ON THE CYCLITOL OXIDIZING ENZYME SYSTEM OF ACETOBACTER SUBOXYDANS*

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The oxidation of various inositols (hexahydroxycyclohexanes) and quercitols (pentahydroxycyclohexanes) and their derivatives by resting cells of Acetobacter suboxydans has been studied by Chargaff and his students (1, 2), who found that the specificity exhibited by the organism toward the cyclitols studied was not adequately described by the Bertrand-Hudson rules (3) for open chain polyalcohols. This led the Columbia workers to formulate new rules describing the steric requirements for cyclitol oxidation, in so far as these could be ascertained with the substrates available. More recently, Posternak and Reymond (4) have repeated Chargaff's work and extended it to the cyclohexanetetrols, the cyclohexanetriols, and lower cyclic alcohols. The Swiss workers, who used a Delft strain of A. suboxydans, largely confirmed the earlier observations on the inositols and quercitols, but found that the oxidation of the tetrols and lower alcohols did not seem to follow either the Bertrand-Hudson or the Chargaff rules consistently.

The experiments with cyclitol oxidation which have been carried out in the authors' laboratory during the last 3 years involve a number of compounds not previously reported. In addition, use has been made of the cell-free preparation of Acetobacter enzymes described by Franzl and Chargaff (5). The results obtained help to define some of the limitations of Chargaff's rules as they apply to the higher cyclitols, and provide an explanation for the supposedly anomalous behavior of 1,cis-2,cis-3-cyclohexanetriol (γ-cyclohexanetriol), which had earlier been studied by Posternak and Ravenna (6).

EXPERIMENTAL

A culture of A. suboxydans, ATCC 621, was kindly furnished by Dr. N. K. Richtmyer of the National Institutes of Health. This strain was

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highly active in oxidizing myo-inositol, and was used in all the work to be described. The bacteria were maintained on yeast extract-agar slants containing 0.5 per cent sorbitol (7). When quantities of cells were desired, the organism was first subcultured on an unbuffered liquid medium containing 1 per cent yeast extract and 5 per cent sorbitol. 800 ml. of the same medium were then distributed among eight 500 ml. Erlenmeyer flasks and the flasks were stoppered and sterilized. Each flask was inoculated with 10 ml. of a 24 to 36 hour-old subculture, and shaken on a Gump rotary shaker¹ for 20 hours at 30°. The bacteria were collected by centrifuging at 0°, washed three times with ice-cold physiological saline, and stored in the frozen state until used. The yield was 10 to 12 ml. of thick suspension containing 6 to 10 mg. of bacterial N per ml.

The oxidation of substrates by the bacterial suspension was carried out in the Warburg apparatus as described by Magasanik and Chargaff (1).

Substrates²

The structural formulas of the cyclitols and cyclitol derivatives used in this investigation are shown in Fig. 1.

Conduritol (VIII) was isolated from commercial condurango bark essentially as described by Kern and Fricke (11). As suggested by these authors, hydrogenation of the mother liquors permitted the recovery of additional amounts of material as dihydroconduritol (IV). Dihydroconduritol was also prepared by hydrogenating isolated conduritol (12, 13). Adams' platinum oxide was used as the hydrogenation catalyst.

muco-Inositol (I) was synthesized from conduritol by the method of Dangschat and Fischer (12). An attempt to prepare allo-inositol (VII) by the method of the same authors failed.

The preparation of myo-inosamine-2 (IX) and scyllo-inosamine (II) has been reported previously (14).

Dl-epi-Inosamine-2 (VIa) (15) and m.-myo-inosamine-4 (IIIa) (16) and their respective N-acetyl derivatives (VIb and IIIb) were synthesized by Mrs. Helga Straube-Rieke of the Institute for Enzyme Research.

Sequoyitol (V) (17) was isolated from redwood (Sequoia sempervirens) by Miss Aurora S. Mamauag of this laboratory.

Pyrogallol was hydrogenated as described by Christian, Gogek, and Purves (18) to yield 1,cis-2,cis-3-cyclohexanetriol (γ-cyclohexanetriol, X).

