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Action of Acetobacter suboxydans upon some 1-desoxy sugar alcohols

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ACTION OF *ACETOBACTER SUBOXYDANS* UPON SOME 1-DESOXY SUGAR ALCOHOLS

by

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In Charge of Major Work

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Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1949

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I. INTRODUCTION

Certain species of the genus Acetobacter can, by selective oxidation, produce chemically pure organic compounds in excellent yields. Historically, the foremost of these species is A. xylinum; more recently A. suboxydans has assumed a highly important position. By the agency of these organisms the production from D-sorbitol of L-sorbose, an invaluable intermediate in the synthesis of ascorbic acid, has been rendered commercially feasible.

The specific action of A. xylinum is elucidated in what has become known as 'Bertrand's rule' (9, 12, 20). In effect, this rule states that for fully hydroxylated sugar alcohols of four or more carbon atoms A. xylinum will promote the oxidation of a secondary alcohol group to a ketone, but only if the hydroxyl group involved is adjacent to a primary hydroxyl group and in cis relation to an adjacent secondary hydroxyl group.

Hann, Tilden, and Hudson (52) have suggested that A. suboxydans is even more specific than A. xylinum. This organism follows the rule of Bertrand but only for those alcohols occurring in what is known in carbohydrate chemistry as a D- series.

The investigations reported in this thesis involve

testing the tenability of the Hann, Tilden, and Hudson modification of 'Bertrand's rule' as applied to 1-desoxy sugar alcohols.

II. HISTORICAL

Fairly complete reviews of the oxidizing action of the various species of Acetobacter have been given by Bernhauer (3) and Butlin (29). While these articles are somewhat dated (1938 and 1936, respectively) they are fairly comprehensive with respect to all excepting the species A. suboxydans with which most subsequent individual publications have dealt. A review concerning the oxidation of polyhydric alcohols by A. suboxydans has recently been published by Fulmer and Underkofler (47). The historical material presented here will be restricted to reviewing the oxidative action on organic compounds by the two species, A. xylinum and A. suboxydans. Wherever possible, the specificity of such action will be emphasized.

Under identical experimental conditions essential to successful oxidations utilizing these two organisms it is to be noted that A. suboxydans inevitably produces yields highly superior to A. xylinum over shorter periods of time. Regarding the time required for maximum oxidation, vigorous aeration, in the case of A. suboxydans, remarkably abbreviates the reaction period with maintenance of excellent yields. A. xylinum grows very slowly, producing a thick zoogloea which retards its oxidizing action, and isolation of the primary

products of oxidation results in comparatively poor yields. Aeration of substrates harboring A. xylinum produces further oxidation and consequently lower yields of the primary products.

A. Oxidation of Carbohydrates and Derivatives

1. Oxidation of sugar alcohols to ketoses.

a. Oxidation of glycerol and derivatives. Bertrand (10, 13, 14) first produced crystalline dihydroxyacetone by the action of A. xylinum in a 5-6 per cent solution of glycerol in yeast water. Bernhauer and Schön (5), Hermann and Neuschul (54), Virtanen and Bärlund (104), and Visser't Hooft (106) reproduced this experiment. Optimum conditions for obtaining very high yields (quantitative, as measured by the amount of reducing compound in solution) of dihydroxyacetone with A. xylinum are recorded by Bernhauer and Schön (5). With intense aeration Butlin (31) obtained an analytically quantitative yield of the ketose under approximately the same conditions but in much shorter time (4-6 days as against 16-20 days) with A. suboxydans. The conditions and yields of Butlin are superior to those given for the same organism by Virtanen and Nordlund (105).

The recovery of product has presented a serious problem. The best recovery yields (77-80 per cent) recorded are to be gained by utilizing the Underkofler and Fulmer (99)

modification of the Neuberg and Hofmann method (77).

Of interest are the reports of Irene Neuberg (78) and Marguerite Cozic (38) concerning the action of the two organisms toward glycerol derivatives. Miss Neuberg introduced the monomethyl and monoethyl ethers of glycerol to the influence of both organisms and was able to recover, though it be in poor yield (10-20 per cent), the substituted dihydroxyacetones.

$$CH_2(OR).CHOH.CH_2OH \longrightarrow CH_2(OR).CO.CH_2OH$$

Miss Cozic measured the oxygen uptake on the Warburg apparatus of the mono- , di- , and triacetyl glycerols in the presence of A. xylinum cells and found evidence of oxidation only in the case of the mono- ester.

Table I

Tetritols and Their Oxidation Products

CH_2OH	CH_2 OH	CH_2OH	CH_2OH
HCOH	HCOH	HCOH	HOCH
HCOH	CO	HOCH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Erythritol	L-Erythrulose	L-Threitol	D-Threitol
I	II	III	IV

b. Oxidation of tetritols. Only one of the three possible diastereoisomeric tetritols has been tested for oxidizability with A. xylinum and/or A. suboxydans. The tetritol involved is the one now known as erythritol (I), the

meso compound. Bertrand (17, 18, 19), using A. xylinum, isolated a sirupy ketose which he named erythrulose (II), and whose structure he proved by reduction to the known L-threitol (III). Subsequent work by Cozic (38), Hermann and Neuschul (54), Müller, Montigel, and Reichstein (74), and Visser't Hooft (106) confirmed Bertrand's results.

For large scale preparation of pure L-erythrulose A. suboxydans has been successfully employed by Whistler and Underkofler (114) to oxidize erythritol. In a medium consisting of 0.5 per cent yeast extract and 4.5 per cent erythritol A. suboxydans was effective in producing practically an analytically quantitative yield of ketose in four days. The recovery of pure ketose by molecular distillation was 87.4 per cent.

It should be noted here that Bertrand (12) expressed a curiosity as to the production of only one ketose. According to the rule of Bertrand as stated in the introduction, one might expect either or both of the secondary hydroxyl groups to be oxidized. It should especially be noticed that the secondary hydroxyl which is oxidized maintains a D- relationship with respect to the adjacent terminal carbon; the remaining secondary hydroxyl group is L- relative to its adjacent terminal carbon. Such a relationship is of utmost importance in consideration of the oxidizability of various glycols by A. xylinum and A. suboxydans. A more detailed discussion will follow in the section on glycols.

It would be of definite interest to investigate the action of A. suboxydans or A. xylinum on the other two tetritols, D-threitol (IV) and L-threitol (III).

c. Oxidation of pentitols. The four possible stereochemical configurations for the pentitols are given in Table II (V, VI, VII, VIII). All of these compounds have been

Table II

Pentitols and Their Oxidation Products

CH_2OH	CH_2OH	CH_2OH	CH_2OH
HCOH	HOCH	HCOH	HCOH
HCOH	HCOH	HOCH	HOCH
HCOH	HCOH	HOCH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Adonitol	D-Arabitol	L-Arabitol	Xylitol
V	VI	VII	VIII

CH_2OH	CH_2OH	CH_2OH
HCOH	HCOH	HOCH
HCOH	HOCH	HCOH
CO	CO	CO
CH_2OH	CH_2OH	CH_2OH
L-Ribulose	L-Xylulose	D-Xylulose
IX	X	XI

tested for oxidizability with either A. xylinum or A. suboxydans. Visser't Hooft (106) demonstrated that A. suboxydans will oxidize a two per cent solution of adonitol (V) to a reducing compound, presumably a ketose (IX). The ketose was not characterized. Reichstein (92), utilizing an Acetobacter

purportedly similar to A. suboxydans, obtained almost a quantitative yield of sirupy L-ribulose (IX) which he fully characterized and identified through the preparation of a number of derivatives.

L-Arabitol (VII) apparently yields a ketose (X) under the oxidative influence of A. xylinum. Bertrand (9, 12, 20) was responsible for this information but the reducing compound was neither isolated nor characterized. With A. suboxydans, Hann, Tilden, and Hudson (52) have indicated the oxidation of L-arabitol to be negligible.

D-Arabitol (VI) yields a ketose when oxidized by A. suboxydans. Identity of the ketose by the reporters of the action, Hann, Tilden, and Hudson (52), with D-xylulose (XI) was suggested by comparison of its specific rotation with that of the known enantiomorph.

According to Bertrand (9, 12, 20) the remaining pentitol, xylitol (VIII), is not oxidized by A. xylinum.

Table III

Hexitols and Their Oxidation Products

CH_2OH	CH_2OH	CH_2OH	CH_2OH
HCOH	HCOH	HOCH	HOCH
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
HCOH	CO	HCOH	CO
CH_2OH	CH_2OH	CH_2OH	CH_2OH
D-Sorbitol	L-Sorbose	D-Mannitol	D-Fructose
XII	XIII	XIV	XV

Table III (Continued)

CH_2OH	CH_2OH	CH_2OH	CH_2OH
HOCH	HCOH	HCOH	HCOH
HCOH	HCOH	HCOH	HOCH
HCOH	HCOH	HCOH	HCOH
HOCH	HCOH	CO	HOCH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Dulcitol	Allitol	L-Allulose	L-Iditol
XVI	XVII	XVIII	XIX

d. Oxidation of hexitols. Perhaps the most important of the Acetobacter oxidations of sugar alcohols is the conversion of D-sorbitol (XII) to L-sorbose (XIII). The latter compound is an important intermediate in the production of synthetic L-ascorbic acid (Vitamin C).

Bertrand (7, 8) was the first to procure crystalline L-sorbose by the action of A. xylinum on D-sorbitol. Further investigations by Böeseken and Leefers (26), Hermann and Neuschul (54), Maurer and Schiedt (71), Razumouskaya (90), Seifert (94), and Visser't Hooft (106) have produced up to 70 per cent yields of crystalline L-sorbose by oxidation of D-sorbitol using A. xylinum. Reichstein and Grüssner (93) are accountable for synthesizing Vitamin C from the L-sorbose thus formed.

Bionda (25), Kluyver and DeLeeuw (66), Iris and Gurria (57), and especially Böeseken and Leefers (26) have revealed that A. suboxydans produces L-sorbose from D-sorbitol much more rapidly and completely than A. xylinum. Fulmer, Dunning,

Guymon, and Underkofler (45) used concentrations of D-sorbitol up to 35 per cent without notably decreasing the final yield (80-86 per cent) of ketose. On a pilot plant scale, using rotary drum fermenters with proper aeration, Ward (111), Wells, Lockwood, Stubbs, Roe, Porges, and Gastrock (112), and Wells, Stubbs, Lockwood, and Roe (113) have used a 20 per cent D-sorbitol medium and obtained up to 90 per cent ketose in 16-32 hours. They realized an 80 per cent recovery of pure L-sorbose.

