formylated in two preparations in the same manner as above with 80% yield, m.p. 168°. A melting point of 169° was reported by Fischer and Schoeller (7).

---

(195) Elks, Elliott, and Hems, J. Chem. Soc., 629 (1944).
(196) Du Vigneaud, Dorfmann, and Loring, J. Biol. Chem., 98, 577 (1932).
(197) Kolb and Toennies, ibid., 144, 193 (1942).

Formyl-DL-alanine. This was prepared exactly as was formyl-DL-phenylalanine, except that the crude product was not washed with hydrochloric acid, but dissolved in warm ethanol, filtered through glass wool, and cooled to give 65% of product, m.p. 143-4°, about 4° lower than that reported by Bilmam et al. (198).

Resolutions of formyl-DL-phenylalanine. Although formyl-DL-phenylalanine can be resolved with brucine, the following experiments gave satisfactory samples of D- and L-phenylalanine by a new procedure, perhaps not so effective as that with brucine, however.

Formyl-DL-phenylalanine (10 g., 0.05 mole) and (-)2-aminobutan-1-ol (4.6 g., 0.05 mole) were dissolved together in 70 ml. warm sec-butanol. After a day of waiting, a crop of crystals, A, weighing 1.22 g., was deposited from the cooled solution. Evaporation of the filtrate to about 40 ml., and cooling, yielded 4.2 g. of crop B. The washing of this precipitate with sec-butanol increased the filtrate volume to about 65 ml. again. Yet more crystals continued to form, and were filtered to give 1.31 g. of crop C. The rotations of these thoroughly dried fractions were taken in 95% ethanol. The ethanol was then evaporated off, and concentrated

---

(198) Bilmann, Jensen and Jensen, Bull. soc. chim., 1, 1661 (1934). Abstracted in C. A., 29, 2917 (1935).

aqueous solutions of the residues were treated with exactly the calculated amount of 5.3N hydrochloric acid, so as to precipitate the free formylphenylalanine. The precipitates were carefully washed with as small as possible successive portions of water, so that the free acid would be representative of the optical proportions existing in the original salt. The rotations of the free acids were taken in 95% ethanol. Table 1 shows the data.

Table 1

Rotations and Antipodal Constitution

of (-)2-Aminobutan-1-ol Formylphenylalanine Fractions

Fraction	Wt. g.	Specific Rotation in 95% ethanol	Specific Rotation of derived formyl-phenylalanine	Optical Proportion in the salt
A	1.22	-47.5°	-66.5°	94.2%-
B	4.2	-15.1	-14.4	61%-
C	1.3	+38.2	+63.0	91.8%+
D	1.1	-42.6		89%-

The resolution appeared to depend upon supersaturation rather than solubility difference. Proof for this is furnished in the next section. Fraction B was recombined with the filtrate, and the solvent 2-butanol evaporated to one-third volume. Fraction D was obtained with 89% (-) formylphenylalanine combined with the amine, as before.

In a large-scale experiment, formyl-DL-phenylalanine

(165 g., 0.84 mole) was dissolved along with (-)2-aminobutan-1-ol (75 g., 0.84 mole) in 600 ml. n-butanol which was charcoaled and filtered. Then 600 ml. each of benzene and Skelly D were stirred in. This particular proportion may not be the best possible combination, but it worked well, as indicated below. The mixture was seeded and stirred; one crop was removed after six hours, another after overnight standing. Both were practically pure (-)2-aminobutan-1-ol formyl-D-phenylalanine salt, m.p. 128-129°, and totalled 58 g., over 50% of theoretical.

Recrystallization from about 200 ml. n-butanol gave 40 g. of pure (-)2-aminobutan-1-ol formyl-D-phenylalanine salt, rosettes of needles, m.p. 129-30°.

$$[\alpha]_D^{29} = -52.5^\circ \pm .2^\circ \text{ (4.4\% in 95\% ethanol)}$$

Anal. Calcd. for $C_{14}H_{22}O_4N_2$: N, 9.93.
Found: N, 9.97(miK).

