THE STEREOCHEMISTRY OF AN ENZYMATIC REACTION:
THE OXIDATION OF \( \text{L-, D-, AND epi-INOSITOL} \)
BY ACETOBACTER SUBOXYDANS*

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The property of certain members of the group of acetic acid bacteria (1)
that brings about the incomplete oxidation of various substrates, stopping
often at the primary oxidation product, is emphasized in the name of the
bacterial type with which this paper deals, *Acetobacter suboxydans* (2).
The action of this organism on polyhydroxy compounds terminates, as a
rule, with the formation of a monoketo compound. Certain sterical limita-
tions, observed experimentally, have found their expression in the well
known rule of Bertrand (3) which, however, does not apply to all substrates
(1, 4).

It occurred to us that the stereochemical specificity of this interesting
enzyme system could be defined with greater precision if a stereoisomeric
series of cyclic polyhydroxy compounds were employed as the substrates of
oxidation, since in this case the lack of free rotation around carbon to car-
bon bonds should permit a clearer correlation between reactivity and the
spatial arrangement of the reactive groups. For this purpose, several iso-
mers of the inositol group and related cyclitols were chosen. The oxidation
rates were studied manometrically and whenever possible the oxidation
products isolated and identified.

The experiments, which in part have been presented very briefly in a
preliminary communication (5), were carried out with resting cells.† Since
the localization of the enzyme system within the bacterial cell is unknown,
it is not yet possible to present an adequate discussion of the reasons for the
differences in oxidation rates found for the several stereoisomers. Should
the speed of permeation of the substrate into the cell prove to be the rate-
determining step, then it would become necessary to consider the bacterial

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cil.

† This report is from a dissertation to be submitted by Boris Magasanik in partial
fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty
of Pure Science, Columbia University.

† The oxidative enzymes of *Acetobacter* have so far not been obtained in a cell-free
state (1).
cell wall as a sterically selective system. If the enzyme is situated at the bacterial surface or if a non-selective diffusion operates, the rates of oxidation would be an expression of the equilibrium conditions governing the formation of the particular substrate-enzyme complex or its reaction with oxygen. (For recent discussions of permeability, cf. (6, 7).) In any event, the selection for enzymatic attack of one or more of the sterically distinct secondary hydroxyl groups of the inositol must derive from the direct action of the bacterial oxidase on the cyclitol. The nature of the Acetobacter oxidase or oxidases, however, still is completely unknown.

While it cannot yet be stated definitely that the same enzyme is responsible for the oxidation of the different stereoisomers discussed here, the preliminary information available would appear to favor this assumption. Mixtures of meso- and d-inositol, each in a concentration sufficient to saturate the enzyme system, were found to be oxidized at a rate identical with that at which either of the components alone was handled. Furthermore, the resting bacteria (cultivated in the presence of d-sorbitol, but in the absence of the inositol isomers) oxidized even as unusual substrates as epi-inositol or d-epi-inosose without a lag phase, as should have been the case if adaptation had been a requisite for enzymatic oxidation.

The planar projections of the cyclitols used as substrates and of some of the oxidation products are shown in structures I to XI. The structure of I is based on the work of Dangschat and Fischer (8) and of Posternak (9), and that of II (10), III (9), IV (11), VI (12), and VII (9) on the series of brilliant studies by Posternak.²

The maximal oxygen consumption of the various substrates is summarized in Table I. In these experiments, carried out with a high concentration of bacteria, the diffusion of oxygen into the suspension became the limiting factor. Veronal buffer could be substituted for the usually employed phosphate buffer of pH 6.0 without changing the nature of the oxidation of meso-inositol.

In the case of meso-inositol (I) the results of Kluyver and Boezaardt (14) were confirmed. The consumption of 1 gm. atom of oxygen per mole of substrate indicated the formation of a monoketo derivative, meso-inosose (VII). This compound has frequently been isolated in different laboratories (14–16).