The fact that D-mannitol (XIV) can be oxidized to D-fructose (XV) through the action of A. xylinum was primarily recognized by the discoverer of the bacterium, A. J. Brown (27). Subsequent reports of this action by Cozic (38), Hermann and Neuschul (54), Vincent and Delachanal (103), and Visser't Hooft (106) have indicated a maximum yield of only 31 per cent D-fructose.

A. suboxydans will produce D-fructose from the same substrate according to Fulmer, Dunning, and Underkofler (46), Kluyver and de Leeuw (66), Visser't Hooft (106), and Ward (111). Fulmer, et alia, (46) have made a systematic study of the oxidative action of A. suboxydans on D-mannitol. They proposed that up to 25 per cent D-mannitol may be used, giving over a period of seven days a 93 per cent yield of D-fructose.

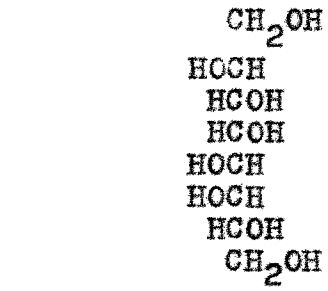
Dulcitol (XVI) is oxidized by neither A. xylinum according to Bertrand (9, 12, 20), Cozic (38), Hermann (53), Seifert (94), and Visser't Hooft (106) nor A. suboxydans

Table IV

Heptitols and Octitols and Their Oxidation Products

CH_2OH HCOH HOCH HOCH HCOH CH_2OH	CH_2OH HCOH HOCH HOCH HCOH CO CH_2OH	CH_2OH HOCH HOCH HOCH HCOH HCOH CH_2OH	CH_2OH HOCH HOCH HOCH HCOH CO CH_2OH
D-Perseitol	L-Perseulose	D-Volemitol	L-Sedoheptulose
XX	XXI	XXII	XXIII
CH_2OH HCOH HCOH HOCH HCOH HCOH CH_2OH	CH_2OH HCOH HCOH HOCH HCOH CO CH_2OH	CH_2OH HOCH HCOH HOCH HCOH HCOH CH_2OH	CH_2OH HOCH HCOH HOCH HCOH CO CH_2OH
D-α-Gluco-heptitol	L-Glucoheptulose	D-β-Gluco-heptitol	D-galaheptulose
XXIV	XXV	XXVI	XXVII
CH_2OH HCOH HCOH HOCH HOCH HCOH CH_2OH	CH_2OH HOCH HCOH HOCH HOCH HCOH CH_2OH	CH_2OH HCOH HCOH HCOH HOCH HCOH HCOH CH_2OH	CH_2OH HCOH HCOH HCOH HOCH HCOH CO CH_2OH
D-α-Galahep-titol	D-β-Galahep-titol	D-α,β-Gluco-octitol	L-altro-L-gluco-2-keto-octose
XXVIII	XIX	XXX	XXXI

Table IV (Continued)



D-α,α-Galaoctitol

XXXII

according to Dunning, Fulmer, and Underkofler (41) and Visser't Hooft (106).

The production of L-allulose (XVIII) in good yield (50-60 per cent) by the oxidation of allitol (XVII) with A. xylinum has been described by Steiger and Reichstein (96). The ketose was fully characterized and identified.

The remaining hexitol that has been tested for oxidizability with an Acetobacter is L-iditol (XIX). Bertrand (20) maintained this hexitol to be indifferent to the action of A. xylinum.

e. Oxidation of heptitols and octitols. The oxidation of naturally occurring perseitol (XX) (D-α-mannoheptitol) to perseulose (XXI) by A. xylinum was first achieved by Bertrand (21, 22). The ketose was easily obtained in crystalline form. This method of preparation of perseulose has been repeated by Cozic (38) and Visser't Hooft (106). The latter also duplicated the oxidation using A. suboxydans as have

more recently Hann, Tilden, and Hudson (52). Employing A. suboxydans in a medium of 3 per cent perseitol, 0.3 per cent potassium dihydrogen phosphate, 0.5 per cent yeast extract, and 0.5 per cent glucose Tilden (98) recovered 65 per cent crystalline perseulose after fermentation in a rotary drum over a period of seven days.

Bertrand (9, 12, 20) was also able to obtain the sirupy ketose, later identified as sedoheptulose (XXIII), by the action of A. xylinum on volemitol (XXII) (D-β-mannoheptitol).

A. xylinum in a medium of 0.5 per cent yeast extract and 3 per cent D-α-glucoheptitol (XXIV) generates over 6-8 weeks from 60-90 per cent crystalline ketose (XXV) according to Bertrand and Nitzberg (23, 24). Hann, Tilden, and Hudson (52) have repeated this oxidation utilizing A. suboxydans.

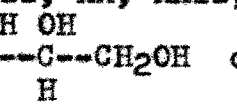
Cozic (38) isolated an unidentified ketose, presumably (XXVII), by subjecting D-β-glucoheptitol (XXVI) to the action of A. xylinum.

The remaining heptitols and octitols listed were exposed to A. suboxydans by Hann, Tilden, and Hudson (52). While D-α-galaheptitol (XXVIII), D-β-galaheptitol (XXIX), and D-α,α-galaoctitol (XXXII) were attacked to a negligible extent, D-α,β-glucooctitol (XXX) yielded an unidentified ketose rotating at -57°, probably (XXXI).

A reconsideration of the sugar alcohols listed above will emphasize the role of configuration relating to the susceptibility of this type of compound to the oxidative

action of both A. xylinum and A. suboxydans. As previously mentioned, such oxidative specificity is expounded in what are known as 'Bertrand's rule' for A. xylinum (9, 12, 20) and the Hann, Tilden, and Hudson modification thereof (52) for A. suboxydans.

For those sugar alcohols of four or more carbon atoms which have been subjected to the action of A. xylinum only those are highly susceptible to oxidation (I, VII, XII, XIV, XVII, XX, XXII, XXIV, XXVI) which contain the grouping



or the mirror image thereof. For A. suboxydans only the grouping shown is readily oxidized, while the mirror image is either not oxidized at all or, at best, at an almost negligible rate.

It should be noted here that the differentiation in the action of the two organisms (A. xylinum's attacking a compound in either a D- or L- series; A. suboxydans' restricting its activity to those compounds of a D- series) hinges on the report of Bertrand (9, 12, 20) concerning one compound, L-arabitol (VII). Bertrand's results with L-arabitol apparently have not been questioned; they certainly have not been substantiated. There is an interesting note on this item in a paper by Votoček, Valentin, and Ráo (110, p. 406):

> Nous avons d'ailleurs observé la passivité vis-à-vis de la bactérie du sorbose encore chez l'arabite (pentite), mais nous avons renoncé à l'étude des pentites aussitôt que l'un de nous a appris par M. G. Bertrand que l'arabite est etudiée sous ce rapport dans son laboratoire. . .

Speculation is tempting but suffice it to say that here is definite indication for the necessity of corroboration of the report of Bertrand concerning the positive action of A. xylinum towards L-arabitol.

2. Oxidation of desoxy sugar alcohols.

a. Oxidation of 1-desoxy sugar alcohols. The replacement of a primary hydroxyl grouping of a sugar alcohol by a methyl group yields a compound which has been identified variously as a methylitol, an ω-desoxy- or 1-desoxy alcohol. In keeping with the suggestion of Pigman and Goepp (80, p. 258) the designation of this type of compound as 1-desoxy alcohols will be adhered to. The variety of synonyms will be included at the introduction of each 1-desoxy alcohol into the discussion.

Sufficient data have been accumulated concerning the mode of attack on the 1-desoxy alcohols by A. xylinum and A. suboxydans to assure a lack of conformation to 'Bertrand's rule'. Contrarily, sufficient evidence of any regularity of action has not been obtained.

The first compound of this type tested as to oxidizability by A. xylinum was the 1-desoxy-L-galactitol (I) (rhodeitol, D-fucitol, D-galactomethylitol). Votoček and Bulíř (107) assigned the relative configuration on carbons four and five of this compound on the basis of the lack of oxidizing action, assuming the applicability of 'Bertrand's

Table V

1-Desoxy Sugar Alcohols and Their Oxidation Products

1-Desoxy-L-galactitol

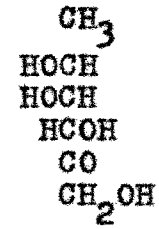
I

1-Desoxy-D-galactitol

II

1-Desoxy-D-mannitol

III

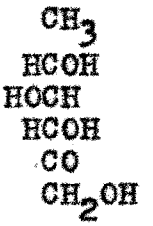


D-Fructomethylose

IV

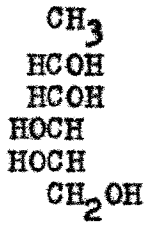
1-Desoxy-D-glucitol

V



L-Sorbomethylose

VI



1-Desoxy-L-mannitol

VII

1-Desoxy-D-gala-L-manno-heptitol

VIII

1-Desoxy-L-altro-L-manno-heptitol

IX

rule'. Such an assumption was unfounded. Later results reported by Hann, Tilden, and Hudson (52) indicated a distinct

oxidation of the enantiomorph of (I), the 1-desoxy-D-galactitol (II) (L-fucitol, L-galactomethylitol) by A. suboxydans. Here is a definite departure from 'Bertrand's rule'. The site of oxidation has not been established.

Votoček, Valentin, and Rác (110) were unable to show oxidation of 1-desoxy-L-mannitol (VII) (L-rhamnitol, L-mannomethylitol) by A. xylinum and therefore suggested that the oxidative susceptibility of compounds to A. xylinum depends upon the homologous series involved. It should be emphasized that the variance illustrated by this compound (VII) is concerned only with D- and L- series, not with the relative configuration at the potential site of attack. Such an observation was strictly fortuitous.

Recently Anderson and Lardy (1) have oxidized the 1-desoxy-D-mannitol (III) (D-rhamnitol, D-mannomethylitol) with A. suboxydans and isolated what is apparently the ketose (IV).

The oxidation of 1-desoxy-D-glucitol (V) (L-gulomethylitol) with A. xylinum was effected by Müller and Reichstein (76). The yield of ketose was poor but the compound was completely characterized and identified as L-sorbomethylose (VI).

The remaining 1-desoxy alcohols (VIII and IX) proved immune to the oxidative action of A. xylinum according to Votoček, Valentin, and Rác (110).