To the filtrate was added another 1000 ml. of Skelly D, which threw out a small amount of oil, leading to the precipitation of 24 g. of 97% pure (-)2-aminobutan-1-ol formyl-L-phenylalanine salt.

Recrystallization from 70 ml. of n-butanol plus 70 ml. benzene gave the pure salt, 20 g., m.p. 106-7°. The resolidified sample melted again at 120-23°.

$[\alpha]_D^{29} = +42.7^\circ \pm .6^\circ$ (1.6% in 95% ethanol)

Anal. Calcd. for $C_{14}H_{22}O_4N_2$: N, 9.93.
Found: N, 9.96(miK).

Neither form was a hydrate, since no weight was lost from air-dried samples on stronger heating. The yield of pure salt of formyl-D-phenylalanine in this first separation was 40% of theory; the yield of the second diastereomer was 17%. However, the original filtrates, when appropriately seeded, continued to deposit crops which were recrystallized to yield another 11 g. of the formyl-D-phenylalanine salt and 20 g. of the formyl-L-phenylalanine salt.

Solubilities of (-)2-aminobutan-1-ol salts of formyl-D- and L-phenylalanine. The pure salts were shaken with a mixture of equal volumes of butanol, benzene, and Skelly D for three days. One set of flasks was brought up to 30° in a constant-temperature bath, and the other set was cooled to 30° from a higher temperature. Seven ml. of filtrate in each case were evaporated to constant weight. The solubility of the salt of formyl-D-phenylalanine was found to be 1.40 ± .01%, 1.56 ± .02% for the diastereomer from formyl-L-phenylalanine. These values indicate that the success of the resolution depends on supersaturation of the salt containing formyl-L-phenylalanine, and not upon

solubility difference. Seeding should be done first with the salt of formyl-D-phenylalanine, as this gives the more effective separation.

Formyl-D-phenylalanine. To liberate the free formyl-phenylalanine, (-)2-aminobutan-1-ol formyl-D-phenylalanine salt (42.3 g., 0.15 mole) was dissolved in about 150 ml. of water and treated with 30 ml. of 5-6N hydrochloric acid to give the free (-)formyl-D-phenylalanine, which was washed with water. A recrystallized sample had m.p. 168-9°.

$$[\alpha]_D^{29} = -70.0^\circ \pm .3^\circ \text{ (2.5\% in 95\% ethanol)}$$

A melting point of 167°, and $[\alpha]_D^{20} = -75.4^\circ$ (in alcohol), were reported by Fischer and Schoeller (7).

D-Phenylalanine. The crude product from above was refluxed with about 500 ml. of 6N hydrochloric acid for five hours, then freed of the latter at water-pump pressure, aided by an extra portion of water. The residue in water was partially neutralized by sodium hydroxide, then with sodium acetate to pH 6, filtered and washed to yield 18 g. of the crude product. This was recrystallized from 250 ml. 20% ethanol to yield 17 g. of D-phenylalanine (65% of theory), found by microbiological assay to be free from the L-antipode.

For maximum rotation, another recrystallization gave 10 g. of pure D-phenylalanine (40% based on the salt).

$$[\alpha]_D^{29} = +35.1^\circ \pm 1.0^\circ \text{ (1\% in water)}$$

Formyl-L-phenylalanine. The (-)2-aminobutan-1-ol formyl-L-phenylalanine salt (22 g., 0.078 mole) was treated in the same manner to give a sharply melting (169°) (+) formyl-L-phenylalanine.

$$[\alpha]_D^{29} = +71.0^\circ \pm .4^\circ \text{ (3.5\% in absolute ethanol)}$$

A melting point of 167° and $[\alpha]_D^{20} = +75.2^\circ$ (in absolute alcohol) were reported by Fischer and Schoeller (7).

