THE OXIDATION OF d-QUERCITOL BY ACETOBACTER SUBOXYDANS*

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A previous communication from this laboratory (1) included the demonstration that d-quercitol (I) was oxidized by resting Acetobacter suboxydans. The maximal oxygen consumption amounted to 2 gm. atoms per mole of substrate. The present paper deals with the isolation and identification of the oxidation product.

\[ \text{[Diagram]} \]

The oxidation was carried out on a preparative scale with resting bacteria in the presence of oxygen by the technique described in the preceding paper (2). Oxygen uptake under these conditions ceased when about 1.3 atoms of oxygen had been taken up per molecule of d-quercitol. The addition of phenylhydrazine to the reaction mixture resulted in the deposition of a dextrorotatory, crystalline, yellow compound, having the analytical composition of the bisphenylhydrazone of a diketo quercitol. The yield was 33 per cent of the theoretical, based on the amount of oxygen consumed. The low recovery of bisphenylhydrazone and the incomplete oxidation (measured by the oxygen uptake) seem to indicate that the attack on a large portion of the substrate stopped with the formation of an intermediate monoketo compound. This finding is in keeping with observations reported previously (1), which showed that the rate of the first oxidation step was considerably greater than that of the second, and that with a smaller quantity of bacteria the total oxygen uptake amounted to only 1 atom per molecule of substrate. Attempts at the isolation from the reaction mixture of the phenylhydrazone of a monoketo compound were, however, not successful.

The absorption spectrum of the bisphenylhydrazone reproduced in Fig. 1

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† William J. Gies Fellow, 1947-48. This report is from a dissertation submitted by Boris Magasanik in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.
is characteristic of osazones (1, 3). The consumption of periodic acid in 66 per cent ethyl alcohol at room temperature corresponded to 2 moles of oxidant per mole of substance. The compound must therefore be the bisphenylhydrazone of an α-diketone, with the three hydroxyl groups situated on adjacent carbon atoms. Structures II and III only are compatible with these results.

\[
\begin{align*}
&\text{N}--\text{NHC}_6\text{H}_5 \\
&\text{C}_6\text{H}_5\text{NH}--\text{N}--\text{NHC}_6\text{H}_5 \\
&\text{C}_6\text{H}_5\text{NH}--\text{N}
\end{align*}
\]

(II) (III)

In order to decide between these two possible structures, the rates of oxidation by periodic acid of this α-bisphenylhydrazone and of the one prepared from d-inositol (IV) (1) were compared.

\[
\begin{align*}
&\text{N}--\text{NHC}_6\text{H}_5 \\
&\text{N}--\text{NHC}_6\text{H}_5
\end{align*}
\]

(IV)

The measurements were carried out in very dilute, strongly acidic, alcoholic solution, in order to allow observation by slowing down the reaction rates sufficiently. The results summarized in Table I reveal that the α-bisphenylhydrazone derived from d-quercitol was oxidized in the early phase of the reaction at a speed about half of that at which the d-inositol derivative was attacked.

It has been shown repeatedly (4, 5) that cyclohexitols possessing two vicinal hydroxyl groups in the cis position are cleaved by periodic acid at an appreciably greater rate than are their trans isomers.

Compound IV (1) does not possess a pair of vicinal cis hydroxyls. A compound having structure III, with one pair of cis hydroxyls, may therefore be expected to be oxidized more rapidly than IV. The observation that the α-bisphenylhydrazone derived from d-quercitol was oxidized at a considerably slower rate than IV favors the assumption that it possesses structure II, in which the vicinal hydroxyl groups are in the trans position. The slower oxidation may be due to the fact that compound II has only three hydroxyl groups, whereas compound IV has four.

The evidence presented indicates that the hydroxyl groups in positions 2 and 3 of d-quercitol (I) are oxidized by Acetobacter suboxydans to give...
Fig. 1. Absorption spectrum (in absolute ethanol) of the α-bisphenylhydrazone of cyclohexane-(1,5) cis-6-triol-2,3-dione (II).

Table I

Rates of Oxidation by Periodic Acid of α-Bisphenylhydrazones Derived from d-Quercitol and d-Inositol

<table>
<thead>
<tr>
<th>Duration of oxidation</th>
<th>Oxidant consumed per mole α-bisphenylhydrazone derived from</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d-Quercitol</td>
<td>d-Inositol</td>
</tr>
<tr>
<td>min.</td>
<td>moles</td>
<td>moles</td>
</tr>
<tr>
<td>1</td>
<td>0.30</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.36</td>
</tr>
<tr>
<td>5</td>
<td>0.68</td>
<td>1.25</td>
</tr>
<tr>
<td>10</td>
<td>0.98</td>
<td>1.48</td>
</tr>
<tr>
<td>30</td>
<td>1.40</td>
<td>1.78</td>
</tr>
<tr>
<td>1440</td>
<td>2.35</td>
<td>3.01</td>
</tr>
</tbody>
</table>

cyclohexane-(1,5) cis-6-triol-2,3-dione, isolated as its bisphenylhydrazone (II).1

1 With respect to the numbering of the carbon atoms, compare a previous discussion (1)
The steric structure of d-quercitol has been discussed in a previous communication (1). It was suggested there that the hydroxyl groups 2 and 3 are situated in the north and south polar planes, respectively, while the remaining hydroxyls are ranged in the equatorial belt. The results reported in this paper are, therefore, in agreement with the previously stated rule for the minimum steric requirements for the oxidation of inositol isomers by Acetobacter suboxydans; viz., that only polar hydroxyl groups are oxidized (1).

