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Studies in the Sphingolipids Series. VII*.
The Configuration of α -Hydroxy-n-hexacosanoic Acid
of Yeast Cerebrin

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It has been established that α -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- α -hydroxy acids.

1. Yeast cerebrin yields on hydrolysis a mixture of α -hydroxy-n-fatty acids, one of which has been identified by Reindel et al.^{2,3} as α -hydroxy-n-hexacosanoic acid (I). More recently Chibnall, Piper and Williams⁴ 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 α -hydroxy-n-hexacosanoic acid with not more than 10% of α -hydroxy-n-tetracosanoic acid (cerebronic acid). The purified acid (I) was found to be optically active showing in pyridine the specific rotations of $+2.1^{03}$ and $+2.45^{04}$ respectively.

2. Recently Mislow and Bleicher⁵ assigned the D-configuration to cerebronic acid (α -hydroxy-n-tetracosanoic acid), one of the hydrolysis products of the sphingolipide cerebrin. 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- α -hydroxy acids. It was suggested also that α -hydroxy-n-hexacosanoic acid of cerebrin might possess the D-configuration.

3. In this paper the experimental evidence for the D-configuration of α -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 Bleicher⁵. For this purpose some derivatives of the yeast acid such as methyl and ethyl ester, amide as well as methyl ester of α -methoxy acid have been prepared and their molecular rotations compared with those of the corresponding derivatives of (D-)cerebronic acid, D-mandelic acid, D-hexamandelic acid and D-lactic acid. All data on molecular rotations are ta-

* Paper VI, see reference¹.

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**

$\begin{array}{c} \text{Acid} \\ \text{R}-\text{CH}-\text{COR}_1 \\ \\ \text{OR}_2 \end{array}$	$\begin{array}{l} \text{R}_1 = \text{CH}_3\text{O}- \\ \text{R}_2 = \text{C}_6\text{H}_5\text{CO}- \end{array}$	$\begin{array}{l} \text{R}_1 = \text{CH}_3\text{O}- \\ \text{R}_2 = \text{H}- \end{array}$	$\begin{array}{l} \text{R}_1 = \text{C}_2\text{H}_5\text{O}- \\ \text{R}_2 = \text{H}- \end{array}$	$\begin{array}{l} \text{R}_1 = \text{NH}_2- \\ \text{R}_2 = \text{H}- \end{array}$	$\begin{array}{l} \text{R}_1 = \text{CH}_3\text{O}- \\ \text{R}_2 = \text{CH}_3- \end{array}$
α -Hydroxy- <i>n</i> -hexacosanoic		+ 8.2 p	+11.7 p	+25.2 p	+48.4 p
(D-)Cerebronic	+ 1.0 p	+10.4 p	+14 p	+29.6 p	+74 p
D-Mandelic	-433 n	-237 m	-211 m	-144 pf	-173 a
D-Hexahydro-mandelic	-38.6 n	-36.8 n	-24.4 n	+75.5 e	
D-Lactic	-40.3 n	+ 8.6 n	+13.6 n	+22.3 w	+112.7 n

** All values here tabulated except those of α -hydroxy-*n*-hexacosanoic acid are taken from the paper of Mislow and Bleicher⁵ 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 Reindel². 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*-tetracosanoic 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. 200–202°/0.2 mm; m. p. 69–70°; $[\alpha]_D^{24} = +1.91^\circ$ (c = 6.8, in pyridine) (lit.² m. p. 70–72°).

*Ethyl α -hydroxy-*n*-hexacosanoate*

Waxy solid, b. p. 200–205°/0.2 mm; m. p. 72–73°; $[\alpha]_D^{26} = +2.65^\circ$ (c = 6.8, in pyridine) (lit.² 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^o, $[\alpha]_D^{34} = + 6.12^o$ (c = 1, in pyridine) (lit.³ m. p. 122—124^o).

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^o/0.15 mm; m. p. 65—66^o; $[\alpha]_D^{23} = + 11.0^o$ (c = 5, in pyridine).

Anal. 8.715 mg. subst.; 24.50 mg. CO₂, 9.72 mg. H₂O
C₂₈H₅₆O₃ (440.73) calc'd: C 76.30; H 12.81^o/_o
found: C 76.72; H 12.48^o/_o

α -Methoxy-n-hexacosanoic Acid

The methyl ester (5 g.) was hydrolyzed 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^o.

Anal. 8.405 mg. subst.: 23.49 mg. CO₂, 9.60 mg. H₂O
C₂₇H₅₄O₃ (426.70) calc'd: C 76.00; H 12.76^o/_o
found: C 76.27; H 12.78^o/_o

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