Similar results were obtained with epi-inositol (II) which yielded a levoro-

² The numbering of the carbon atoms is similar to the one adopted by Ebel ((13) p. 619). The carbon atoms are numbered clockwise, 1 and 6 chosen with their hydroxyls in trans position. The structure is arranged in such a manner as to have as many hydroxyl groups as possible cis to the one in position 1. Where this scheme allows two possibilities, that arrangement is chosen in which the carbon atoms with hydroxyls cis to the one in position 1 have the lowest positional numbers. The same system is used for keto and desoxy derivatives.
The study of this derivative, included in the experimental part, served to establish its structure as either VIII or IX, that of \( \textit{epi}-\text{inositol} \) as II, and that of the \( \textit{inosose} \) obtained by the action of
nitric acid on meso-inositol (17) as the dl compound VIII + IX; but since analogous findings were in the meantime presented independently by Posternak (10), who proved the structure of the levorotatory ketone to be VIII, a detailed discussion appears superfluous.

dl-epi-Inosose (VIII + IX), formed by the action of nitric acid on I, was found to consume 0.25 mole of oxygen per mole of substrate. Inasmuch as l-epi-inosose (VIII), the end-product in the oxidation of epi-inositol, is re-

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximal Oxygen Consumption</strong></td>
</tr>
</tbody>
</table>

The Warburg vessels in each experiment contained 0.5 cc. of the suspension of resting *Acetobacter suboxydans* (about 5 mg. of bacterial N) and 2 cc. of 1/15 M phosphate buffer of pH 6.0 in the main compartment, 0.5 cc. of the substrate solution in the side arm, and 0.2 cc. of 10 per cent KOH in the center well. The experiments were carried out at 38° in the presence of air.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Oxygen consumption, moles per mole substrate</th>
<th>Duration of oxygen uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>meso-Inositol</td>
<td>6.6</td>
<td>0.47, 0.50</td>
</tr>
<tr>
<td></td>
<td>13.9</td>
<td>0.50, 0.50</td>
</tr>
<tr>
<td></td>
<td>13.9*</td>
<td>0.51, 0.47</td>
</tr>
<tr>
<td>l-Inositol</td>
<td>6.6</td>
<td>1.01, 1.03, 1.00</td>
</tr>
<tr>
<td>d-Inositol</td>
<td>10.0</td>
<td>1.00, 0.97, 0.99</td>
</tr>
<tr>
<td>Scyllitol</td>
<td>5.8</td>
<td>0</td>
</tr>
<tr>
<td>epi-Inositol</td>
<td>8.7</td>
<td>0.51, 0.49</td>
</tr>
<tr>
<td>dl-epi-Inosose</td>
<td>6.8</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>13.4</td>
<td>0.27, 0.27</td>
</tr>
<tr>
<td></td>
<td>17.7</td>
<td>0.25, 0.26</td>
</tr>
<tr>
<td>Quebrachitol</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>Pinitol</td>
<td>7.8</td>
<td>0</td>
</tr>
<tr>
<td>d-Quercitol</td>
<td>7.0</td>
<td>0.95, 0.97, 0.96, 1.01</td>
</tr>
<tr>
<td></td>
<td>7.0†</td>
<td>0.50, 0.47</td>
</tr>
</tbody>
</table>

* In this experiment veronal buffer (pH 6.0) was substituted.
† The first gm. atom of oxygen was consumed within about 7 minutes.
‡ This experiment was performed with only about 0.7 mg. of bacterial N per vessel.

sistant to further oxidation, one may conclude that each mole of *d*-epi-inosose (IX) reacted with 0.5 mole of oxygen, giving rise to a diketo compound. Attempts at the isolation of this derivative have not yet been successful. The second carbonyl group probably is not vicinal to the one present in IX, since such α-diketones readily give rise to bisphenylhydrazones; it more probably occupies position 2 of IX, by analogy to the oxidation of epi-inositol. The oxidation product was found to have acidic properties, which is in agreement with the expected behavior of an enolized β-diketone.
The two optically active stereoisomers, \textit{l}-inositol (IV) and \textit{d}-inositol (V), each reacted with 2 gm. atoms of oxygen per mole of substrate. From 5 to 20 day-old culture fluids of \textit{Acetobacter subox}dans containing these substances the oxidation products could be isolated by means of phenylhydrazine, but only in the case of the \textit{l} compound was the yield good. Both products obtained in this manner from \textit{l}- and \textit{d}-inositol respectively proved to be bisphenylhydrazones of diketo inositols; they formed yellow needles with identical melting points, identical absorption spectra (Fig. 1) characteristic for osazones \((18)\), and optical rotations equal but opposite in sign. When equal amounts of the two substances were permitted to crystallize together, a new compound with a lower melting point, but an unchanged absorption spectrum, was obtained. The two bisphenylhydrazones clearly were enantiomorphs. The consumption of periodic acid in 66 per cent alcohol at room temperature corresponded to 3 moles of the oxidant per mole of bisphenylhydrazone \((19)\). These findings serve to establish these compounds as \textit{\alpha}-bisphenylhydrazones. The reaction products obtained by the