Again let it be emphasized that further experimentation is definitely indicated before it may be ascertained whether or not 'Bertrand's rule' for A. xylinum should be identified

with the Hann, Tilden, and Hudson modification for A. suboxydans.

b. Oxidation of 2-desoxy sugar alcohols. Regna (91) claimed that 2-desoxy-D-sorbitol, a by-product from the electroreduction of D-glucose, can be oxidized by A. suboxydans to a ketose identified as 5-desoxy-L-sorbose. Reduction of the ketose gave the sorbitol and iditol derivatives. Only the former alcohol responded to A. suboxydans oxidation, apparently in compliance with 'Bertrand's rule'.

3. Oxidation of cyclitols.

Posternak and Ravenna (89) noted that the cis-1,2-cyclohexanediol is easily oxidized by the Acetobacters while the trans compound is relatively inert.

Of the four stereoisomeric forms of 1,2,3-cyclohexanetriol (I, II, III, IV, Table VI), Posternak and Ravenna (89) were able to oxidize all but one form (IV) with A. xylinum and A. suboxydans. The one meso form which is oxidized (I) apparently is attacked on one of the outer hydroxyl groups. This site of oxidation is suggested by the isolation of an optically active triol (II or III) on sodium amalgam reduction of the ketose formed.

Both optically active triols (II and III) are attacked by A. suboxydans and A. xylinum, although the oxidation of one of these isomers proceeds much more rapidly. Presumably this

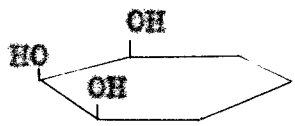
action is analogous to the preferential attack by the organisms upon compounds belonging to a D- series in the straight chain alcohols. Posternak and Ravenna (89) suggested that in order to become susceptible to the action of A. suboxydans cyclitols should possess vicinal hydroxyl groups which are cis in relation to each other.

The first keto-inositol or inosose (Table VII) was obtained by Posternak (84) who oxidized meso-inositol (VII) with nitric acid. Before Posternak submitted a proof of structure, Kluyver and Boezaardt (65) reported the production of an inosose, claimed identical with that of Posternak, by the oxidation of meso-inositol with A. suboxydans. Posternak conclusively demonstrated later (84, 85, 86) that the two methods of oxidation give entirely different inososes. By nitric acid oxidation the dl-epi-ms-inosose (IX + XI) is obtained while A. suboxydans oxidation of meso-inositol yields the scyllo-ms-inosose (VIII).

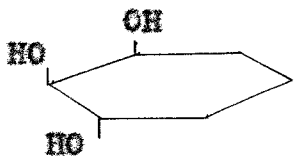
Kluyver and Boezaardt (65) obtained a low yield (18-25 per cent) of inosose and Dunning, Fulmer, and Underkofler (41) proved the inability of A. suboxydans to dissimilate (i.e., utilize as a growth substrate) the meso-inositol to any great extent. The latter investigators showed that supplying the organism with a small amount (0.05 per cent) of an available (or dissimilable) carbon source, such as sorbitol, allowed the responsible enzyme system to oxidize the meso-inositol to a much greater extent, with consequent increase in yield of

Table VI

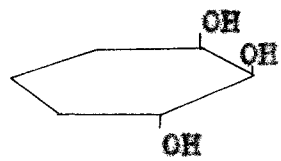
Schematic Representation of Some Cyclitols



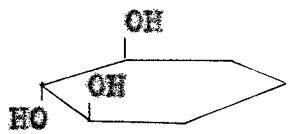
I



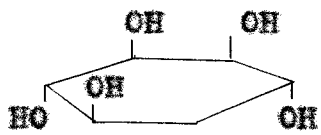
II



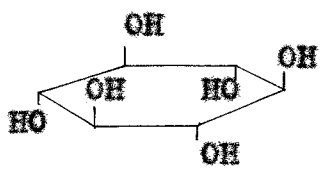
III



IV



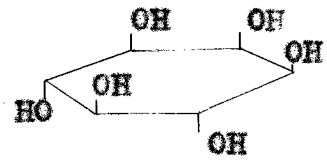
V



VI

Table VII

Schematic Representation of Some Inositols and Their Oxidation Products



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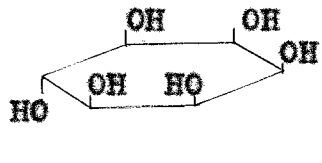

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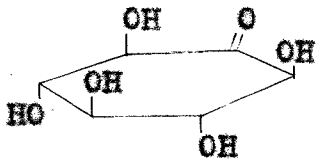

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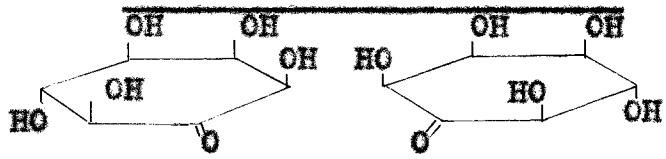

IX XI

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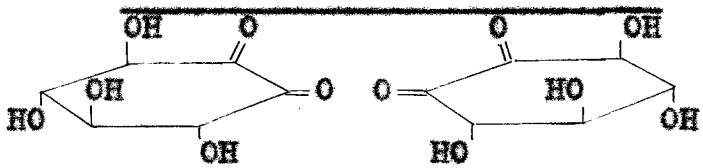

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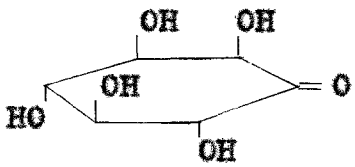

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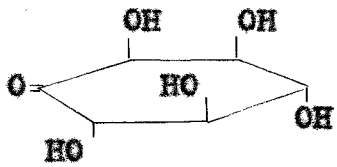

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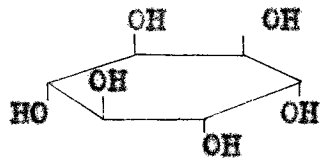

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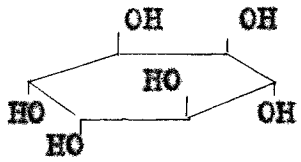


XIII

inosose (average of 50 g. inosose per 100 g. inositol).

It should be mentioned here that Dunning (39), Dunning, Fulmer, Guymon, and Underkofler (40), and Pitcher (82) have claimed the production of a substantial amount of an unidentified diketo-inositol by A. suboxydans oxidation of meso-inositol. A close repetition of the conditions used by Dunning, et alia by Carter, Belinskey, Clark, Flynn, Lytle, McCasland, and Robbins (34) gave predominantly the scyllo-ms-inosose (VIII).

By the oxidation of epi-inositol (X) with A. suboxydans Posternak (88) procured a new inosose, l-epi-ms-inosose (XI), the configuration of which he conclusively proved. Magasanik and Chargaff (69) substantiated these findings.

A rather thorough series of investigations concerning the oxidative action of A. suboxydans on the various inositols has been published by Chargaff and Magasanik (36, 68, 69, 70).

Using a modified Warburg technique, Magasanik and Chargaff (69) determined quantitatively the amount of oxygen a number of the inositols and inososes would consume in the presence of A. suboxydans. On the basis of these data they predicted the formation of corresponding inososes and diketo-inositols and finally isolated, characterized, and identified some of the reducing compounds.

Such experiments indicated only a monoketo compound can be derived from meso-inositol. The scyllo-ms-inosose of Kluyver and Boezaardt (65) was so identified. The same

indication was obtained with epi-inositol and the inosose was proved identical with the l-epi-ms-inosose of Posternak (88).

The combination of the fact that l-epi-ms-inosose (XI) consumes no oxygen in the presence of A. suboxydans and that the dl-epi-ms-inosose (IX + XI) obtained by Posternak's nitric acid oxidation of meso-inositol does take up a definite amount led to the conclusion that the d-epi-ms-inosose could be further oxidized, presumably to a diketo-inositol. The latter compound has yet to be isolated.

Both d- and l-inositol (XII and XIII) were shown to give diketo-inositols (XVI and XVII) although in both instances monoketo compounds (XIV and XV) could be isolated. The diketo-inositols were isolated as the bisphenylhydrazones and structure was established by identification of the dl-bisphenylhydrazone with that obtained by Carter, Clark, Flynn, Lytle, and Robbins (35) by oxidation of the phenyl-hydrazone of scyllo-ms-inosose (VIII) with phenylhydrazine.

Neither quebrachitol, the monomethyl ether of l-inositol, pinitol, the monomethyl ether of d-inositol, nor scyllitol (VI) were oxidized under the same conditions.

Magasanik and Chargaff (69) have proposed a unique method for showing the specificity of A. suboxydans toward the inositols. Assuming such compounds to exist in the chair form the resultant model will show six carbon constituents forming a belt around the molecule. Such atoms are designated as equatorial. Of the remaining six constituents, three will

be on top of the model, or north polar, and three on the under side, or south polar. Examination of models of the inositols oxidized shows that only those hydroxyl groups which are polar are attacked. The relationship to 'Bertrand's rule' is indicated by proposing that a polar group between two equatorial groups corresponds to a secondary hydroxyl situated between a primary and adjacent cis hydroxyl group.

4. Oxidation of aldoses to aldonic acids.

By far the most searching examination of Acetobacter oxidation of aldoses has been concerned with the conversion of D-glucose to D-gluconic acid.

A. J. Brown (27) employed the production of acid from D-glucose as a characterization of his A. xylinum. Bertrand (11, 16, 20) isolated the acid and identified it as D-gluconic acid. Bernhauer and Schön (6) obtained an 80 per cent yield of D-gluconic acid as the calcium salt by culturing A. xylinum on a medium consisting of 0.5 per cent yeast extract, 5 per cent D-glucose, and calcium carbonate. A yield of 58.6 per cent acid was obtained by Porges, Clark, and Gastrock (83) from the same medium. Upon aeration of such a medium for four days Porges, et alia, (83) reduced the yield of acid to 28.5 per cent. Aeration evidently causes further degradation of the acid in question. In fact, Banning (2) observed a rather prolific production of oxalic acid when a two per cent D-glucose solution was subjected to A. xylinum

over fourteen days.

With A. suboxydans such secondary oxidation is quite negligible. On aeration of a concentrated D-glucose solution (30-35 per cent) over a three day period in the presence of A. suboxydans Butlin and Wince (32) procured a 95 per cent yield of D-gluconic acid, isolated as the calcium salt.