It may be of interest to point out that Bertrand's rule (6), which describes the action of the microorganism on straight chain polyhydroxy compounds, would have led to the expectation that a monoketo compound should be formed from d-quercitol.

EXPERIMENTAL

**Material**

d-Quercitol was a commercial preparation, melting at 239–240°; \([\alpha]_b^{23} = +23.9°\). Acetobacter suboxydans was cultivated by the methods described previously (1).

**Formation of Compound II from d-Quercitol**

To a solution of 1.76 gm. (10.7 mm) of d-quercitol in 25 cc. of \(\frac{1}{5}\) phosphate buffer of pH 6.0 a suspension of washed Acetobacter suboxydans (about 0.5 gm., dry weight) in 25 cc. of 0.9 per cent aqueous sodium chloride was added. The flask was attached to a graduated reservoir filled with oxygen and the mixture agitated with a magnetic stirrer in a room maintained at 37°. The consumption of oxygen, rapid at first, ceased when 167 cc. (about 6.7 mm) of oxygen had been taken up in 230 minutes. The bacteria were removed by centrifugation and filtration through infusorial earth and 4 cc. of phenylhydrazine in 8 cc. of 50 per cent acetic acid were added to the clear, yellow filtrate. The precipitate (1.45 gm.) weighed, after being washed with cold ethanol, 750 mg. (2.2 mm, 33 per cent of the theoretical yield, based on the amount of oxygen consumed) and formed pale yellow crystals. The bisphenylhydrazone of cyclohexane-(1,5) cis-6-triol-2,3-dione (II), after two recrystallizations from aqueous methyl cellosolve and boiling ethanol, melted (with decomposition) at 199–200°. The optical rotation in 1:1 ethanol-pyridine (\(c = 0.357, l = 0.5\) dm., \([\alpha]_b^{25} = +0.11°\)) was found as \([\alpha]_b^{25} = +62°\).

**C\(_1\)H\(_{25}\)O\(_2\)N\(_4\) (340.4)**

Calculated. C 63.5, H 5.9, N 16.5

Found. " 63.1, " 5.6, " (Dumas) 16.6

*The melting points, reported without correction, were determined with an electrically heated stage (Fisher-Johns). The intact crystals were placed on the stage which was preheated to about 5–7° below the melting point.*
The various mother liquors yielded, on evaporation, red amorphous powders which were very soluble in alcohol.

Absorption Spectrum—The absorption spectrum of II (0.0288 mM solution in absolute ethanol) is reproduced in Fig. 1. The band at 258 m\(\mu\) (\(\varepsilon = 20,900\)) has the same center of absorption as the corresponding bands of the bisphenylhydrazones derived from \(l\)- and \(d\)-inositol (1). The other two bands of II at 308 m\(\mu\) (\(\varepsilon = 11,400\)) and 389 m\(\mu\) (\(\varepsilon = 17,700\)) are at slightly lower wave-lengths than the corresponding bands of the inositol derivatives.

Action of Periodic Acid on Compound II

Total Consumption—The \(\text{HIO}_4\) consumption was determined by treating 20 cc. portions (each containing about 60 micromoles of the bisphenylhydrazone in 60 per cent ethanol) with 234 micromoles of periodic acid in 0.5 cc. of water at room temperature for 1 hour (1, 7). The excess periodic acid was determined with 0.1 N sodium arsenite in the usual manner. The average consumption (per mole of compound) of periodic acid was 2.1 moles.

Rate of Periodic Acid Oxidation of Compounds II and IV—To 10 cc. of an aqueous solution containing 2.18 micromoles of periodic acid and 0.5 cc. of 4 N sulfuric acid, 0.5 micromole of the bisphenylhydrazone in 0.5 cc. of ethanol was added. The reaction was stopped by the addition of 2 cc. of a saturated solution of potassium bicarbonate and some solid potassium iodide. The excess periodic acid was determined in the customary manner with 0.01 N sodium arsenite with the aid of a micrometric burette (8). The results are summarized in Table I.

We are indebted to Miss R. Rother for the microanalyses.

SUMMARY

Following the oxidation of \(d\)-quercitol (I) by resting \textit{Acetobacter suboxydans}, the \(\alpha\)-bisphenylhydrazone (II) of a diketo quercitol was isolated and its structure determined. The results show that hydroxyl groups 2 and 3 of \(d\)-quercitol, which are situated in polar planes, were oxidized. This is in agreement with the previously stated rule for the minimum steric requirements for the oxidation of inositol by \textit{Acetobacter suboxydans}.

BIBLIOGRAPHY

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