L-Phenylalanine. Most of the formyl-L-phenylalanine was hydrolyzed and treated as above to yield 4 g., of L-phenylalanine.

$$[\alpha]_D^{30} = -35.0^\circ \pm 1.0^\circ \text{ (1\% in water)}$$

In view of the success of the above procedure, limited investigations were carried out on other acylated amino acids, with (-)2-aminobutan-1-ol.

Attempted resolution of formyl-DL-valine. Formyl-DL-valine (6 g., 0.04 mole) and (-)2-aminobutan-1-ol (3.67 g., 0.04 mole) were combined in about 12 ml. of sec-butyl alcohol. When scratching of the glass failed to start crystallization, the solution was seeded with crystals prepared from a pure formyl-D-valine antipode. Even with this, the crystallization was extremely delayed. The

resulting hygroscopic solid was dissolved in a very little water, and treated with concentrated hydrochloric acid to yield a precipitate of formylvaline, which was washed with two one-cc. portions of water, with stirring, so as to get the maximum benefit from a small water volume. Hydrolysis with concentrated hydrochloric gave DL-valine only. This result would indicate that the two diastereomers of (-)2-aminobutan-1-ol salt of formylvaline are of about equal solubility.

Attempted resolution of benzoyl-DL-methionine. Equimolar portions of (-)2-aminobutan-1-ol and benzoyl-DL methionine were mixed together in a small volume of 2-butanol. Crystals from this solution amounted to 20% of the total weight, were treated with a slight excess of hydrochloric acid, and the liberated benzoylmethionine was found to be completely inactive.

Attempted resolution of miscellaneous acylated amino acids. All attempts to produce crystalline salts from (-)2-aminobutan-1-ol and formyl-DL-alanine, formyl-DL-methionine and benzoyl-DL-tryptophan were unsuccessful, in spite of the use of a variety of solvents, including iso-amyl alcohol, dioxane, ether, butanone, and ethyl acetate. The salts were thrown out of most solutions by ether as oils.

Benzoyl-L-tryptophan anilide. Limited experiments

on the benzoyl-DL-tryptophan, using the papain-anilide procedure (199), gave 85% yield of anilide in optically pure form. However, benzoyl-D-tryptophan was not obtained from the filtrate, and hydrolysis of the L-anilide in an atmosphere of carbon dioxide led to some racemization, although a 70% yield of tryptophan was obtained without serious decomposition.*

It is, of course, possible that the pronounced tendency of the 2-aminobutan-1-ol salts to supersaturate could be developed, with further effort, into resolutions similar to that of formylphenylalanine. However, the samples of L- and D-phenylalanine were sufficient for the main objective of the work.

New resolution of DL-glutamic acid. In view of the extra carboxyl group on glutamic acid, it was of interest to determine whether (-)2-aminobutan-1-ol could be used to resolve this unacylated amino acid. A few preliminary experiments were performed with pure L- and pure D-glutamic acid in which only the salt of D-glutamic acid crystallized. Then DL-glutamic acid (7.3 g., 50 millimoles) and (-)2-aminobutan-1-ol (4.45 g., 50 millimoles) were mixed with

---

* Recrystallization of this product gave an equilibrium mixture of DL- with about 80% L-tryptophan, having the same specific rotation as an artificially prepared, saturated solution of DL- and L-varieties.

(199) Halverson, unpublished work in this laboratory.

only two ml. of water to form a paste, which gradually cleared up with heating to about 70° on a hot water bath. The almost clear syrup was dissolved in 100 ml. of absolute ethanol, and seeded with a little of the salt of D-glutamic acid prepared from the pure antipode. After frequent shaking and overnight standing, a copious fine powdery precipitate was obtained, filtered, and washed with absolute alcohol. It was immediately redissolved by heating again with two ml. of water and reprecipitated as before with 100 ml. of absolute alcohol.

