Studies in the Sphingolipids Series. VII.
The Configuration of \( \alpha \)-Hydroxy-n-hexacosanoic Acid of Yeast Cerebrin

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It has been established that \( \alpha \)-hydroxy-n-hexacosanoic acid of yeast cerebrin has the D-configuration. This was concluded on the basis of molecular rotations of some of its derivatives which were compared with those of the corresponding derivatives of D-\( \alpha \)-hydroxy acids.

1. Yeast cerebrin yields on hydrolysis a mixture of \( \alpha \)-hydroxy-n-fatty acids, one of which has been identified by Reindel et al.\(^2,3\) as \( \alpha \)-hydroxy-n-hexacosanoic acid (I). More recently Chibnall, Piper and Williams\(^4\) have investigated the yeast hydroxy acids in detail and concluded on the basis of X-ray data and melting point determinations of the n-fatty acids obtained on oxidation, that the cerebrin hydroxy acid is \( \alpha \)-hydroxy-n-hexacosanoic acid with not more than 10\% of \( \alpha \)-hydroxy-n-tetracosanoic acid (cerebronic acid). The purified acid (I) was found to be optically active showing in pyridine the specific rotations of \(+\)2.1\(^{o}\) and \(+\)2.45\(^{o}\) respectively.

2. Recently Mislow and Bleicher\(^5\) assigned the D-configuration to cerebronic acid (\( \alpha \)-hydroxy-n-tetracosanoic acid), one of the hydrolysis products of the sphingolipide cerebron. This was established through application of the Freudenberg's Displacement Rule (Rule of Shifts, Verschiebungssatz)\(^6\). It states that two asymmetric molecules of similar structure undergo shifts in molecular rotations in the same direction when both are chemically altered in the same way. The rotations of derivatives of cerebronic acid have been compared with those of corresponding derivatives of some D-\( \alpha \)-hydroxy acids. It was suggested also that \( \alpha \)-hydroxy-n-hexacosanoic acid of cerebron might possess the D-configuration.

3. In this paper the experimental evidence for the D-configuration of \( \alpha \)-hydroxy-n-hexacosanoic acid of yeast cerebrin is presented. This was established on the basis of the Displacement Rule following the experience of Mislow and Bleicher\(^5\). For this purpose some derivatives of the yeast acid such as methyl and ethyl ester, amide as well as methyl ester of \( \alpha \)-methoxy acid have been prepared and their molecular rotations compared with those of the corresponding derivatives of (D-)cerebronic acid, D-mandelic acid, D-hexahydromandelic acid and D-lactic acid. All data on molecular rotations are ta-

* Paper VI, see reference\(^1\).
bulated in Table I. The parallelism in rotations showing successively more positive shifts in the above sequence of compounds is evident. In the light of these data the d-configuration should be assigned to the yeast acid.

**TABLE I**

*Comparison of Molecular Rotations [M]D of Derivatives of α-Hydroxy-n-hexacosanoic Acid with those of α-Hydroxy Acids which Belong to the d-Series***

<table>
<thead>
<tr>
<th>Acid</th>
<th>R—CH—COR1</th>
<th>R1 = CH3O—</th>
<th>R2 = C18H36CO—</th>
<th>R1 = CH3O—</th>
<th>R2 = H—</th>
<th>R1 = CH3O—</th>
<th>R2 = H—</th>
<th>R1 = NH2—</th>
<th>R2 = H—</th>
<th>R1 = CH3O—</th>
<th>R2 = CH3—</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Hydroxy-n-hexacosanoic</td>
<td></td>
<td>+ 8.2 p</td>
<td></td>
<td>+ 11.7 p</td>
<td>+ 25.2 p</td>
<td>+ 48.4 p</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(D)-Cerebroinic</td>
<td>+ 1.0 p</td>
<td>+ 10.4 p</td>
<td>+ 14 p</td>
<td>+ 29.6 p</td>
<td>+ 74 p</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>D-Mandelic</td>
<td>—433 n</td>
<td>—237 m</td>
<td>—211 m</td>
<td>—144 pf</td>
<td>—173 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Hexahydro-mandelic</td>
<td>—38.6 n</td>
<td>—36.8 n</td>
<td>—24.4 n</td>
<td>+ 75.5 e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Lactic</td>
<td>—40.3 n</td>
<td>+ 8.6 n</td>
<td>+ 13.6 n</td>
<td>+ 22.3 w</td>
<td>+ 112.7 n</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*** All values here tabulated except those of α-hydroxy-n-hexacosanoic acid are taken from the paper of Mislow and Bleicher where also a survey on corresponding references is to be found. The letters following the rotations refer to the solvent: n = no solvent; p = pyridine; m = methanol; pf = 1:1 pyridine-formic acid; a = acetone; e = ethanol; w = water.

**EXPERIMENTAL**

The melting points are uncorrected.

α-Hydroxy-n-hexacosanoic Acid (I)

The acid was isolated from yeast cerebrin following the procedure by Reindel. The acid was purified through the potassium salt and recrystallized from acetic acid. It melted at 101—103° and contained probably a small quantity of α-hydroxy-n-tetraicosanoic acid.

Methyl α-hydroxy-n-hexacosanoate

The ester was prepared in the usual manner by saturating the methanol solution of the acid with dry hydrogen chloride and refluxing the solution for 6 hrs. The waxy solid was distilled in vacuo; b. p. 200—205/0.2 mm; m. p. 69—70°; [α]D = + 1.91° (c = 6.8, in pyridine) (lit. m. p. 70—72°).

Ethyl α-hydroxy-n-hexacosanoate

Waxy solid, b. p. 200—205/0.2 mm; m. p. 72—73°; [α]D = + 2.65° (c = 6.8, in pyridine) (lit. m. p. 73—74.5°).

α-Hydroxy-n-hexacosanoic Acid Amide

A suspension of the methyl ester (2 g.) in absolute methanol (20 ml.) was saturated with anhydrous ammonia at 0° and allowed to stand at room temperature
for 48 hrs. The colorless solid was filtered off and recrystallized three times from methanol; m.p. 125°C, [α]_D^{24} = +6.12° (c = 1, in pyridine) (lit. m.p. 122—124°C).

**Methyl α-methoxy-n-hexacosanoate**

A suspension of methyl α-hydroxy-n-hexacosanoate (6.2 g.) and silver oxide (6 g.) in methyl iodide (20 ml.) were refluxed for 24 hrs. After cooling the mixture was treated with warm ether and undissolved inorganic material removed by filtration. The residue after evaporating the ether was distilled in vacuo to give 5.3 g. of a waxy solid; b. p. 201—203°C/0.15 mm; m.p. 65—66°C; [α]_D^{23} = +11.0° (c = 5, in pyridine).

**Anal.** 8.715 mg. subst.; 24.50 mg. CO₂, 9.72 mg. H₂O
C_{28}H_{56}O₃ (440.73) calc'd: C 76.30%; H 12.81%
found: C 76.72%; H 12.48%

**α-Methoxy-n-hexacosanoic Acid**

The methyl ester (5 g.) was hydrolized with 2 N ethanolic solution of potassium hydroxide (50 ml.) under reflux for 1 hr. The solution was then poured into 500 ml. of N sulphuric acid, the colorless solid extracted with ether and crystallized from acetone. Yield 3.7 g., m.p. 98—100°C.

**Anal.** 8.405 mg. subst.: 23.49 mg. CO₂, 9.60 mg. H₂O
C₂₁H₄₆O₃ (426.70) calc'd: C 76.00%; H 12.76%
found: C 76.27%; H 12.78%

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