¹ Purchased from the B. F. Gump Company, Chicago, Illinois.
² In this communication, all cyclitols having four or more hydroxyls are named and numbered according to the system of Fletcher, Anderson, and Lardy (8). The suggestions of Epstein and Rossini (9) regarding alkyleycloalkanes have been used in the systematic naming of the cyclohexanetriols.
Fig. 1. Conventional planar formulas of the cyclitols used. The vertical lines represent hydroxyl groups; a small circle at the end of a vertical line indicates a polar hydroxyl (polar amino groups are underlined). The term polar is used in this communication to indicate conformation, as suggested by Pitzer and Beckett (10). It applies to a group which projects above or below the average plane of the puckered cyclohexane ring, in contrast to an equatorial group, which is in that plane.
DL-1, cis-2, trans-3-Cyclohexanetriol (DL-β-cyclohexanetriol) was also isolated from the reaction mixture as its tribenzoate.

For the preparation of those inositols and derivatives which have also been tested with *A. suboxydans* by Chargaff and coworkers, the reader is referred to their papers and to the general literature.

### Table I

**Maximal Oxygen Consumption**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>No. of runs</th>
<th>Amount taken</th>
<th>Oxygen consumption</th>
<th>Duration of oxygen consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μM</td>
<td>gm. atoms per mole</td>
<td>gm. atoms per mole</td>
</tr>
<tr>
<td>muco-Inositol (I)*</td>
<td>28†</td>
<td>24 - 59</td>
<td>2.27</td>
<td>1.98 - 2.56</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.1 - 3.5</td>
<td>2.40</td>
<td>2.38 - 2.42</td>
</tr>
<tr>
<td>sceyllo-Inosamine (II)</td>
<td>3</td>
<td>7.5 - 9.2</td>
<td>0.06</td>
<td>0.05 - 0.07</td>
</tr>
<tr>
<td>nL-myo-Inosamine-4 (IIIa)</td>
<td>3</td>
<td>5.8 - 13.1</td>
<td>0.44</td>
<td>0.40 - 0.50</td>
</tr>
<tr>
<td>N-Acetyl-nL-myo-inosamine-4 (IIIb)</td>
<td>3</td>
<td>5.6 - 7.3</td>
<td>0.69</td>
<td>0.67 - 0.72</td>
</tr>
<tr>
<td>Dihydroconduritol (IV)†</td>
<td>4</td>
<td>8.2 - 15.0</td>
<td>1.13</td>
<td>1.06 - 1.18</td>
</tr>
<tr>
<td>Sequoyitol (V)</td>
<td>2</td>
<td>5.1</td>
<td>0.16</td>
<td>0.14 - 0.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>14.3 - 15.1</td>
<td>0.27</td>
<td>0.22 - 0.32</td>
</tr>
<tr>
<td>DL-epi-Inosamine-2 (VIa)</td>
<td>3</td>
<td>11.6 - 20.6</td>
<td>0.09</td>
<td>0.04 - 0.12</td>
</tr>
<tr>
<td>N-Acetyl-DL-epi-inosamine-2 (VIib)</td>
<td>2</td>
<td>4.1 - 6.9</td>
<td>0.02</td>
<td>0.01 - 0.03</td>
</tr>
<tr>
<td>Conduritol (VIII)‡</td>
<td>4</td>
<td>11.4 - 13.5</td>
<td>1.00</td>
<td>0.83 - 1.12</td>
</tr>
<tr>
<td>myo-Inosamine-2 (IX)</td>
<td>3</td>
<td>7.5 - 7.7</td>
<td>0.07</td>
<td>0.05 - 0.09</td>
</tr>
<tr>
<td>1,cis-2,cis-3-Cyclohexanetriol</td>
<td>1</td>
<td>12.1</td>
<td>1.14</td>
<td></td>
</tr>
</tbody>
</table>

Each Warburg flask contained a suspension of resting cells of *A. suboxydans* (3 to 5 mg. of bacterial N), 0.5 ml.; 0.15 M phosphate buffer, pH 6.0, 2.2 ml.; substrate (in the side arm), 0.3 ml.; and 10 per cent KOH (in the center well), 0.2 ml. Incubation was at 37°C with air as the gas phase.

* Preliminary data on the oxidation of this compound were reported by R. F. Franz in a footnote (2).
† One flask per run.
‡ These compounds are also reported by Posternak and Reymond (4).
§ The second run was continued to 240 minutes, at which time oxidation was still proceeding at an undiminished rate.