It was not possible to use simple hot-to-cold recrystallization of the salt, due to its great insolubility in hot alcohol. The tendency toward slow crystallization was, however, admirably adapted to a throwing-out method. The dried, mildly hygroscopic (-)2-aminobutan-1-ol D-glutamate weighed four g., 70% of theory, m.p. 146-7°.

$$[\alpha]_D^{22} = -3.2° \pm .1° \text{ (5\% in water)}$$

Anal. Calcd. for $C_9H_{20}O_5N_2$: N, 11.85.
Found: N, 11.91(MiK).

D-Glutamic Acid. The four g. of salt was dissolved in 25 ml. of water and treated with the theoretical 3 ml. of 6N hydrochloric acid. The precipitated glutamic acid was washed with alcohol, and recrystallized from water to give 1.5 g. of pure D-glutamic acid, m.p. 218-9°.

$$[\alpha]_D^{25} = -30.4^\circ \pm .2^\circ \text{ (5.5\% in 3N hydrochloric acid)}$$

L-Glutamic acid. After somewhat more of the original salt had precipitated from its diastereomer, the filtrate was treated with an insufficient amount of hydrochloric acid, plus an excess of chloroacetic acid. The latter has its pK near to the isoelectric point of glutamic acid, and therefore a slight excess would result in a well-buffered solution. The refrigerated mixture was filtered, and the washed precipitate recrystallized from 25 ml. of water to yield 0.85 g. of L-glutamic acid.

$$[\alpha]_D^{24} = +29.7^\circ \pm .2^\circ \text{ (6\% in 3N hydrochloric acid)}$$

The over-all yield of D-glutamic acid was 42%, while that of the L-antipode was 23%. If this procedure were to be used on a larger preparative scale, these yields could undoubtedly be greatly increased.

The evaluation of strong organic bases in salt formation with amino acids or carbamino acids

Method. The following general scheme was applied to the quaternary hydroxides with D- and L-phenylalanine and some other amino acids.

(a) The asymmetric quaternary halide was dissolved in methanol and vigorously shaken with carefully prepared sodium-and nitrate-free silver oxide until the precipitate

acquired the dark gray-brown color of excess oxide. (+)2-Guanidinobutan-1-ol was liberated from the sulfate with barium hydroxide. An aliquot of the clear solution was titrated to the brom thymol blue end point to determine its normality.

In the case of methylbrucinium hydroxide, it was necessary to establish that the hydroxide ion would not split an amide-type bond at another point in the molecule. If this were the case, the resulting carboxyl group would immediately neutralize hydroxide, leaving a betaine with no basic groups stronger than an aromatic amine and a carboxyl anion.

Two lines of evidence indicated the methylbrucinium hydroxide was satisfactorily stable, at least in the cold. Any weak basic groups such as those mentioned above should be titrated in moving from pH 6.5 (brom thymol blue) to pH 2.0 (thymol blue). The volume of hydrochloric acid actually required for this transition was the same as in a blank. The same weak bases should also make it impossible to obtain a sharp methyl red end point (pH 5.0). Actually the end point was sharp.

(b) Although some of the experiments were carried out with DL-phenylalanine, in most cases a pure antipode was dissolved in the equivalent portion of the quaternary hydroxide solution in a micro Kjeldahl flask. In experiments

on carbamino salt formation with carbon dioxide, the amino acid was dissolved with two equivalent portions of base, since there are two acidic groups in a carbamino acid. The methanol was evaporated at water-pump pressure. Addition and evaporation of dry dioxane aided the removal of small amounts of water as azeotropes. If a precipitate was obtained here with only one amino acid antipode, it was considered a promising technique for resolution.

(c) In attempts to prepare carbamino salts, the solution was then treated with just enough isoamyl alcohol to prevent solidification of dioxane when cooled in ice; then dry carbon dioxide was bubbled in. Again, the appearance of a precipitate with only one antipode would be a good indication of possible success in resolution.

Preliminary results. The above technique was used on the following combinations with the result indicated.