![Absorption spectrum](http://www.jbc.org/)

\textbf{Fig. 1.} Absorption spectrum (in absolute ethanol) of the \textit{\alpha}-bisphenylhydrazone of cyclohexane-(1,5)cis-tetrol-3,4-dione (XII) obtained from \textit{l}-inositol.
action of periodic acid on these substances are discussed in another com-
munication (20).

The exact configurations of the \( \alpha \)-bisphenylhydrazones obtained from the
biological oxidation products of \( l \)- and \( d \)-inositol were determined by com-
paring their racemic mixture with an \( \alpha \)-bisphenylhydrazone of a diketo
inositol obtained by Carter et al. (21) by the action of phenylhydrazine on
the phenylhydrazone of meso-inosose (VII).\(^3\) In this compound the second
phenylhydrazono group must have been introduced into positions 1 and 3
respectively, leading to a racemic mixture of XII and XIII.

Both racemic mixtures exhibited the same absorption spectrum, identi-
cal with the one shown in Fig. 1, and identical melting points. Their mix-
ture showed no depression of the melting point. Structure XII can, there-
fore, be assigned to the dextrorotatory compound obtained from \( l \)-inositol,
structure XIII to the levorotatory substance produced from \( d \)-inositol.

\( l \)-Inositol (IV) has thus been shown to be oxidized by \textit{Acetobacter suboxy-
dans} in positions 1 and 6 to yield the \( \alpha \)-diketone X; \( d \)-inositol (V), attacked
in the corresponding mirror image positions 2 and 3, affords the \( \alpha \)-diketone
XI. This appears to be the first instance of the production by this micro-
organism of a dicarbonyl compound from a polyhydric alcohol.\(^4\)

\( \text{Quebrachitol} \) and \( \text{pinitol} \), the naturally occurring monomethyl ethers of
\( l \)- and \( d \)-inositol respectively, were not oxidized by \textit{Acetobacter}. The po-
sition of the methoxyls in these compounds is not known. \( \text{Scyllitol} \) (III)
likewise was not attacked.

\( d \)-\textit{Quercitol} (VI) consumed 2 gm. atoms of oxygen per mole of substrate.
The 2nd gm. atom was taken up at a much slower rate. With a lower con-
centration of bacteria, the oxygen uptake terminated with the consumption
of the first half mole, a phenomenon not observed with the optically active
inositols.

The \textit{rates of oxygen uptake} by equal quantities of the various substrates,

\(^3\) We should like to thank Dr. H. E. Carter of the University of Illinois for a speci-
men of this compound and for a personal communication concerning the mode of
formation of this osazone.

\(^4\) The production by \textit{Acetobacter suboxydans} of a diketo derivative from \textit{meso-}
inositol has been reported by one group of workers (22-25). This product does not
appear to have been observed in other laboratories (14-16) nor is its formation com-
patible with the manometric measurements presented previously (5, 14) and in this
paper.
insufficient in concentration to give maximal oxidation rates, are presented in Table II. When, as is done in Table II, these rates are related to the rate of oxygen consumption by meso-inositol at the same bacterial concentration, it will be seen that under the experimental conditions the various substrates were oxidized at widely differing rates, d-inositol being the most slowly and epi-inositol the most rapidly oxidized stereoisomer. The slow oxidation of d-inositol may be responsible for the low yield of oxidation product obtained in the culture experiments.5

### Table II

#### Comparison of Oxidation Rates

The Warburg vessels in each experiment (carried out at 38°) contained 0.5 cc. of the suspension of resting bacteria and 2 cc. of 1/15 M phosphate buffer of pH 6.0 in the main compartment, 0.5 cc. of solution containing 7 micromoles of substrate in the side arm, and 0.2 cc. of 10 per cent KOH in the center well.