**Results**

In addition to the compounds listed in Table I, D-inositol, L-inositol, pinitol, quebrachitol, and 2-desoxy-myo-inositol were tested as substrates for resting cells of *A. suboxydans*. The results obtained by Chargaff *et al.* (1, 2) with these inositols were completely confirmed. The data pertaining to the hitherto unreported inositols and derivatives are presented in Table I.
In each run described, two flasks were used for each substrate. In addition, two flasks without substrate and two flasks containing about 12 µM of myo-inositol as the substrate were included. The myo-inositol was uniformly oxidized in 15 to 30 minutes, with an average oxygen consumption of 1.10 gm. atoms per mole. With one exception, shaking was continued until substrate oxidation had ceased. The oxygen consumption values in Table I are corrected for endogenous uptake.

Fig. 2. Oxidation by a cell-free enzyme preparation from A. suboxydans. The enzyme was obtained as described by Franzl and Chargaff (5). Each Warburg vessel contained: enzyme, 1.0 ml. (≤ 0.35 mg. of N); 1/15 M phosphate buffer, pH 6.3, 1.7 ml.; substrate, 0.3 ml. (in the side arm, 11.3 to 18.3 µM); and 10 per cent KOH, 0.2 ml. (in the center well). In the cases indicated, 0.5 ml. of heat-killed bacterial suspension was added at the expense of buffer. Incubation was at 37°, with air as the gas phase.

The behavior of γ-cyclohexanetriol with the cell-free preparation is shown in Fig. 2. In accordance with the observation of Franzl and Chargaff (5), the oxidation of cyclitols is minimal in the absence of added heat-killed bacteria. On the addition of boiled cells, the oxidation of myo-inositol and of the tetrod dihydroconduritol is markedly activated, but the oxidation of γ-cyclohexanetriol is not increased. It therefore seems clear that the latter oxidation is not catalyzed by the oxidase or oxidases which attack the higher cyclitols, but by a separate enzyme.

It may be noted from Table I that four of the cyclitols tried (II, VIa, VIb, and IX) were not oxidized by resting cells of A. suboxydans. It was
of interest to see whether these compounds would inhibit the oxidation of myo-inositol. To this end, the free amines II, VIa, and IX were incorporated in the buffer by dissolving them in 85 per cent phosphoric acid and diluting and neutralizing to pH 6, and the oxidation of myo-inositol in these buffers was then tested as described above. The following levels were used (micromoles per flask): scyllo-inosamine (II) up to 425; dL-epi-inosamine-2 (VIa) up to 206; and myo-inosamine-2 (IX) up to 79. No inhibition of the oxidation rate was observed. In some cases, the total oxygen uptake was slightly increased.

Oxidation Products

Efforts were made to characterize the product of the bacterial oxidation of muco-inositol by treatment with phenylhydrazine and by oxidation with nitric acid. The phenylhydrazine derivative was amorphous and resisted purification. A calcium salt of the nitric acid oxidation product was obtained, but attempts to identify it met with failure.

The Acetobacter oxidation of N-acetyl-DL-myo-inosamine-4 is being studied in connection with another project now under way in this laboratory, and will be described in a later communication.

Chemical studies of the oxidation of dihydroconduritol were carried out on material obtained by fermentation (6). Immediately after removal of the bacterial cells, the oxidation product was treated with hydrogen and platinum catalyst under acid conditions. The purpose of the hydrogen treatment was to convert the keto group to methylene (19), leaving a stable cyclohexanetriol to be identified. If no rearrangements occurred, one of the known 1, cis-2, trans-3-cyclohexanetriols would be formed by hydrogenolytic removal of the keto group resulting from bacterial oxidation at C-1 or C-4. If the oxidation took place at C-5 or C-6, hydrogenolysis would give a 1, trans-2, cis-4-cyclohexanetriol. Positions 1 and 4 are equivalent in dihydroconduritol, if one neglects optical isomerism, and positions 5 and 6 form a second equivalent pair.