A. xylinum also oxidizes D-galactose to the corresponding aldonic acid. Bertrand (11, 16, 20) made the original observation of this reaction. His work was corroborated by Visser't Hooft (106) and Hermann and Neuschul (54, 56).

Although Cozic (38) was unable to show any oxygen uptake by A. xylinum in the presence of L-arabinose on the Warburg apparatus, it would appear quite evident that A. xylinum is able to oxidize this aldose. Bertrand (11, 16, 20) identified L-arabonic acid produced under such conditions. Visser't Hooft (106) reproduced these findings and Hermann and Neuschul (54) isolated L-arabonic acid in 46 per cent yield after allowing A. xylinum to act upon a solution containing two per cent L-arabinose and 0.5 per cent yeast extract for three and one half months. It is strange that Cozic (38) was unable to show positive results with her Warburg experiments when one also considers the fact that Banning (2) disclosed that A. xylinum could produce a substantial amount of oxalic acid from L-arabinose. Further discrepancies of the same sort will be noted in future sections.

Visser't Hooft (106) also obtained L-arabonic acid from L-arabinose using A. suboxydans.

It has also been demonstrated by Bertrand (11, 15, 16, 20), Cozic (38), and Visser't Hooft (106) that either A. xylinum or A. suboxydans is capable of oxidizing D-xylose to D-xylonic acid.

In media containing D-xylose or L-arabinose Fred, Peterson, and Anderson (44) obtained singular results with A. xylinum. With both aldoses they isolated substantial amounts of carbon dioxide, ethanol, and acetone, the percentage of ethanol and acetone increasing with the age of the culture employed. The experiments were repeated many times but no other investigators have recorded similar results.

As measured on the Warburg apparatus by Cozic (38), D-mannose in the presence of A. xylinum causes a substantial uptake of oxygen. Presumably D-mannonic acid is formed.

Data on L-rhamnose are inconclusive. With A. xylinum Banning (2) found oxidation of the aldose to proceed to such an extent that an appreciable amount of oxalic acid could be isolated. Visser't Hooft (106) showed acid production from L-rhamnose by the action of A. suboxydans but attempted no isolation of product. However, Dunning, Fulmer, and Underkofler (41) failed to produce oxidation of this compound in the presence of varying amounts of sorbitol with A. suboxydans.

Three derivatives of aldoses whose stereochemical configurations might lead one to predict oxidation by either

Acetobacter according to 'Bertrand's rule', have been reported unattacked by such organisms. Hann, Tilden, and Hudson (52) failed to obtain any reducing compound by growing A. suboxydans in the presence of D-mannose diethyl mercaptal. Identical negative results have been published by Iselin (58) concerning D-glucose diethyl mercaptal and D-glucose dimethyl acetal. The latter compounds do not inhibit the organism's enzyme system. This fact was ascertained by Iselin (58) by demonstrating that A. suboxydans can oxidize meso-inositol in the presence of either the D-glucose diethyl mercaptal or dimethyl acetal. It has become fairly obvious that Acetobacter action is defined by the substrate involved and that no rule is universally applicable.

5. Oxidation of aldonic acids.

The fact that the oxidation of D-glucose by either A. xylinum or A. suboxydans proceeds beyond the formation of D-gluconic acid to yield the 5-ketogluconic acid has long been recognized. Bertrand (20), using A. xylinum, was able to isolate the crystalline calcium salt of this keto-acid. With the same organism Bernhauer and Schön (6) produced up to 65 per cent of the 5-keto acid from a slightly alkaline five per cent solution of D-glucose containing calcium carbonate.

It is interesting to note regarding the transformation of the aldose to the 5-keto acid that the aldose is preferentially oxidized to the acid. Further oxidation of the aldonic acid

to the 5-keto acid apparently occurs only when no more aldose is available to the organism. Kluyver and Boezaardt (64) observed this phenomenon with A. suboxydans by measuring the oxygen uptake on the Warburg apparatus and correlating with it the presence or absence of reducing compounds.

While at first it was assumed that the production of this 5-keto acid indicated the probability of aldonic acids' conforming to the rule of Bertrand, such is not the case. Fortunate for isolation and misleading as to the specificity of attack the calcium 5-ketogluconate precipitates quite completely from a medium containing D-glucose and calcium carbonate or a calcium gluconate medium. Hermann and Neuschul (54) found a substantial amount of what they defined as the calcium 5-ketogluconate in solution after oxidizing a solution containing five per cent D-glucose and two per cent calcium carbonate with A. xylinum. That this soluble salt is predominantly calcium 2-ketogluconate has been indicated by Bernhauer and Knobloch (4). By adjusting conditions to the use of either calcium or potassium gluconate the latter were able to isolate, in a yield of 75 per cent, the potassium 2-ketogluconate.

Once more it is quite evident that the stereochemistry of the molecules attacked is not sufficient to define the specificity of the organisms under consideration.

Of the few other aldonic acids investigated Votoček, Valentin, and Rác (110) reported that the L-rhamnonic acid is

not attacked by A. xylinum nor are the D-α,α-galaoctonic and D-_-galaheptonic acids oxidized by A. suboxydans according to Hann, Tilden, and Hudson (52).

6. Oxidation of ketoses.

Investigations concerning the susceptibility of the ketoses to the action of either A. xylinum or A. suboxydans have been concerned only with noting the presence or absence of growth of the organisms on media containing such compounds.

Fructose has been shown by Banning (2), Visser't Hooft (106), Hermann and Neuschul (54), and Cozic (38) to promote good growth of A. xylinum. Hermann and Neuschul (54) could isolate no definite oxidation products but Banning (2) obtained a definite amount of oxalic acid after subjecting fructose to A. xylinum.

Cozic (38) and Visser't Hooft (106) have demonstrated a good growth of A. xylinum on a medium containing dihydroxyacetone. A. xylinum also grows well on media containing erythrulose or sorbose according to Visser't Hooft (106). On substrates with any one of the four mentioned ketoses (fructose, dihydroxyacetone, erythrulose, sorbose) present, Visser't Hooft (106) reported a negligible growth of A. suboxydans. These data are a further indication of the greater oxidizing intensity of A. xylinum compared to A. suboxydans.

B. Oxidation of Other Organic Compounds

1. Oxidation of aliphatic monohydroxy alcohols.

a. Oxidation of primary alcohols to acids. Cozic (38) reported the absence of oxygen uptake on the Warburg apparatus by A. xylinum in the presence of methyl alcohol. Visser't Hooft (106) obtained a very small amount of formic acid when he cultivated A. suboxydans on a medium containing two per cent methyl alcohol.

As a characteristic of A. xylinum Brown (27) noted the complete oxidation of ethyl alcohol through acetic acid to carbon dioxide and water. Such action has been substantiated by Bertrand (8, 20) and Cozic (38).

On a medium containing two per cent ethyl alcohol A. suboxydans gives quantitative conversion to acetic acid according to Visser't Hooft (106).

n-Propyl alcohol yields 42 per cent and 75 per cent propionic acid when oxidized, respectively, by A. xylinum and A. suboxydans. These data are recorded by Hermann and Neuschul (54) for A. xylinum and Visser't Hooft (106) for A. suboxydans.

Visser't Hooft (106) isolated the corresponding butyric acid in 60 per cent yield after the oxidation of n-butyl alcohol with A. suboxydans. While Cozic (38) could show no appreciable oxygen uptake by A. xylinum in the presence of

n-butyl alcohol, Banning (2) recorded the production of good growth by the organism under the same conditions.

By the action of A. suboxydans on isobutyl alcohol Visser't Hooft (106) was able to isolate up to 55 per cent isobutyric acid.

Amyl alcohol does not support the growth of A. xylinum according to Banning (2) and Cozic (38), nor of A. suboxydans according to Visser't Hooft (106).

b. Oxidation of secondary alcohols to ketones. Over a period of 1-3 weeks either A. xylinum or A. suboxydans form 'remarkable quantities' of acetone (identified as the p-nitrophenylhydrazone) from a two per cent solution of isopropyl alcohol. The only recorded evidence of such a transformation is that of Visser't Hooft (106).

c. Oxidation of tertiary alcohols. Visser't Hooft (106) reported the failure of A. suboxydans to utilize tert.-butyl alcohol.

2. Oxidation of glycols.

a. Oxidation of ethylene glycol. The evidence concerning the oxidation of ethylene glycol by either organism in question is quite contradictory. The early work of Bertrand (9, 12, 20) with A. xylinum indicated an absence of oxidation. Cozic (38) could not show any appreciable uptake of oxygen in the Warburg apparatus with the same organism in the presence of the glycol. However, Banning (2) claimed

good growth of A. xylinum in the presence of this compound. More indicative of positive action by both A. xylinum and A. suboxydans upon ethylene glycol are data given by Visser't Hooft (106). Over a period of three weeks both organisms produced substantial quantities of an acid which was recovered in 25 per cent yield and identified as the calcium salt of glycollic acid. Visser't Hooft also detected the presence of a volatile aldehyde, possibly glycolaldehyde. The isolation of such an aldehyde would be quite suggestive of the path of oxidation.

b. Oxidation of propylene glycol to acetol. When Kling (61, 62) first produced acetol by the oxidation of DL-propylene glycol with A. xylinum he obtained a very poor yield (10 per cent). After the fermentation his examination showed the residue consisted of some dextrorotary glycol and unoxidized DL-glycol. The low yields and positive rotation of residual glycol led Kling to state that A. xylinum prefers the laevorotary form and, consequently, the maximum yield would be 50 per cent.