<table>
<thead>
<tr>
<th>Bacterial N</th>
<th>Substrate</th>
<th>QO₂ (N)</th>
<th>QO₂ (N) (substrate)</th>
<th>QO₂ (N) (meso-inositol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.36</td>
<td>meso-Inositol</td>
<td>-370</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>epi-Inositol</td>
<td>-620</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>meso-Inositol</td>
<td>-530</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>epi-Inositol</td>
<td>-880</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d-epi-Inosose*</td>
<td>-280</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td>d-epi-Indosose*</td>
<td>-230</td>
<td>0.4†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>d-Quercitol</td>
<td>-240</td>
<td>0.4†</td>
<td></td>
</tr>
<tr>
<td>0.89</td>
<td>d-epi-Indosose*</td>
<td>-330</td>
<td>0.5†</td>
<td></td>
</tr>
<tr>
<td>0.94</td>
<td>meso-Indositol</td>
<td>-740</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t-Inositol</td>
<td>-430</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>d-Inositol</td>
<td>-170</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 14.0 micromoles of dl-epi-inosose were used.
† The corresponding values for meso-inositol were interpolated.

The maximal rates of oxidation of meso- and d-inositol are compared in Table III. In these experiments the enzyme system was saturated with respect to substrate. The maximal rates of oxygen uptake were found identical for both isomers and also for their equimolar mixture (Table III, Preparation 2). But the meso compound was oxidized at the maximal rate at a concentration at which the oxidation of d-inositol had not yet reached its maximum (Table III, Preparation 1). Some of the possible explanations of this difference in saturation levels have been mentioned above. If the influence of the cell wall on the speed of diffusion of the two isomers is dis-

5 A much better yield of derivative XIII could be obtained in preparatory experiments with resting bacteria, as will be described on a later occasion.
regarded, the results shown in Table III seem to indicate that the equilibrium conditions governing the combination between enzyme and substrate are responsible for the differences.

The steric specificity of the enzyme system responsible for the oxidations that form the subject of this paper can now be discussed succinctly. It is, of course, evident that the structural formulae I to XIII of this series of cyclohexane derivatives do not describe the actual positions in space of the various atoms, but merely represent planar projections based on the conventions introduced into stereochemistry by Emil Fischer (26).

### Table III

**Maximal Rates of Oxidation**

The Warburg vessels in each experiment (38°) contained 0.5 cc. of the bacterial suspension and 2 cc. of 1/15 M phosphate buffer of pH 6.0 in the main compartment, 0.5 cc. of substrate solution in the side arm, and 0.2 cc. of 10 per cent KOH in the center well.

<table>
<thead>
<tr>
<th>Bacterial preparation No.</th>
<th>Bacterial N</th>
<th>meso-Inositol</th>
<th>d-Inositol</th>
<th>( Q_{O_4}(N) )</th>
<th>( Q_{O_4}(N) ) (substrate)</th>
<th>( Q_{O_4}(N) ) (meso-inositol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.47</td>
<td>28.2</td>
<td>28.2</td>
<td>-990</td>
<td>-1100</td>
<td>-340 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56.4</td>
<td>56.4</td>
<td></td>
<td></td>
<td>680 0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.2</td>
<td></td>
<td></td>
<td>940 0.85</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>225</td>
<td>225</td>
<td>-1700</td>
<td>-1570</td>
<td>-1730 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>450</td>
<td>450</td>
<td></td>
<td></td>
<td>-1920 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>225</td>
<td></td>
<td></td>
<td>-1680 1.1</td>
</tr>
</tbody>
</table>

Recent work based on electron diffraction studies (27) and spectroscopic (28) and thermodynamic (29) properties has led to the conclusion that at room temperature cyclohexane exists predominantly in the chair configuration. If the conceptions applied to cyclohexane and its mono- and dimethyl derivatives (29, 30) are extended to the inositols, it will be seen that six of the substituents (hydroxyl or hydrogen) surround the ring of carbon atoms in what has been called an equatorial belt; the six others are perpendicular to the plane formed by the carbon ring: three above (north polar), three below (south polar). Each carbon atom will, therefore, carry 1 polar and 1 equatorial substituent. A twist of the 6 carbon atoms through a single plane to the opposite chair form renders the equatorial substituents polar and vice versa (30).
Consequently, there will exist two geometrically tautomeric forms for each inositol isomer. By analogy to the methyl-substituted cyclohexanes (29) it may, however, be assumed that the tautomer possessing the smaller number of polar hydroxyls will predominate. The constellations of compounds I to XI, based on the postulates outlined here, are compared in Table IV.