(+)α-Ethylcholine + DL-phenylalanine	Oil produced
(-)o-Chlorobenzyl ether of α-ethylcholine + D-phenylalanine	No ppt.
(-)o-Chlorobenzyl ether of α-ethylcholine + L-phenylalanine	No ppt.
Methylbrucinium hydroxide + D-phenylalanine	No ppt.
Methylbrucinium hydroxide + L-phenylalanine	A few very small rosettes of needles
Methylbrucinium hydroxide + DL-phenylalanine	No ppt.
Methylcinchoninium hydroxide + DL-tryptophan	Oil produced

Methylcinchoninium hydroxide + L-tryptophan	Oil produced
Methylcinchoninium hydroxide + L-phenylalanine	No ppt.
Methylcinchoninium hydroxide + D-phenylalanine	Copious solid ppt.
Methylcinchoninium hydroxide + D-leucine	Solid ppt.
Methylcinchoninium hydroxide + L-leucine	Solid ppt.
(+)Dimethylaminobutan-1-ol (large excess*) + D-phenylalanine + carbon dioxide	Ppt.**
(+)α-Ethylcholine + L-phenylalanine + carbon dioxide	Ppt.**
(+)α-Ethylcholine + D-phenylalanine + carbon dioxide	Ppt.
(-)α-Ethyl-N-benzylcholine + D-phenylalanine + carbon dioxide	Ppt.
(-)α-Ethyl-N-benzylcholine + L-phenylalanine + carbon dioxide	Ppt.
(+)o-Chlorobenzyl ether of N-benzylcholine + D-phenylalanine + carbon dioxide	Ppt.**
(+)o-Chlorobenzyl ether of N-benzylcholine + L-phenylalanine + carbon dioxide	Ppt.
Methylbrucinium hydroxide + D-phenylalanine + carbon dioxide	Ppt.**
Methylbrucinium hydroxide + L-phenylalanine + carbon dioxide	Ppt.**

---

* Addition of two volumes of 60% alcohol was necessary to dissolve the amino acid.

** These precipitates were isolated and dissolved in hot water, in which any carbamino salt would be decomposed to amino acid and the carbonate of the base. In all five cases, titration to the methyl red end point indicated too little base to support a carbamino salt formula for the precipitates, which were evidently phenylalanine in most cases.

In none of the last nine combinations was any solid precipitate obtained prior to carbon dioxide addition. The oils obtained with (+)α-ethylcholine and phenylalanine antipodes were redissolved by treatment with a small proportion of absolute alcohol before carbon dioxide addition.

Some experiments with amine solvents. Because of the suggestion of success with methylbrucinium hydroxide, a larger run was made with DL-phenylalanine (3.3 g., 0.02 mole) and the stoichiometric quantity of methylbrucinium hydroxide. A new technique was tried, based on the idea that in an amine solvent, an amino acid should display somewhat better acid properties, and might form a better precipitate with the methylbrucinium ion. Thus the alcohol solvent was evaporated and replaced with about 25-30 ml. of anhydrous pyridine. Cooling at this point did not yield any precipitate, but the addition of about an equal volume of n-butylamine gave, after overnight refrigeration, a satisfactory precipitate. The precipitate, washed with a little pyridine, then with ether, proved to be extremely hygroscopic and melted almost immediately on exposure to the air. Addition of 1.5 cc. of water, then 0.5 ml. of glacial acetic acid, precipitated 900 mg. of phenylalanine which, however, was completely inactive. That the hygroscopic solid was actually a methylbrucinium-phenylalanine salt was strongly indicated by a second preparation, from

which DL-phenylalanine (washed free of any brucine derivatives) was recovered in 68% yield, which is typical of phenylalanine recoveries from salt solutions. Addition of hydriodic acid to the methylbrucinium acetate solution precipitated methylbrucinium iodide in 92% yield, m.p. 290° (dec.). (See section on methylbrucinium iodide preparation.)