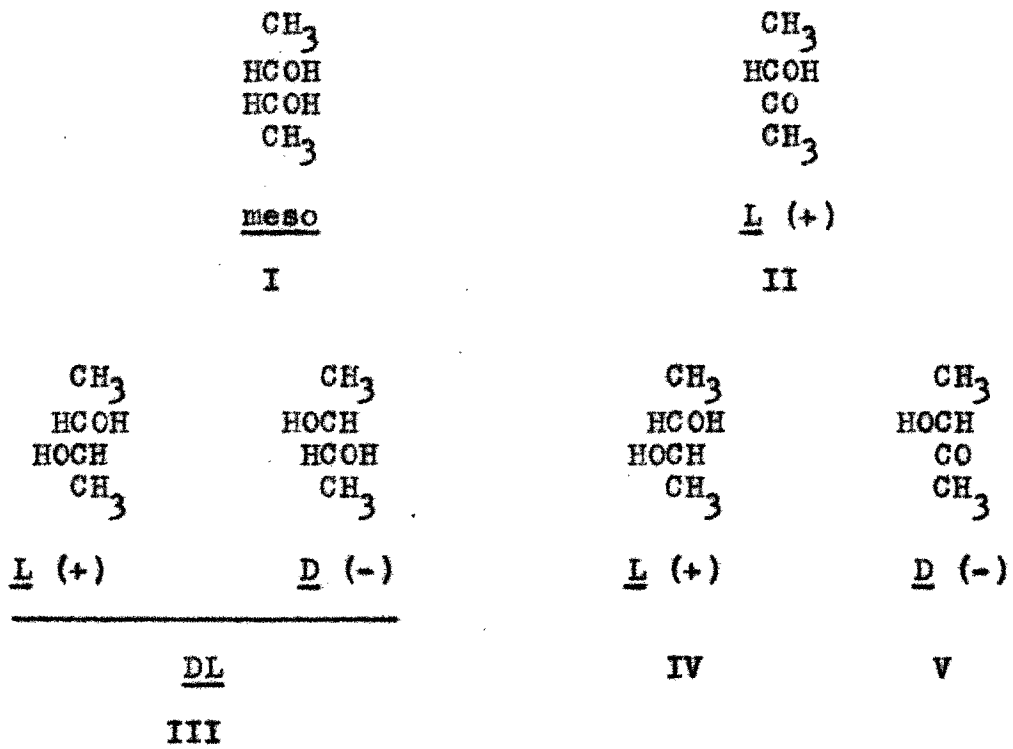
Eventually Visser't Hooft (106) proved such a conclusion erroneous. By oxidizing the DL-propylene glycol with A. xylinum and A. suboxydans Visser't Hooft obtained yields of acetol amounting to 66.0 per cent and 69.5 per cent, respectively.

Corroboration of the non-specific action of A. suboxydans on the DL-glycol was later given by Butlin and Wince (33).

Cultured on a medium consisting of 0.5 per cent yeast extract, 0.5 per cent potassium dihydrogen phosphate, 0.5 per cent glycerol or glucose, and 5-10 per cent glycol with intense aeration A. suboxydans produced over a period of three days a quantitative yield of acetol.

Table VIII

2,3-Butylene Glycols and Their Oxidation Products



c. Oxidation of 2,3-butylene glycol to acetylmethylcarbinol. A rather comprehensive evaluation of the susceptibility of both the racemic and meso diols to A. xylinum was reported by Grivsky (50). The oxidation of the meso glycol

(I) was found to yield L-(+)-acetylmethylcarbinol (II) quantitatively over 75 days. In approximately the same period of time the racemic glycol (III) was about 50 per cent oxidized. With extension of time to more than three months further oxidation occurred. In the case of the DL- form the acetylmethylcarbinol formed was identified as the D-(-) isomer (V). The residual substances were unoxidized DL- and L-(+) diol (IV).

Grivsky's results indicated A. xylinum will oxidize the 2,3-butylene glycols in a preferential manner. The meso glycol (I) is attacked on that carbon the hydroxyl group of which maintains a D- relationship to its adjacent terminal methyl group. Of the racemic glycol (III) the D- component is primarily oxidized, although with this organism (A. xylinum) the L- isomer is also attacked to a certain extent.

A. suboxydans restricts its activity towards these diols even more, the L-diol being inviolable. Underkofler, Fulmer, Bantz, and Kooi (100) and Fulmer, Underkofler, and Bantz (48) have conclusively demonstrated this specificity. They have shown that this bacterium promotes yields of 95 per cent of the corresponding acetylmethylcarbinols (II and V) from the meso and D-(-) glycols. The L-(+) glycol under the same conditions was oxidized to a very slight extent, such oxidation probably being due to the presence of meso contaminant in the L-(+) glycol used.

d. Oxidation of 3,4-hexanediols. H. Van Risseghem (102) has proved that A. xylinum attacks the meso and racemic forms of this glycol in a manner quite comparable to that shown by Grivsky (50) for the 2,3-butylene glycols.

e. Oxidation of other glycols. Kling (63) was unsuccessful in producing oxidation of $CH_3CH_2CHOHCH_2OH$ and $C_6H_5CHOHCH_2OH$ with A. xylinum.

3. Oxidation of acids.

a. Aliphatic monobasic acids. Banning (2) reported good growth of A. xylinum in a medium containing one per cent sodium formate; growth of A. suboxydans on an agar plate in the presence of calcium formate is negligible, according to Visser't Hooft (106).

That acetic acid is completely converted to carbon dioxide and water by A. xylinum has been established by Brown (27) and Hermann and Neuschul (55). According to Visser't Hooft (106) A. suboxydans grows very poorly in liquid medium containing calcium acetate.

Propionic acid, in the form of its sodium salt, promotes very little growth of A. xylinum according to Banning (2). A similar effect was noted by Visser't Hooft (106) for A. suboxydans in the presence of calcium propionate. The same results have been recorded by the same investigators for butyric acid.

While calcium isobutyrate will not support the growth of

A. suboxydans, according to Visser't Hooft (106), Banning (2) recorded good growth of A. xylinum in a 0.5 per cent solution of the sodium salt of this acid.

Again according to Banning (2) a 0.5 per cent solution of potassium valerate supports good growth of A. xylinum.

b. Aliphatic polybasic acids. Banning (2) reported good growth of A. xylinum in the presence of sodium succinate and no growth of the same organism in the presence of malonic acid.

As listed by Visser't Hooft (106) the following salts provoke good growth of A. suboxydans on agar plates: ammonium oxalate, the calcium salts of malonic, fumaric, glutaric, and aconitic acids. A. suboxydans grows poorly in the presence of calcium succinate.

c. Keto- and hydroxy-acids. Both A. xylinum and A. suboxydans grow very poorly if at all in media containing glycollic acid according to Banning (2) and Visser't Hooft (106), respectively.

The oxidation of lactic acid by both organisms has been more thoroughly investigated with regard to isolation of products. A. xylinum grows very well on a medium containing sodium DL-lactate. Banning (2) was able to isolate no oxalic acid from such a medium. However, Hermann and Neuschul (55), after exposing a medium containing 2.5 per cent of the same salt to A. xylinum over a period of two months, were able to isolate and identify small amounts of laevorotary

acetylmethylcarbinol (0.102 per cent), acetic acid (0.18 per cent), and carbon dioxide (0.38 per cent). While Visser't Hooft (106) indicated a lack of growth of A. xylinum on a medium containing the free acid, Cozic (37) obtained 8-20 per cent of pyruvic acid, identified as the dinitrophenylhydrazone, under the same conditions. After cultivating A. xylinum in a 2.5 per cent solution of calcium lactate Visser't Hooft (106) was able to isolate calcium acetate in 32 per cent yield.

A. suboxydans grows well in media containing either DL-lactic acid or the calcium salt of the acid. Visser't Hooft (106) isolated 60 per cent acetic acid and 76 per cent calcium acetate from the respective media.

According to Visser't Hooft (106) the calcium salts of α- and β-hydroxybutyric acids promote only weak growth of A. suboxydans.

Banning (2) reported that sodium malate supports growth of A. xylinum and Visser't Hooft (106) reported that calcium malate supports the growth of A. suboxydans. No products were identified.

Using the same salts of citric acid for the organisms concerned the same investigators found insignificant growth.

The calcium salt of levulinic acid promotes good growth of A. suboxydans, according to Visser't Hooft (106).

A. xylinum on a medium containing two per cent of α-ketoglutaric acid and five per cent calcium carbonate over three days produces identifiable succinic acid in 15 per cent

yield according to Iwatsuru (60).

d. Polyhydroxy acids. Banning (2) reported but little growth of A. xylinum in the presence of sodium tartrate.

A. suboxydans attacks calcium glycerate, according to Visser't Hooft (106), but no products were identified.

Visser't Hooft (106) reported that the calcium salts of D-tartaric, L-tartaric, mucic, and saccharic acids all support the growth of A. suboxydans.

e. Amino acids. Banning (2) reported that A. xylinum grows only slightly in solutions containing either glycine or leucine.

Miyaji (73) has claimed a slight conversion of D-glutamic acid to succinic acid by A. xylinum.

III. METHODS

A. General Methods of Preparing 1-Desoxy Sugar Alcohols

For the preparation of the 1-desoxy sugar alcohols one has recourse to a number of methods. Perhaps most suitable for obtaining small amounts (2-3 grams) of the desired compounds is the hydrogenolysis of mercaptal acetates with Raney nickel after the procedure of Wolfrom and Karabinos (118). The classical desoxydation of the terminal primary hydroxyl group of aldoses as exemplified by the work of Hann, Ness, and Hudson (51) can be quite tedious. Reduction of ω-desoxy-aldoses is evidently a rapid method for the preparation of the 1-desoxy alcohols. However, the rarity of the essential aldoses limits the usefulness of such a method.

Recently Wolfrom and Brown (117) have developed the synthesis of 1-desoxy-ketoses by the addition of diazomethane to aldonic acid chlorides or their esters followed by treatment with hydriodic acid. Similar results have been obtained by Wolfrom, Weisblat, Zophy, and Waisbrot (121) using acyclic sugar esters. Subsequent reduction of the ketoses will furnish two diastereoisomeric 1-desoxy alcohols.

Various Grignard reagents have been shown by Gätzi and Reichstein (49) and by English and Griswold (42) to add to acyclic sugar derivatives. By the addition of methyl

magnesium iodide to 2,3:4,5-diacetone-aldehydo-D-arabinose Gätzi and Reichstein (49) obtained in 78 per cent yield an easily separable mixture of the 1-desoxy-D-glucitol and 1-desoxy-D-mannitol derivatives. English and Griswold (42) used an identical procedure with other Grignard reagents but made no attempt to identify the products as to their configuration.

Because of the availability of certain aldoses the procedure of Wolfrom and Karabinos (118) involving the hydrogenolysis of corresponding mercaptal acetates was frequently employed during this investigation. However, subsequent developments indicated that perhaps the preferable method for obtaining 1-desoxy alcohols is the addition of methyl magnesium iodide to an aldehydo acetate. The yields reported herein for such a reaction are not satisfactory enough to warrant its use preferentially. However, developmental work is indicated before discarding it as a potentially general reaction for the preparation of the 1-desoxy compounds.

B. Microbiological Procedures

The culture of Acetobacter suboxydans, listed as No. 621, was secured from the American Type Culture Collection. The stock cultures were carried by subculturing in a 0.5 per cent yeast extract--5 per cent sorbitol medium using 10 ml. of medium in each 50 ml. Erlenmeyer flask. Before inoculation

into media containing material to be tested the bacteria were activated by transferring every twenty-four hours for three days on the same medium. All media were sterilized in the autoclave at fifteen pounds steam pressure for fifteen minutes. In all cases cultures were incubated at the optimum temperature of 28° C.