The inspection of atomic models of the inositols shows the position of the hydroxyl groups to be very unfavorable to the formation of cyclic compounds, such as benzylidene or isopropylidene derivatives. This is in line with the great difficulty with which the condensation of meso-inositol with acetone is brought about (8).

Recent work on the configuration and the biological activity of the hexachlorocyclohexanes also provides interesting analogies to the inositols. Among the structures, assigned by Slade (31) to the different isomers, \( \gamma \)-hexachlorocyclohexane corresponds to configuration I in Table IV, the \( \alpha \) isomer to a racemic mixture of IV and V, and the \( \beta \) compound to III. The biological antagonism of \( \gamma \)-hexachlorocyclohexane to meso-inositol as a growth factor for yeast (32) also is suggestive of a spatial similarity between these compounds. Moreover, the melting points of the two series of cyclohexane derivatives show a parallel trend. meso-Inositol, \( dl \)-inositol, and scyllitol melt at 218°, 253°, and 345° respectively; the respective melting

**Table IV**

**Spatial Configurations of Inositol Isomers and Related Substances**

<table>
<thead>
<tr>
<th>Planar projection</th>
<th>Compound</th>
<th>Position of hydroxyls on carbon atoms</th>
<th>Position of hydroxyls oxidized by Acetobacter suboxydans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Equatorial</td>
<td>North polar</td>
</tr>
<tr>
<td>I</td>
<td>meso-Inositol</td>
<td>1, 3, 4, 5, 6</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>epi-Inositol</td>
<td>1, 3, 5, 6</td>
<td>2, 4</td>
</tr>
<tr>
<td>III</td>
<td>Scyllitol</td>
<td>1, 2, 3, 4, 5, 6</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>L-Inositol</td>
<td>2, 3, 4, 5</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>D-Inositol</td>
<td>1, 4, 5, 6</td>
<td>2</td>
</tr>
<tr>
<td>VI</td>
<td>d-Quercitol</td>
<td>1, 5, 6</td>
<td>2</td>
</tr>
<tr>
<td>VII</td>
<td>meso-Inosose</td>
<td>1, 3, 4, 5, 6</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>L-epi-Inosose</td>
<td>1, 3, 5, 6</td>
<td>4</td>
</tr>
<tr>
<td>IX</td>
<td>D-epi-Inosose</td>
<td>1, 3, 5, 6</td>
<td>2</td>
</tr>
<tr>
<td>X</td>
<td>Cyclohexane -(1,5)cis -tetrol-3,4-dione</td>
<td>1, 2, 5, 6</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>Cyclohexane -(1,3)cis -tetrol-4,5-dione</td>
<td>1, 2, 3, 6</td>
<td></td>
</tr>
</tbody>
</table>

* 2 gm. atoms of oxygen taken up per mole of substrate.
† 1 gm. atom of oxygen taken up per mole of substrate.
points of the $\gamma$, $\alpha$, and $\beta$ isomers of hexachlorocyclohexane are at 112°, 158°, and 309°.

The specific steric requirements for oxidation can, on the basis of the structures assigned to the inositols (Table IV), be expressed as follows: **Only polar hydroxyl groups are oxidized.** In the case of $\textit{epi}$-inositol (II), where two hydroxyls are situated in the same polar plane, only one is attacked by the enzyme to yield $\textit{l-epi}$-inosose (VIII). The enantiomorph $\textit{d-epi}$-inosose (IX), however, is further oxidized. Additional work will be necessary to show whether the relative position of the polar hydroxyls with respect to the hydrogen atom in the same polar plane is of importance for the extent of oxidation.

The study of the oxidation of aliphatic polyhydric alcohols by $\textit{Acetobacter}$ has led to the conclusion that only those hydroxyl groups are attacked that are situated between a primary hydroxyl and a secondary one in cis position (3, 4). Of the enantiomorphs, the isomer possessing the $\textit{D}$ configuration with respect to the secondary cis hydroxyls is oxidized more readily (4). A similar relationship appears to apply to cyclic enantiomorphs of the inositol series. In $\textit{l}$-inositol, which is much more rapidly oxidized than the $\textit{d}$ isomer, the hydroxyl groups that are oxidized and the neighboring cis hydroxyl groups have the $\textit{D}$ configuration.