When the pyridine-butylamine solvent combination was tried on the (+)2-guanidinobutan-1-ol salt of DL-phenylalanine, no precipitate was obtained.

Resolution of unsubstituted DL-phenylalanine. DL-Phenylalanine (38 g., .23 mole) was dissolved in 720 ml. of 0.32N methylcinchoninium hydroxide solution (prepared from the iodide and silver hydroxide in methanol. The methanol was mostly evaporated, then replaced by dioxane, which was also partly evaporated to about 200 ml. volume, when the methanol was believed to be sufficiently removed. The precipitate was filtered, and the filtrate was further evaporated to yield still more precipitate, which was combined with the first crop, and dissolved in methanol. Recrystallization was accomplished in the same manner with dioxane, giving, after washing with dioxane, then with ether, 50 g. of methylcinchoninium D-phenylalanine salt* (theory 54 g.), m.p. 174°(dec.) or

---

* This particular sample was later found to contain some of the methylcinchoninium L-phenylalanine, which did not affect the neutral equivalent.

above, depending on the rate of heating.

Anal. Calcd. for $C_{29}H_{35}O_3N_3 \cdot H_2O$: neut. equiv., 492; H_2O, 3.66.

Found: neut. equiv., 506; H_2O, 3.5.

The diastereomer was further characterized by recovery of both phenylalanine and methylcinchoninium iodide as described later.

The diastereomer (50 g., 0.1 mole) was dissolved in 200 ml. of ethanol-methanol mixture, and treated with oxalic acid dihydrate (6.7 g., 0.1 equivalent plus a slight excess) dissolved in alcohol. The precipitated phenylalanine was washed practically free of oxalate ion, and weighed 16.5 g. Recrystallization by repeated hot extraction with 100 ml. of water, and final addition of two volumes of alcohol gave 13.5 g. of D-phenylalanine (theory 16.5 g.). However, this material had a specific rotation of $+29.9^\circ \pm 0.7^\circ$ (in water), not much greater than that of an equilibrium-saturated mixture of D- and DL-phenylalanine (determined separately). Thus recrystallization of the amino acid was not likely to achieve optical purity.

However, the material was purified by recombination with the resolving agent, since no other derivatization is necessary. For this purpose, the alcoholic solution of methylcinchoninium oxalate, resulting from liberation of

free D-phenylalanine, was shaken with successive (excess) portions of pure calcium hydroxide. After each shaking, the base normality increased and the oxalate test became fainter, until after four portions had been added and filtered, a solution was obtained completely free of both calcium and oxalate, and containing 90% of the original methylcinchoninium ion judging by titration of hydroxide. Addition of hydriodic acid to a small sample precipitated the original cinchonine methiodide in 58% yield after recrystallization; this did not depress the melting point of an authentic sample on mixing.

The 13.5 g. of phenylalanine was redissolved in the calculated amount of this solution, plus about 5% excess, and precipitated, then recrystallized twice, all by the same original technique. A 34 g. sample of diastereomer was obtained, $[\alpha]_D^{27} = +163^\circ$. Addition of an alcohol solution of oxalic acid dihydrate (4.4 g., 70 milliequivalents) to a methanol-ethanol solution of the diastereomer as before precipitated phenylalanine which, after two recrystallizations for the sake of optical purity, weighed 3.8 g. and had $[\alpha]_D^{29} = +35.5^\circ$ (2% in water). Although this over-all yield is only 20% of theory (based on the D-component of the original DL-phenylalanine), some improvements, as brought out in the discussion section, are expected to improve the process.

The filtrate from the first precipitation of the methylcinchoninium D-phenylalanine salt, after some standing, deposited another four g. of salt; the filtrate was checked, by titration, for base content; then an alcoholic solution of oxalic acid dihydrate (8.2 g., 130 milliequivalents) was added to precipitate 17.5 g. of crude L-phenylalanine which, after recrystallization from water, weighed 13.5 g., and had $[\alpha]_D^{30} = -28.2^\circ \pm .6^\circ$ (1.6% in water). Thus it is evident that, although the initial yields seemed practically quantitative, the diastereomer separation is the step which must be improved in order to obtain optically pure free amino acids.