The compounds to be tested for action by A. suboxydans were usually employed in concentrations of 100 mg. per 100 ml. of medium. Ten ml. of medium in each 50 ml. Erlenmeyer flask were used in all cases and all media, except where otherwise noted, contained 0.5 per cent yeast extract.

For inoculation into flasks containing compounds to be tested the cells of an active (twenty-four hour) culture of the organism were centrifuged, washed twice with sterile isotonic salt solution, and finally suspended in 10 ml. of sterile saline solution. The inoculum for each flask containing 10 ml. of medium consisted of 1 ml. of the latter suspension.

After periods of incubation at 28° C. of five and ten days 1 ml. samples were removed from the flasks and the presence of reducing substances was tested for by the Underkofler, Guymon, Rayman, and Fulmer modification (101) of the Shaffer-Somogyi sugar titration method (95). In the case of ten day analyses all test media were made up to 10 ml. to replace loss by evaporation before removing sub-samples for testing.

When the presence of a reducing compound was indicated by analysis, a positive oxidative action towards the compound originally in the medium was tentatively assigned to A. suboxydans. A more conclusive proof of such action would be the isolation and identification of such reducing compounds. With such a consideration in mind these experiments must be designated as preliminary in scope for the availability of the compounds tested made their quantities so small that no attempt was made at any isolation of products.

IV. EXPERIMENTAL RESULTS

A. Preparation of 1-Desoxy Sugar Alcohols

1. By the hydrogenolysis of mercaptal acetates with Raney nickel.

a. Preparation of 1-desoxy-L-arabitol (L-lyxomethylitol). To a solution of 12 g. of L-arabinose diethyl mercaptal tetraacetate, prepared according to Wolfrom and Newlin (119), in 500 ml. of 70% ethanol were added 150 g. of Raney nickel, prepared after the procedure of Pavlic and Adkins (79). The mixture was refluxed for six hours. After the removal of heat the nickel was allowed to settle and then the supernatant liquor was decanted. The nickel residue was refluxed with five successive 150 ml. portions of absolute ethanol and the combined extracts were concentrated to dryness under vacuum at the water pump. The residue was boiled with 20 ml. of absolute ethanol, filtered hot, and the filtrate allowed to cool. The crystalline product which readily formed was recrystallized from absolute ethanol to a constant melting point of 115° C. and $[\alpha]_D^{30}$ -26.37 ± 2.00° ($CHCl_3$) (1, 2; c, 1.29). The yield of the 1-desoxy-L-arabitol tetraacetate was 4.3 g. (50%).

Anal. Calc'd. for $C_{13}H_{20}O_8$: C, 51.31; H, 6.58.

Found : C, 51.37; H, 6.57.

A solution of 1 g. of 1-desoxy-L-arabitol tetraacetate in 15 ml. of absolute methanol was refluxed for 15 minutes in the presence of 0.01 ml. of 0.5N barium methylate. After cooling, anhydrous ether was added dropwise to the clear solution until crystals started to form on the sides of the reaction flask. Refrigeration for 24 hours gave a product melting at 129-131° C. Repeated recrystallization from absolute methanol-absolute ether mixture gave no change in melting point. The 1-desoxy-L-arabitol so obtained had a specific rotation $[\alpha]_D^{30}$ -1.46 ±2.00° (H_2O) (1, 2; c, 1.02). The yield of the pure compound was 0.42 g. (94%).

Anal. Calc'd. for $C_5H_{12}O_4$: C, 44.12; H, 8.82.

Found : C, 44.02; H, 8.92.

b. Preparation of 1-desoxy-D-arabitol (D-lyxomethylitol). In a manner similar to that given above for the enantiomorph, 2.35 g. (27%) of the 1-desoxy-D-arabitol tetraacetate were obtained from 12 g. of D-arabinose diethyl mercaptal tetraacetate. The latter compound was prepared according to the method of Wolfrom, Weisblat, Zophy, and Waisbrot (121). The physical constants were m. p. 115-116° C.; $[\alpha]_D^{30}$ +27.30 ±2.00° ($CHCl_3$) (1, 2; c, 1.00).

Anal. Calc'd. for $C_{13}H_{20}O_8$: C, 51.31; H, 6.58.

Found : C, 51.59; H, 6.77.

Hydrolysis of the tetraacetate (1.5 g.) was effected by refluxing an absolute methanol solution (15 ml.) in the presence of 0.01 ml. of 0.5N barium methylate for 15 minutes. Addition of anhydrous ether to the cooled reaction mixture gave 0.60 g. (90%) of the 1-desoxy-D-arabitol of m.p. 131-132° C. and $[\alpha]_D^{29}$ +2.46 ± 2.00° (H_2O) (1, 2: c, 1.01). Further recrystallizations from absolute methanol-absolute ether mixture did not change the above constants.

Anal. Calc'd. for $C_5H_{12}O_4$: C, 44.12; H, 8.82.

Found : C, 44.25; H, 9.21.

c. Preparation of 1-desoxy-D-galactitol (L-fucitol). By the same general procedure 1-desoxy-D-galactitol was prepared from the D-galactose diethyl mercaptal pentaacetate of Wolfrom (116) in an overall yield of 41% (1.36 g. of desoxy alcohol from 10 g. mercaptal acetate). The physical constants of m.p. 155-156° C. and $[\alpha]_D^{30}$ +3.00 ± 2.00° (sat'd. borax soln.) (1, 2; c, 1.00) compared favorably with those recorded for L-fucitol by Votoček and Potměšil (109). These are m.p. 153-154° C. and $[\alpha]_D^{20}$ +4.7 (borax) (c, 3).

d. Preparation of 1-desoxy-D-glucitol (L-gulomethylitol). In the same manner 12 g. of D-glucose diethyl mercaptal pentaacetate, prepared according to Wolfrom (115), yielded 1.32 g. (33%) 1-desoxy-D-glucitol of m.p. 134° C. and $[\alpha]_D^{28}$ +2.10 ±2.00° (H_2O) (1, 2; c, 1.00). The recorded constants for this compound are given by Müller and Reichstein (75) as

m.p. 131-132° C. and $[\alpha]_D^{20}$ +3.95 ± 1.5° (H_2O).

e. Preparation of 1-desoxy-D-mannitol (D-mannomethylitol, D-rhamnitol). The 1-desoxy-D-mannitol was obtained from D-mannose diethyl mercaptal pentaacetate, prepared according to Pirie (81), by an identical method in 36% yield (0.60 g. desoxy alcohol from 5 g. mercaptal acetate). The product melted at 120-121° C. and had an $[\alpha]_D^{28}$ -10.0 ± 2.00° (H_2O) (l, 2; c, 1.00). A m.p. of 123° C. and an $[\alpha]_D$ -12.4 (H_2O) have been reported for this compound by Votoček, Valentin, and Rác (110).

2. By the addition of methyl magnesium iodide to aldehydo compounds.

a. Preparation of 1-desoxy-L-glucitol (D-gulomethylitol) and 1-desoxy-L-mannitol (L-mannomethylitol, L-rhamnitol). After the procedure of English and Griswold (42), 5 g. of 2,3:4,5-diacetone-aldehydo-L-arabinose were prepared from 17 g. of 2,3:4,5-diacetone-L-arabinose diethyl mercaptal (29% yield).

A solution of 5 g. (0.02 mole) of 2,3:4,5-diacetone-aldehydo-L-arabinose in 50 ml. of anhydrous ether was added dropwise during ten minutes to an excess (0.04 mole) of methyl magnesium iodide (prepared from 0.96 g. atoms of magnesium and 5.96 g. or 0.042 mole of methyl iodide) in 150 ml. of anhydrous ether. After refluxing the solution for half an hour, the complex was hydrolyzed by pouring into 300 ml. of an ice cold saturated ammonium chloride solution. The ethereal layer

was separated and the aqueous layer extracted five times with 75 ml. portions of ether. After drying the combined extracts over anhydrous sodium sulfate and filtering, the solvent was removed by evaporation and 4.2 g. (78.5%) of light yellow sirup resulted. The crude sirup was dissolved in 10 ml. of Skelly A and refrigerated for one month. No crystalline product was obtained in this manner. The Skelly A solution was then immersed in a dry ice-acetone bath and, with the aid of scratching with a glass rod, crystals were obtained. This product was filtered immediately after removal from the freezing mixture and washed with equally cool Skelly A. At this point the solid product was easily crystallizable at room temperature from Skelly A. The melting point remained constant at 62-64° C.; $[\alpha]_D^{28}$ 0 ± 2.00° (MeOH) (1, 2; c, 1.00). Gätzi and Reichstein (49) give for the 3,4:5,6-diacetone-1-desoxy-D-mannitol, the enantiomorph of the product obtained above, a m.p. 66.5-67° C. and $[\alpha]_D^{19}$ +1.0 ± 1.5° (MeOH) (c, 1.4). The yield of 3,4:5,6-diacetone-1-desoxy-L-mannitol was 1.5 g. (28%).

Anal. Calc'd. for $C_{12}H_{22}O_5$: C, 58.54; H, 8.94.

Found : C, 58.68; H, 8.95.

From the mother liquor of the mannitol derivative was obtained 2.1 g. (39%) of a light yellow sirup of $[\alpha]_D^{28}$ -1.00 ± 2.00° (MeOH) (1,2; c, 1.00). This compound was considered as the 3,4:5,6-diacetone-1-desoxy-L-glucitol. Gätzi and

Reichstein (49) give an $[\alpha]_D^{19}$ +3.0 ± 2.00° (MeOH) (c, 0.68) for the enantiomorph.

Anal. Calc'd. for $C_{12}H_{22}O_5$: C, 58.54; H, 8.94.

Found : C, 58.56; H, 8.97.

The 1-desoxy-L-mannitol (L-rhamnitol, L-mannomethylitol) was obtained in 74% yield (0.5 g.) by warming 1 g. of the diacetone derivative in 15 ml. of 10% acetic acid at 100° C. for four hours. The solvent was removed in vacuo, the sirupy residue dissolved in absolute ethanol, and acetone added to incipient turbidity. After a few hours the 1-desoxy-L-mannitol crystallized. The product melted at 120-121° C. and had a specific rotation of $[\alpha]_D^{28}$ +9.50 ± 2.00° Z(H_2O) (1, 2; c, 1.00). Such constants were in agreement with a m.p. of 123° C. and $[\alpha]_D^{20}$ +12.4 (H_2O) given by Fischer and Piloty (43) for the same compound.