Treatment of the residue from the hydrogenolysis with benzoyl chloride gave a mixture of benzoates from which a small amount of constant melting (138–139°) material was obtained by repeated recrystallization. This substance, after saponification, consumed 2 molar equivalents of periodate and is therefore the tribenzoate of a 1,2,3-cyclohexanetriol. Mixed melting point determinations were made with all the three possible 1,2,3-cyclohexanetriol tribenzoates. Depressions were obtained with the dextro-1, cis-2, trans-3 (+β) isomer and the 1, cis-2, cis-3 (γ) isomer, but not with the 1, trans-2, cis-3 (α) isomer. The isolated material is not quite pure, since

3 Synthesized by the method of Posternak and Ravenna (6).
4 M.p. 139–140°; kindly furnished by Professor C. B. Purves of McGill University.
it has a small (+) optical rotation (α-cyclohexanetriol is a meso compound), and its carbon content is low, suggesting that the impurity is a tetrol tetrabenzoate. The melting point data conclusively identify it, however, as 1, trans-2, cis-3-cyclohexanetriol tribenzoate, and, since this configuration does not exist preformed in dihydroconduritol, it must be an artifact. The determination of the position at which A. suboxydans attacks dihydroconduritol therefore depends upon the results of future work.

Further evidence as to the identity of the isolated triol tribenzoate was obtained by comparing its infra-red spectrum with the spectra of the three authentic triol tribenzoates. The spectra are distinctive, and that of the α-trienzoate (1, trans-2, cis-3) is identical with the spectrum of the isolated compound.

Professor T. Posternak of Geneva (private communication) has recently found D-1, 2-dideoxy-myo-inositol in the mixture resulting from the hydrogenation under neutral conditions of the Acetobacter oxidation product from dihydroconduritol. This finding would seem to exclude positions 4 and 5 as sites for the oxidation.

With regard to conduritol, oxidation at position 1 or 4 would give an α, β-unsaturated ketone, while oxidation at position 5 or 6 would give a β, γ-unsaturated ketone. The supernatant fluid from a blank incubation (buffer only, no substrate) of Acetobacter cells has a considerable end-absorption in the ultraviolet, but, when oxidized conduritol is present, the shape of the curve is altered and the absorption is greater. A plot of the difference between the two curves levels off at 215 με, and the ordinate at this point corresponds to a molar extinction of about 7000. These data are consistent with the formulation of this oxidation product as an α, β-unsaturated ketone. More definite evidence will of course be required before a firm conclusion can be drawn.

The authors are indebted to Mr. Donald R. Johnson and Professor A. L. Wilds of the Department of Chemistry for help in the preparation and interpretation of the absorption spectra.

DISCUSSION

Chargaff's rules (2) relating to the specificity of the cyclitol-oxidizing system of A. suboxydans are based upon the assumption that the preferred conformation of a cyclitol is that chair form which has the minimal number of polar hydroxyls or equivalent groups. The greater stability of the
form with the fewer polar substituents is well established in the case of the alkyl- and halogen-substituted cyclohexanes and similar derivatives (20, 21). More recently, Reeves (22) has obtained data indicating that the pyranose derivatives of the sugars conform to the same principle. In the light of this work, Chargaff's assumption seems most reasonable, since the inositols bear a very close structural resemblance to the pyranosides.

According to the rules cited, the necessary conditions for the oxidation by the organism of a hydroxyl group of a cyclitol are (1) that the hydroxyl group must be polar, and (2) that, when oriented as shown in Fig. 3, the cyclitol must have an equatorial hydroxyl in the clockwise meta position d. It was shown that oxidation proceeds, regardless of whether the hydroxyls in positions a, b, and e are polar or equatorial. The presence of a keto group or the absence of an oxygen function in these positions was likewise shown to be qualitatively without effect. However, it is possible that a hydroxyl group, to be oxidized, must meet another condition, i.e., (3) that an equatorial hydroxyl be present in the para position c. There is little information on this point, since, of the sixteen higher cyclitols tried by previous workers, only pinitol may be oriented so that there is anything other than an equatorial hydroxyl at this position. One orientation of pinitol places the (equatorial) methoxyl group of the compound at position c, but, as suggested by Magasanik et al. (2), the failure of pinitol to undergo oxidation may be due to an effect of the methoxyl group which is not position-specific. The idea that the methoxyl group inhibits oxidation even in a non-critical position is strengthened now that the structure of quebrachitol is known (23). Quebrachitol, which is not oxidized, has its (equatorial) methoxyl at position a in one of the Chargaff orientations.