An attempt was made to resolve DL-leucine by a procedure similar to the above. It was indicated in the preliminary trials that both diastereomers precipitated from dioxane. Although addition of about one per cent of ethanol led to a difference between solubilities of the pure diastereomers, application of this to the resolution led only to the eventual recovery of DL-leucine.

DISCUSSION AND CONCLUSIONS

The amines used in this work were not strong enough, even in large excess, to form stoichiometric salts with unsubstituted amino acids or to break up the racemic dipolar ion. The quaternary hydroxides, however, were easily able to bring amino acids into methanol solution. Many of these salts were so soluble in organic media as to suggest that they are unlikely ever to crystallize conveniently, at least for a "simplified" resolution.

The reputation for crystallinity of alkaloid derivatives is well illustrated here, since only methylated brucine and cinchonine gave solid amino acid salts.

Two improvements in the methycinchonine method are worth trying. First, the weakness of amino acids might lead to some solvolysis of the quaternary salt, leaving some free amino acid, even with a stoichiometric quantity of strong base. (The odor of hydrogen cyanide from sodium cyanide is a parallel situation.) Since this reaction would probably precipitate the DL-amino acid from organic solution, imperfect resolutions could result. The use of excess base should correct this situation.

Also there are many quaternary ions which will not precipitate in any form with amino acids. These could be

used in conjunction with the rather expensive methylcinchoninium hydroxide so that only enough methylcinchoninium ion to bring down the D-amino acid need be used. The easy recovery of the resolving agent with the oxalic acid-calcium hydroxide method removes the objection of expense.

Apparently in those cases in which a carbamino salt does not form, the free amino acid is precipitated instead. In view of the instability of carbamino salts (except for the heavy metal salts) one would obtain only the racemate as a final result.

In order to induce the 2-aminobutan-1-ol derivatives to form crystalline precipitates, it would evidently be necessary to substitute still larger groups on the oxygen or nitrogen. Unfortunately some of the polar substituents which tend to raise melting points would be unstable in the strong basic solution.

In almost any amino acid resolution the chief factor one must keep in mind is the smaller solubility of racemic compounds. The difficulty caused by this can be understood by consideration of phenylalanine, whose active antipodes are about two and a half times as soluble in water as the DL-compound (when both are present in the same solution). If one attempted to recrystallize a crude sample of L-phenylalanine containing only 10% D-antipode (actually 20%

DL-phenylalanine), an amount of water sufficient to retain the 20% of racemate would also retain two and a half times this weight of the L-antipode. The yield of the latter could not be better than 30% at best, just for the recrystallization alone. On the other hand, the unique lack of DL-compound formation in the case of glutamic acid is largely responsible for the ease with which the new resolution yielded optically pure antipodes, as described in the experimental section.

SUMMARY

The literature has been reviewed for all existing types of amino acid resolution as well as for many experiments in other fields which could have a direct or indirect bearing on the problem of resolving unsubstituted amino acids.

Twelve miscellaneous new compounds have been characterized for developmental purposes on synthetic methods.

Five new optically active amines and four of their bioxalates have been characterized.

Ten new quaternary halides and two new substituted guanidine sulfates (all optically active) have been characterized.

Three new resolutions of amino acids have been described, involving four new diastereomeric salts.

Twenty other strong organic base-amino acid combinations also were investigated, as well as miscellaneous other methods including the use of optically active solvents or adsorbents; none of them resulted in resolution.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. S. W. Fox for his advice and encouragement; to the Williams-Waterman Foundation for their financial support of a part of this project; to Dorothy DeFontaine and Armand McMillan for nitrogen analyses on new compounds; and to the Commercial Solvents Company for samples of 2-aminobutan-1-ol.