The findings reported here permit the inference that, in the aliphatic polyhydroxy compounds attacked by the $\textit{Acetobacter}$-oxidase system, the position in space of a secondary hydroxyl group between a primary alcohol group and another secondary cis hydroxyl corresponds to the position of a polar hydroxyl group between two equatorial ones in the inositol molecule.

It should be pointed out that the studies on the enzymatic oxidation of inositol isomers, which are being continued, may prove of more general biological interest in several respects. The great importance of $\textit{meso}$-inositol as a growth factor for certain microorganisms ((33) p. 207), the discovery of a deficient mutant of $\textit{Neurospora crassa}$ requiring inositol (34), and the dietary significance of this compound in animal nutrition (35) all require a better insight into the ways in which $\textit{meso}$-inositol and its isomers are dealt with by living cells. Moreover, one of the constituents of streptomycin has been shown to be a 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane (36, 37). In view of what has been said above about the oxidation of $\textit{d-epi}$-inosose (IX) by $\textit{Acetobacter}$, it is possible that additional work will lead to the preparation of 1,3-substituted inositol derivatives of biological interest.

**EXPERIMENTAL**

**Material**

$\textit{Acetobacter suboxydans}$ was obtained from the American Type Culture
Collection (No. 621). The culture methods employed will be discussed below.

d-Quercitol and meso-inositol were purified commercial preparations.

A crude preparation of pinitol was obtained through the courtesy of Professor R. C. Elderfield of this University. It was purified by repeated decolorization of its aqueous solution with charcoal (Darco G-60) and crystallization from 75 per cent ethanol. The pure pinitol, melting at 185–186°, \([\alpha]_D^{25} = +66.4°\), yielded, on treatment with hydriodic acid (38), d-inositol (86 per cent of the theoretical yield, crystallized from 72 per cent ethanol) which had a melting point of 249–250° and \([\alpha]_D^{25} = +65.0°\) (in water).

The crude quebrachitol, obtained some years ago from the British Rubber Research Association, London, was freed of traces of sulfate by treatment with barium hydroxide and crystallized by the addition of 1.8 parts of hot acetone to the hot aqueous solution. The pure monomethyl ether melted at 195°. It yielded, when treated with hydriodic acid, l-inositol (83 per cent of the theory, crystallized from 70 per cent ethanol) which melted at 249–250° and had \([\alpha]_D^{25} = -63.8°\) (in water).

Scyllitol\(^7\) was prepared from meso-inositol by the method of Posternak (15), m.p. 345° (with decomposition); its hexaacetyl derivative melted at 296–297°. dl-epi-Inosose, m.p. 221° (with decomposition), was prepared by the action of nitric acid on meso-inositol (17). epi-Inositol, m.p. 285° (with decomposition), was obtained by the catalytic hydrogenation of dl-epi-inosose (17).

Oxidation of Inositol Stereoisomers and Related Substances by Acetobacter suboxydans

Cultivation of Bacteria—The cells were grown at 30° in a medium containing 50 gm. of d-sorbitol and 10 gm. of yeast extract (Difco) in 1 liter of tap water. When larger quantities of resting organisms were required, the cultivation was carried out in Roux bottles containing 150 cc. of the liquid medium and 4.5 gm. of agar. A 3 cc. portion of a 3 day-old liquid culture served as the inoculum for each Roux bottle. After 70 hours the cells were collected in physiological saline, centrifuged in the cold at 4000 R.P.M., and washed three times with ice-cold saline. They were then again suspended in physiological saline. In all experiments bacteria collected the same day were used.

\(^6\) The melting points, reported without correction, were determined with an electrically heated stage (Fisher-Johns). Unless noted otherwise, the intact crystals were placed on the stage preheated to about 5–7° below the melting point.

\(^7\) The melting points of scyllitol and hexaacetyl scyllitol were determined in capillaries.
**Maximal Oxygen Consumption**—When 0.5 cc. of a bacterial suspension in physiological saline containing about 5 mg. of bacterial nitrogen was permitted to act on 5 to 18 micromoles of the various substrates in a total volume of 3 cc., very rapid oxidation took place. The reaction was considered as terminated when the amount of oxygen consumed in a 5 minute interval was no greater than that taken up in the same period by the resting bacteria alone (i.e. about 2 microliters). Table I summarizes the results.