The Bertrand-Hudson generalizations have been very useful as a guide to the use of A. suboxydans for the production of ketoses from open chain

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**FIG. 3.** Hypothetical sketch of an oxidation-susceptible cyclitol oriented on the oxidizing enzyme, as seen from above. The open circle represents a south polar hydroxyl (i.e., one which projects downward); hydroxyls connected to the ring by bond lines are equatorial.

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*Quebrachitol is 1-methyl-L-inositol.*
sugar alcohols. Rules such as those of Chargaff have equal potentialities in cyclitol chemistry, and it is important to establish their limits of applicability. The work of Posternak and Reymond (4) shows that they presently describe the situation only with respect to cyclitols having five or six hydroxyls or related substituents. In the case of the lower cyclitol, \( \gamma \)-cyclohexanetriol (X), the reason for non-applicability is made clear by the results of the present investigation. If the preferred conformation of this compound is "minimum polar," then the hydroxyl at position 2 is the only polar one. This hydroxyl does not meet condition (2) above; hence, no oxidation would be predicted. *A. suboxydans* attacks the compound, however, and, more important, attacks it at one of the positions 1 (equivalent to positions 3) (6). But since, as shown under "Results," the oxidizing enzyme is different from that which oxidizes the higher cyclitols, the rules for the latter compounds cannot be expected to apply. No decision as to whether the triol follows Hudson's rule can be made until the optical configuration of the product is established.

However, the above finding emphasizes the fact that our lack of knowledge concerning the number of individual oxidases present in the cells of *A. suboxydans* is responsible for some of our confusion about the validity of generalizations regarding the oxidative specificity of this organism. The cyclohexanetetrols and lower cyclitols may well be a mixed group of substrates, some of which are attacked by the higher cyclitol oxidase and some by the lower cyclitol oxidase, which may or may not be identical with the enzyme responsible for the oxidation of sorbitol and the open chain polyalcohols. The tetrols which showed oxidation counter to prediction in the experiments of Posternak and Reymond would be examples of the latter class, while dihydroconduritol (IV) would be an example of the former. This tetrol was shown in the present study (Fig. 2) to behave like *myo*-inositol, in that its oxidation by the cell-free enzyme preparation depends upon the presence of boiled cells. The oxygen uptake with whole cells is that predicted by the Chargaff rules, but only when the structure and configuration of the oxidation product are known can it be decided whether the attack took place at the predicted position.

For maximal utility, rules such as those under discussion should not only describe the minimal conditions for oxidation of a substrate; they should also permit one to predict whether a given hydroxyl in a potential substrate will be oxidized. The results of the present investigation, summarized in Table II, show that this higher criterion is not met. Of the compounds listed in Table II, four (I, II, IIIa, and IIIb) behaved essentially as predicted, three (V, VIa, and VIIb) failed to live up to the predictions made for them, and three (IV, VIII, and IX) do not come within the purview of the rules. Some of these compounds warrant further comment.

*muco*-Inositol (I) and *N*-acetyl-\( DL \)-*myo*-inosamine-4 (IIIb) are peculiar
in that their oxygen consumption was higher than that predicted by a fraction of a gm. atom. The cyclic ketoses resulting from the oxidation of the inositol are sensitive compounds, and this extra oxidation may be non-enzymatic. Or, it is possible that we may be dealing with additional examples, in these cases slow and incomplete, of enzymatic attack on hydroxyls which do not meet the minimal qualifications laid down by the rules.

The situation with regard to sequoyitol (V) is not yet completely clear. The bacterial oxidation of this myo-inositol methyl ether will be discussed in a forthcoming paper on the structure of the compound.