Hydrolysis of the diastereoisomeric 1-desoxy-L-glucitol derivative (1.2 g.) in the same manner gave 0.54 g. (62.5%) of the 1-desoxy-L-glucitol of m.p. 130-132° C. and $[\alpha]_D^{28}$ -2.30 ± 2.00° (H_2O) (1, 2; c, 1.01). The constants recorded by Müller and Reichstein (75) for the enantiomorph are m.p. 131-132° C. and $[\alpha]_D^{20}$ +3.95 ± 1.5° (H_2O).

Anal. Calc'd. for $C_6H_{14}O_5$: C, 43.37; H, 8.43.

Found : C, 43.52; H, 8.49.

b. Preparation of 1-desoxy-D-iditol (D-idomethylitol) and 1-desoxy-D-gulitol (L-glucomethylitol, L-epirhamnitol, L-isorhamnitol). The aldehydo-D-xylose tetraacetate used in this reaction was prepared according to the directions of Wolfrom, Olin, and Evans (120) in 74.5% yield (10 g. from 15 g. of D-xylose diethyl mercaptal tetraacetate).

A solution of 10 g. (0.031 mole) of aldehydo-D-xylose tetraacetate in 50 ml. of anhydrous benzene was added with stirring in a nitrogen atmosphere to 250 ml. of an ethereal solution of excess (0.372 mole) of methyl magnesium iodide (prepared from 9.0 g. atoms of magnesium and 54 g. or 0.38 mole of methyl iodide) over 10 minutes. After completing the addition the mixture was refluxed for half an hour. The complex was hydrolyzed by pouring into an ice cold 5% sulfuric acid solution of 500 ml. volume. After removal of ether and benzene in an air stream, the iodide ion was precipitated by the addition of silver carbonate. Filtration was followed by treatment with hydrogen sulfide to remove excess silver ions. Following aeration to remove excess hydrogen sulfide an excess of barium hydroxide was added to the heated solution and the mixture was gently boiled for an hour. The magnesium hydroxide and barium sulfate were then centrifuged off and excess of barium ions removed from the supernatant by exactly neutralizing with dilute sulfuric acid. Centrifugation of the barium sulfate was followed by concentration of the filtrate to dryness in vacuo to give 5 g. (96%) of crude sirupy product.

This sirup was dissolved in 150 ml. of dry pyridine, 75 ml. of acetic anhydride added, and the solution allowed to stand at room temperature for three days. After removal of solvents in vacuo the residual sirup was dissolved in 60 ml. of ether and extracted five times with 20 ml. portions of 10% HCl, saturated potassium carbonate, and water. The ethereal layer was then dried over anhydrous sodium sulfate. After filtration, evaporation of ether gave 1.9 g. (16.1%) of a sirupy mixture of acetates. The sirup was dissolved in Skelly A and the solution cooled in a dry ice-acetone bath. The resultant gummy crystals were filtered rapidly, washed with cold Skelly A, and recrystallized several times from Skelly A at room temperature. The crystalline 1-desoxy-D-iditol pentaacetate thus obtained amounted to 1.1 g. (9.2%), melted at 100° C. and had a specific rotation, $[\alpha]_D^{28}$ +10.5 ± 2.0° ($CHCl_3$) (1,2; c, 1.00). The constants given by Meyer and Reichstein (72) for the enantiomorphic compound are m.p. 102-103°; $[\alpha]_D^{19}$ -13.1 ± 1° ($CHCl_3$).

Anal. Calc'd. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.43.

Found : C, 51.18; H, 6.38.

The 1-desoxy-D-iditol pentaacetate (800 mg.) was hydrolyzed by adding to a methanolic solution of the compound 1 ml. of 0.5N barium methylate and allowing the solution to stand in the refrigerator for 24 hours. The barium was removed by the addition of anhydrous ether, filtering, evaporating to dryness, dissolving the residue in absolute

methanol, and repeating the process until 200 mg. (56.5%) of a clear, light yellow sirup were obtained. $[\alpha]_D^{28}$ +1.43 ± 2.00° (H_2O) (l, 2; c, 1.00). For the enantiomorph Meyer and Reichstein (72) reported a sirup of $[\alpha]_D^{17}$ -2.6 ± 0.5° (H_2O).

Anal. Calc'd. for $C_6H_{14}O_5$: C, 43.37; H, 8.43.

Found : C, 43.49; H, 8.50.

The sirupy mother liquors from the 1-desoxy-D-iditol pentaacetate (625 mg.) were hydrolyzed in a similar manner. The 1-desoxy-D-gulitol (200 mg., 79%) obtained as a sirup had a specific rotation $[\alpha]_D^{28}$ +7.21 ± 2.00° (H_2O) (l, 2; c, 1.00). Votoček and Mikšič (108) give an $[\alpha]_D^{20}$ +9.18° (H_2O) for this compound. The amount of material available was too small to allow further purification by means of a solid derivative.

3. Miscellaneous preparations

a. Preparation of 1-desoxy-L-allitol (D-allomethylitol). D-Allomethylose (5.2 g.) was prepared in an overall yield of 26% from L-rhamnose (20 g.) according to the procedure of Levene and Compton (67). Reduction of the aldose to the desired 1-desoxy-L-allitol with sodium amalgam was effected in accordance with the directions of Iwadare (59). The 1-desoxy-L-allitol thus prepared had a melting point of 60° C. and $[\alpha]_D^{30}$ -8.62 ± 2.00° (H_2O) (l, 2; c, 1.00). The constants recorded by Iwadare (59) for this compound are m.p. 62-63° C.

and $[\alpha]_D^{16}$ -11 (H_2O).

b. Preparation of L-threitol. L-Threose was prepared in 68% yield from 1,3-monobenzylidene-L-arabitol (0.37 g. from 1.1 g.) by the procedure of Steiger and Reichstein (97). Sodium amalgam reduction of 0.37 g. of L-threose gave 0.10 g. (26%) of L-threitol of m.p. 86.5-87.5° C. and $[\alpha]_D^{28}$ -3.50 ± 2.00° (H_2O) (l, 2; c, 1.00). The constants recorded for L-threitol by Bertrand (19) are m.p. 88-89° C.; $[\alpha]_D$ -4.46° (H_2O).

B. Action of Acetobacter suboxydans upon Compounds Prepared for Testing

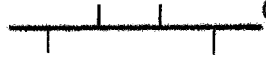
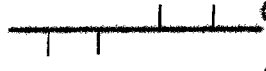
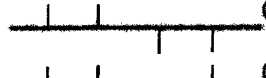
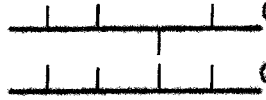
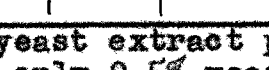
1. Action of Acetobacter suboxydans upon 1-desoxy sugar alcohols.

The results of the tests for the production of reducing compounds by the action of A. suboxydans upon ten 1-desoxy sugar alcohols prepared for this investigation are recorded in Table IX. A consideration of the apparent effect of the configuration of this type of compound relative to the susceptibility to the oxidative action of A. suboxydans will be given below in the section reserved for discussion.

In order to facilitate the isolation of sufficient material for a further study of the specific compound formed by the action of A. suboxydans upon the 1-desoxy-D-galactitol an attempt was made to increase the yield of reducing product.

Table IX

Production of Reducing Compounds by the Action of *Acetobacter suboxydans* upon Some l-Desoxy Sugar Alcohols

Compound	Configuration	mg. reducing compound per 100 mg. alcohol		
		Days		
		5	10	21
l-desoxy-L-arabitol		9.50	17.7	25.0
l-desoxy-D-arabitol		82.0	85.7	--
l-desoxy-D-glucitol		60.0	85.2	--
l-desoxy-D-galactitol		6.57	14.3	27.0
l-desoxy-D-mannitol		26.4	87.5	--
l-desoxy-L-mannitol		no reduction		
l-desoxy-L-glucitol		no reduction		
l-desoxy-L-allitol*		14.2	21.4	
l-desoxy-D-gulitol*		2.40	1.94	
l-desoxy-D-iditol*		-- no reduction		

*Media contained 0.5% yeast extract plus 0.025% sorbitol; all other media contained only 0.5% yeast extract.

Dunning, Fulmer, Guymon, and Underkofler (40) showed in their work on inositol that the production of reducing compound from inositol could be substantially increased by supplying *A. suboxydans* with small amounts of an available carbon source. The addition of small amounts of sorbitol serves very well in this capacity. In Table X are recorded data on the effect of variation of the concentration of sorbitol and l-desoxy-D-galactitol upon the production of reducing compound by the

Table X

Effect of Variation of Concentration of Sorbitol and 1-Desoxy-D-galactitol on the Action of Acetobacter suboxydans on 1-Desoxy-D-galactitol

Mg. sorbitol per 100 ml.	Mg. 1-desoxy-D-galactitol per 100 ml.	Mg. reducing compound per 100 mg. alcohol	
		5 days	10 days
25	100	22.5	43.1
25	200	23.2	34.2
25	300	----	4.2
50	100	32.2	41.1
50	200	26.3	29.4
50	300	----	15.5
75	100	30.1	43.0
75	200	18.0	24.8
75	300	----	19.1

action of A. suboxydans on 1-desoxy-D-galactitol. Concentrations of sorbitol ranged from 25 mg. to 75 mg. in 10 ml. of medium containing 100 mg., 200 mg., and 300 mg. of 1-desoxy-D-galactitol. Optimum analytical yields of up to 43 mg. of reducing compound per 100 mg. of alcohol were obtained with a one per cent solution of 1-desoxy-D-galactitol in the presence of the varying amounts of sorbitol used over a period of ten days.

A further attempt to increase the yields of reducing

compounds from both 1-desoxy-D-galactitol and 1-desoxy-L-arabitol consisted of re-inoculating the test media at given intervals with 1 ml. of A. suboxydans cells suspended in sterile saline containing 0.025% sorbitol. The results of this experiment, as seen in Table XI, show the possibility of producing up to 68% reducing compound from 1-desoxy-D-galactitol and 47% reducing compound from 1-desoxy-L-arabitol.