**Comparison of Oxidation Rates**—The rates at which the several substrates were dehydrogenated were compared in a series of experiments in each of which 7 micromoles of substrate and small amounts of resting bacteria (0.36 to 0.94 mg. of bacterial nitrogen) were employed. These experiments are presented in Table II. For the calculation of the $Q_{O_2}$ (N), the time interval for which the oxygen uptake was linear was used.

**Maximal Rates of Oxidation**—In the preceding experiments the enzyme system was not saturated with respect to substrate. The saturation levels for meso- and $d$-inositol were compared in another experiment reproduced in Table III.

**Formation of Compound X from $l$-Inositol**

*Preparation*—*Acetobacter suboxydans* was grown for 3 days at 30° in a medium consisting of 0.2 gm. of $D$-sorbitol, 0.2 gm. of yeast extract (Difco), and 0.5 gm. of $l$-inositol in 10 cc. of tap water. The culture was then transferred under sterile conditions to a 2.5 liter Fernbach flask containing a sterilized solution of 4.5 gm. of $l$-inositol in 90 cc. of tap water. Following incubation for 5 days at 30° the bacteria were removed by filtration through infusorial earth and a solution of 10 cc. of freshly distilled phenylhydrazine in 20 cc. of 50 per cent acetic acid was added to the filtrate. Crystals soon made their appearance at room temperature and transformed the mixture which was cooled overnight to a semisolid paste. The crystals which at this stage were contaminated with a red oil were filtered off, washed with ice-cold water, and spread on a porous plate. To their filtered solution in 75 cc. of hot pyridine, 200 cc. of hot water were added slowly. The chilled mixture deposited 4.2 gm. of reddish yellow crystals (42 per cent of the theoretical yield) which were recrystallized from a 1:1.6 mixture of water and methyl cellosolve (ethylene glycol monomethyl ether), following the treatment of the methyl cellosolve solution with 5 gm. of activated charcoal (Darco G-60). The crystals (2.9 gm.) were recrystallized once more from 3 parts of methyl cellosolve and 2 parts of water when 2.6 gm. of the $a$-bisphenylhydrazone of cyclohexane-$($1,5$)-cis-tetrol-3,4-dione (XII) were obtained as long yellow needles with a greenish sheen melting (with decomposition) at 217°. The optical rotation in 1:1 ethanol-pyridine ($c = 0.793, l = 0.5$ dm., $\alpha_p = +0.95°$) was found as $[\alpha]_b^{25} = +240°$ (initial); after 24 hours ($\alpha_p = +0.85°$) it was $[\alpha]_b^{25} = +214°$. 
The absorption spectrum of the levorotatory bisphenylhydrazone corre-

8 The crude crystals were found to contain, in addition to the osazone, a phenyl-
hydrzone of a monoketo derivative of d-inositol which could be separated by virtue of its complete insolubility in hot absolute ethanol. Several recrystallizations from aqueous pyridine yielded colorless plates which melted (with decomposition) at 197–199°. Calculated for C_{13}H_{16}O_{4}N_{4} (268.3), C 53.7, H 6.0, N 10.4; found, C 53.3, H 5.7, N 10.0.
sponded completely with that exhibited by the dextrorotatory derivative (Fig. 1) obtained from D-inositol.

This substance, when examined as described before in this paper, was found to consume 2.9 moles of periodic acid.

**Racemic Inosazone**

A mixture of equal amounts of the D- and L-inosazones, described in the preceding sections, was recrystallized from 2:3 water-methyl cellosolve. The yellow crystals of the racemic mixture melted (with decomposition) at 205° when placed on the stage at 200°.

The properties of these racemic crystals were compared with those of the phenylosazone obtained by the action of phenylhydrazine on the phenylhydrazone of meso-inosose (21). This compound, recrystallized from 2:3 water-methyl cellosolve, likewise exhibited a melting point (with decomposition) of 205° (stage preheated to 200°). Both substances gave the same absorption spectrum, which was identical with the one reproduced in Fig. 1.