**TABLE II**

*Predicted and Observed Behavior of Cyclitols and Cyclitol Derivatives*

<table>
<thead>
<tr>
<th>Cyclitol</th>
<th>Positions of polar hydroxyls</th>
<th>Oxidation predicted at</th>
<th>Oxygen consumed</th>
<th>Positions oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td>muco-Inositol (I)</td>
<td>2, 3, 4</td>
<td>2, 3</td>
<td>2.25</td>
<td>Not known</td>
</tr>
<tr>
<td>scylo-Inosamine (II)</td>
<td>None</td>
<td>None</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>dl-myo-Inosamine-4 (IIIa)</td>
<td>2</td>
<td>2 (b form only)</td>
<td>0.5</td>
<td>Not known</td>
</tr>
<tr>
<td>N-Acetyl-dl-myo-inosamine-4 (IIIb)</td>
<td>2</td>
<td>2 &quot; &quot; &quot;</td>
<td>0.75</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Dihydroconduritol (IV)</td>
<td>1, 6 or 4, 5</td>
<td>1 (Possibly 5; not both)</td>
<td>1</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Sequoyitol (V)</td>
<td>2</td>
<td>2 (Possibly)</td>
<td>0</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>dl-epi-Inosamine-2 (VIa)</td>
<td>4</td>
<td>4 (L form only)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-dl-epi-inosamine-2 (VIb)</td>
<td>4</td>
<td>4 &quot; &quot; &quot;</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Conduritol (VIII)</td>
<td>None</td>
<td>?</td>
<td>1</td>
<td>Not known</td>
</tr>
<tr>
<td>myo-Inosamine-2 (IX)</td>
<td>None</td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* See Table I.

The introduction of an amino group into an inositol molecule might well be expected to alter its behavior in any given enzyme system, and the non-oxidation of L-epi-inosamine-2 (L-VIa) and its N-acetate (L-VIb) (both tried in the L form) would therefore not be surprising if it were not for the fact that the epimeric amines myo-inosamine-4 (IIIa) and N-acetylmyo-inosamine-4 (IIIb) are readily attacked. One can only conclude that the oxidizing enzyme is not completely uncritical with regard to the groups present at positions a, b, and e.

Conduritol (VIII) and myo-inosamine-2 (IX) are of interest because they have structural features not previously occurring in *Acetobacter* sub-

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Posternak and Reymond (4) found that D epi inosose 2 and D 2 desoxy epi inositol are slowly attacked by their strain of *A. suboxydans*. The polar hydroxyls in these compounds do not fulfil the requirements listed above.
strates. Conduritol differs from all of the other known cyclitols in being a derivative of cyclohexene. The molecular geometry of cyclohexene apparently does not differ radically from that of cyclohexane, however (24, 25). Assuming that the hydroxyl groups in conduritol do not cause any major alteration of the basic cyclohexene geometry, it seems likely that the orientation of conduritol on the oxidizing enzyme is governed by the same factors which are operative in the case of its dihydro derivative.

*myo*-Inosamine-2 represents *myo*-inositol in which the oxidation-susceptible polar hydroxyl is replaced by a polar amino group. If the enzyme were able, as some are, to attack amino as well as hydroxyl groups, then *myo*-inosamine-2 would be oxidized to *myo*-inosose-2 and ammonia. Actually, no oxidation takes place.

In conclusion, it should be pointed out that the failure of certain inositols to be oxidized by *A. suboxydans* when oxidation is predicted by the rules of Magasanik and Chargaff in no way argues against the suggestion of the Columbia authors (2) that the polar hydroxyl being oxidized, the equatorial hydroxyl at position d (Fig. 3), and possibly the equatorial hydroxyl at position c, are the groups through which inositols and quercitols are bound to the higher cyclitol oxidase or oxidases which occur in this organism.

**SUMMARY**

1. Ten cyclitols and cyclitol derivatives which had not previously been reported as substrates for *Acetobacter suboxydans* were tested with suspensions of resting cells of this organism. In addition, 1, *cis*-2, *cis*-3-cyclohexanetriol (*r*-cyclohexanetriol) was tested with a cell-free cyclitol oxidase preparation.

2. The enzyme which oxidizes the triol must be different from that which oxidizes the higher cyclitols, since the triol is not attacked in the cell-free system. The fact that Chargaff's rules do not correctly predict the course of the oxidation of *r*-cyclohexanetriol is therefore irrelevant.

3. Chargaff's rules appear to be valid as a description of the minimal steric requirements of the higher cyclitol oxidase, but certain cyclitols which fulfil these minimal requirements are not oxidized.

4. Some inosamines which are not oxidized by *A. suboxydans* were checked for ability to inhibit the oxidation of *myo*-inositol. No inhibition was observed in any case.

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