Table XI

Effect of Re-inoculating Media on the Action of Acetobacter suboxydans on 1-Desoxy-D-galactitol and 1-Desoxy-L-arabitol

Time in days of re-inoculation*	Total time in days before analysis	Mg. reducing compound per 100 mg. alcohol	
		1-desoxy-D-galactitol	1-desoxy-L-arabitol
5	10	55.5	34.2
10	15	61.8	42.5
10	20	68.0	47.4

*Re-inoculated with 1 ml. saline suspension of organism containing 0.025% sorbitol.

2. Action of Acetobacter suboxydans upon L-threitol and some mercaptals.

Incidental to the main theme of this research a number of mercaptals and the interesting L-threitol were also subjected to the oxidative action of A. suboxydans. Data concerning

the production of reducing compounds obtained from these substrates are given in Table XII.

Table XII

Production of Reducing Compounds by the Action of *Acetobacter suboxydans* upon L-Threitol and Some Mercaptals

Compound*	Configuration	Mg. reducing compound per 100 mg. substrate compound	
		5 days	10 days
L-threitol		----	1.05
Mercaptals			
D-xylose		17.1	17.4
D-arabinose		23.7	30.3
L-arabinose		15.3	17.0
D-galactose		15.4	17.2
D-glucose		----	22.2
D-mannose		17.7	25.4

*All media contained 0.5% yeast extract and 0.025% sorbitol.

V. DISCUSSION

Included in the number of six carbon l-desoxy sugar alcohols that were investigated were all those compounds previously subjected to the action of A. suboxydans by other workers. The results obtained with the l-desoxy alcohols involved (Table IX), namely, l-desoxy-D-mannitol, l-desoxy-L-mannitol, and l-desoxy-D-galactitol, substantiate former reports. Specifically, the production of a prolific amount of reducing compound by the action of A. suboxydans on l-desoxy-D-mannitol corroborates the findings of Anderson and Lardy (1). Similarly, the apparent immunity of l-desoxy-L-mannitol to oxidation by A. suboxydans reported herein is in agreement with the report of Dunning, Fulmer, and Underkofler (41). The production of a reducing compound by the organism from l-desoxy-D-galactitol is a verification of the same reaction by Hann, Tilden, and Hudson (52).

Concerning the last mentioned oxidation it will be noted (Table IX) that the amount of reducing compound produced from the l-desoxy-D-galactitol, though definite (27 mg. per 100 mg. alcohol), was too small for identification purposes. Fortification of the medium containing 100 mg. per 10 ml. of medium with from 25-75 mg. of sorbitol per 10 ml. of medium materially increased the yield of reducing compound (Table X) (43 mg. per

100 mg. of alcohol). As a further developmental procedure (Table XI) re-inoculation of media containing 100 mg. of 1-desoxy-D-galactitol and 25 mg. of sorbitol per 10 ml. of medium with additional cells of A. suboxydans and sorbitol raised the analytical yield of reducing compound from the 1-desoxy-D-galactitol to 68 mg. per 100 mg. of alcohol. Such a yield should render possible a program directed towards the identification of the reducing compound in question.

Of the remaining 1-desoxy alcohols tested only the 1-desoxy-D-glucitol has been reported as having been subjected to the action of an Acetobacter. Using A. xylinum, Müller and Reichstein (76) obtained unsatisfactory yields (1 mg. per 100 mg. of alcohol) of ketose from the 1-desoxy-D-glucitol. In Table IX is recorded an analytical yield of 85 mg. of ketose from 100 mg. of the same alcohol by oxidation with A. suboxydans. These comparative yields once more lend emphasis to the greater rapidity of action of A. suboxydans over A. xylinum.

Two other known 1-desoxy alcohols, 1-desoxy-D-gulitol and 1-desoxy-L-allitol, have been tested for response to the oxidative action of A. suboxydans for the first time. As shown in Table IX the 1-desoxy-D-gulitol apparently remains unattacked by A. suboxydans. Contrarily, 1-desoxy-L-allitol gave a small but definite amount of reducing compound (21.4 mg. from 100 mg. of alcohol).

Of the newly prepared and characterized 1-desoxy sugar

alcohols the 1-desoxy-D-iditol and 1-desoxy-L-glucitol yielded no significant amounts of reducing compound while the enantiomorphic 1-desoxy arabitols both produced reducing compounds when acted upon by A. suboxydans.

Recapitulating, the compounds tested that gave the highest yields of reducing compounds are notable for their configurational similarity. These 1-desoxy-alcohols, 1-desoxy-D-arabitol, 1-desoxy-D-glucitol, and 1-desoxy-D-mannitol, all belong to a D- series and possess a cis pair of hydroxyls adjacent to a primary hydroxyl group. The negligible action of A. suboxydans upon 1-desoxy-L-mannitol, 1-desoxy-L-glucitol, 1-desoxy-D-gulitol, and 1-desoxy-D-iditol might be explained by the absence in these compounds of one of the two mentioned configurational characteristics. Superficially, these results are in conformation with 'Bertrand's rule'.

Of special interest are the results obtained by the action of A. suboxydans on 1-desoxy-L-allitol, 1-desoxy-L-arabitol, and 1-desoxy-D-galactitol. The oxidation of the 1-desoxy-L-allitol is perhaps not too anomalous. A cautious statement concerning the oxidative specificity of A. suboxydans will indicate a preference of the organism for a member of a D-series with the proper configuration. The same might hold for the 1-desoxy-L-arabitol were it not for the fact that this compound may be considered as deriving from the same homomorphous series as the 1-desoxy-D-galactitol. In view of such a possible relationship, the reaction of the remaining member

of such a series, the 1-desoxy-L-altritol, would prove of definite interest.

Considering all the 1-desoxy sugar alcohols tested, with the exception of the 1-desoxy-D-galactitol, one might rationalize that A. suboxydans attacks this type of compound in accordance with a loosely applied 'Bertrand's rule'. However, if any rule may be applied to the oxidation of the 1-desoxy sugar alcohols by A. suboxydans the production of definite amounts of a reducing compound from 1-desoxy-D-galactitol precludes its being the rule of Bertrand or any present modification thereof. The necessity for at least a preliminary testing of the remaining eight 1-desoxy alcohols of the six carbon series is quite evident.

In Table XII evidence is given for the absence of any reducing compound produced by A. suboxydans from a medium containing L-threitol. This result in no way suggests the possible specific action A. suboxydans has toward the tetritols. The enantiomorph must be tested before deciding whether the organism acts according to 'Bertrand's rule', necessitating two secondary cis hydroxyl groups, or follows the pattern of glycol oxidation, wherein a secondary hydroxyl needs only be D- relative to its adjacent terminal carbon atom.

Apparently no configurational pattern is determinative for A. suboxydans oxidation of the mercaptals (Table XII). Although analysis showed relatively slight oxidation in all cases, definite formation of reducing compounds was obtained

with all the mercaptals tested. It is of interest to reiterate that Hann, Tilden, and Hudson (52) failed to detect any reducing compound when they used D-mannose diethyl mercaptal as a substrate for A. suboxydans, nor did Iselin (58) using D-glucose diethyl mercaptal. The age of the culture may be a possible explanation for such a discrepancy. Butlin (28, 30) and Kluyver and Boezaardt (64) showed in a rather conclusive manner, using the Warburg apparatus, that 24 hour and 48 hour cultures of A. suboxydans possess high enough oxidizing intensities to produce briefly carbon dioxide from glucose. Such a power disappears in 3-4 day cultures. As mentioned previously, cultures used in this work were 24 hours old. In the papers of Iselin (58) and Hann, Tilden, and Hudson (52) the age of the culture is not given. The possibility of the presence of sorbitol in the media used in these experiments being responsible for the production of reducing compounds is rendered negligible by the fact that Iselin (58) applied the same technique. Hann, Tilden, and Hudson (52) used small amounts of glucose in their basal medium. This compound might serve the same purpose as the sorbitol. The only conclusive argument would be the isolation and identification of any reducing compounds produced from the mercaptals. Once more further investigations are indicated.

VI. SUMMARY AND CONCLUSIONS

1. Ten 1-desoxy sugar alcohols have been prepared and subjected to the action of A. suboxydans, the extent of such action being determined by analysis for the production of reducing compounds at five and ten day intervals.
2. Four new 1-desoxy alcohols, 1-desoxy-D-arabitol, 1-desoxy-L-arabitol, 1-desoxy-D-iditol, and 1-desoxy-L-glucitol, have been prepared. The first three were characterized by their crystalline acetates. A sirupy diacetone derivative of the 1-desoxy-L-glucitol was also prepared.
3. Of those 1-desoxy alcohols tested, the following gave highly conclusive evidence (production of at least 80 mg. of reducing compound per 100 mg. of alcohol) of having been oxidized by A. suboxydans: 1-desoxy-D-glucitol, 1-desoxy-D-mannitol, and 1-desoxy-D-arabitol.
4. The production of reducing compounds from the following 1-desoxy alcohols was insignificant: 1-desoxy-L-glucitol, 1-desoxy-L-mannitol, 1-desoxy-D-iditol, and 1-desoxy-D-gulitol.
5. The 1-desoxy-D-galactitol, 1-desoxy-L-arabitol, and 1-desoxy-L-allitol gave definite but not high yields of reducing compounds.

6. Addition of varying amounts of sorbitol (25, 50, and 75 mg. per 10 ml. of medium) to media containing 1, 2, and 3 per cent of 1-desoxy-D-galactitol showed that over a period of ten days the amount of reducing compound produced from the 1-desoxy-D-galactitol by A. suboxydans could be definitely increased. A maximum yield of 47 mg. of reducing compound per 100 mg. of alcohol was obtained by utilizing 0.025 mg. of sorbitol and 100 mg. of 1-desoxy-D-galactitol per 10 ml. of medium.
7. Re-inoculation of media containing 0.025 mg. of sorbitol and 100 mg. of 1-desoxy-D-galactitol per 10 ml. of medium with additional bacteria and sorbitol at five and ten day periods gave further increase (68 mg. per 100 mg. of alcohol) in analytical yield of reducing compound.
8. Similarly, from the 1-desoxy-L-arabitol a maximum yield of 45 mg. of reducing compound per 100 mg. of alcohol was obtained, using re-inoculation and sorbitol.
9. Insufficient evidence was collected to allow any generalization of the oxidative specificity of A. suboxydans towards the 1-desoxy sugar alcohols.
10. Several mercaptals yielded minor amounts of reducing compounds when acted upon by A. suboxydans. No configurational specificity was indicated.
11. The action of A. suboxydans upon L-threitol produced no detectable amount of reducing compound over a ten day period.

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