For the determination of mixed melting points, the effect of crushing on the melting points of the various substances had to be studied. The finely powdered preparations were found to decompose, without truly melting, at temperatures somewhat lower than the melting points of the crystals themselves. The powders of the dextrorotatory (XII) and levorotatory (XIII) osazones both had decomposition points of 210°; their mechanical mixture decomposed at 203°–206°. The finely powdered racemic mixture obtained by joint crystallization of the two osazones decomposed at 199°, the osazone described by Carter et al. (21) at 198°–199°. No depression of the decomposition point was observed with a mixture of the two racemic osazones.

**Formation of L-epi-Inosose (VIII) from epi-Inositol (II)**

5 cc. of a 3 day-old culture of *Acetobacter suboxydans* in the sorbitol-yeast extract medium were added to a sterile solution of 5 gm. of epi-inositol, 0.85 gm. of yeast extract (Difco), and 0.17 gm. of D-sorbitol in 170 cc. of tap water. The mixture was incubated in a large Fernbach flask at 30° for 7 days. The culture fluid, which showed heavy growth, was filtered through infusorial earth and the filtrate concentrated to dryness in vacuo. The solution of the residue in 10 cc. of water deposited 3.0 gm. of crystalline material on the addition of 90 cc. of ethanol. The crude product was dissolved in 15 cc. of water and converted to the phenylhydrazone by the addition of 3 cc. of phenylhydrazine in 6 cc. of 50 per cent acetic acid.

The phenylhydrazone was washed with water and ethyl alcohol (weight 3.17 gm.; decomposition point 197°) and decomposed in the customary manner by refluxing its suspension in 65 cc. of water with 4.8 cc. of freshly distilled benzaldehyde and a small amount of benzoic acid for 20 minutes.
The filtrate from the chilled reaction mixture was extracted with ether and evaporated to dryness in vacuo. To the solution of the residue in 25 cc. of hot water 100 cc. of hot ethanol were added. The mixture, following filtration while hot, deposited, on being chilled, 524 mg. of \textit{l-epi-inosose} (VIII), white needles melting (with decomposition) at 194–196°. Another preparation of this ketone, obtained in a similar manner, melted (with decomposition) at 198°. The substance was levorotatory in water \(c = 2.54, l = 2\) dm., \(\alpha_d = -0.26^\circ \pm 0.02^\circ\); \(\alpha_d^{27} = -5.1^\circ \pm 0.4^\circ\). It showed no mutarotation. Its solution reduced Benedict's solution in the cold.

\[ \text{C}_9\text{H}_{10}\text{O}_5 (178.1). \text{ Calculated, C 40.5, H 5.7; found, C 40.4, H 5.7} \]

For reduction, 50 mg. of \textit{l-epi-inosose} in 6 cc. of water were shaken with four 300 mg. portions of 2.5 per cent sodium amalgam. The reaction mixture was kept acid by the addition of four portions of 0.37 cc. of \(\text{N}\) acetic acid. The dry evaporation residue of the supernatant was heated under a reflux with 2 cc. of acetic anhydride for 20 minutes. The excess reagent was decomposed with 5 volumes of water. The chilled mixture deposited 77 mg. of crystalline material which was extracted with 0.8 cc. of boiling ethanol. The insoluble residue was recrystallized from 6 cc. of absolute ethanol, when 22 mg. of \textit{hexaacetyl meso-inositol} were obtained, melting at 216–217°. Admixture of an authentic specimen of this hexaacetate produced no depression of the melting point.

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**SUMMARY**

The mechanisms of oxidation by \textit{Acetobacter suboxydans} of several isomers of the inositol group and related cyclitols have been studied. The extent and rate of oxygen uptake by the different substrates have been determined and the oxidation products or their derivatives isolated and identified. \textit{epi-Inositol} has been shown to yield a monoketone, \textit{l-epi-inosose}; \textit{l-} and \textit{d-inositol} gave rise to \(\alpha\)-diketones whose configurations have been ascertained. Scyliitol and the monomethyl ethers quebrachitol and pinitol were not attacked. On the basis of these results and in the light of recent work on the stereochemistry of cyclohexane, the minimum steric requirements for the oxidation of inositol isomers by \textit{Acetobacter suboxydans} can be described in the statement that only those hydroxyl groups are oxidized that are situated in a polar plane.

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