

CCA-191

547.392.6.07:541.634

On the *erythro* and *threo*-2,3-Dihydroxytetracosanoic Acids

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Received July 24, 1960

The *erythro* and *threo*-2,3-dihydroxytetracosanoic acids have been prepared by hydroxylation of *trans*-2-tetracosenoic acid (I) with both peracetic acid and potassium permanganate. A procedure for the preparation of *trans*-2-tetracosenoic acid (I) is also described.

Long-chain 2,3-dihydroxy fatty acids belong to the poorly investigated compounds. Only a few synthetic representatives have been prepared so far^{1,2}. However, the optically active 2,3-dihydroxytetracosanoic acid, m. p. 116—117°, $[\alpha]_D = +2.69^\circ$ (in pyridine), has been reported to occur in nature. It has been liberated in the hydrolysis with alcoholic sulphuric acid of hydroxycerebrin — a peculiar phytosphingolipid isolated recently by Oda from the mycelium of penicillin producing mold Q 176³. The stereochemistry of the acid which contains two asymmetric carbon atoms is unknown.

In endeavour to carry out certain stereochemical studies we have prepared 2,3-dihydroxytetracosanoic acid synthetically. The only way for the preparation of such kind of compounds seemed to proceed through the corresponding 2,3-unsaturated fatty acids as the intermediates.

The hitherto undescribed *trans*-2-tetracosenoic acid (I) has been prepared essentially by the Ponzio's method of synthesis of unsaturated acids⁴ with the improvements given recently by Sweet and Estes⁵ and Myers⁶.

The starting material was commercial grade behenic acid (docosanoic acid) which was purified most satisfactorily by treatment with boiling ether. In this manner all lower and unsaturated acids have easily been removed. Docosanoic acid was converted in a series of classical reactions — through the intermediates ethyl docosanoate, 1-docosanol, 1-bromodocosane, diethyl *n*-docosylmalonate — into *n*-docosylmalonic acid. The bromination of the latter furnished in excellent yield *n*-docosylbromomalonic acid which was decarboxylated to give 2-bromo-tetracosanoic acid. Treatment of the latter with potassium iodide in ethanol followed by the action of alcoholic potassium hydroxide on the resulting solution of 2-iodotetracosanoic acid gave a mixture of *trans*-2-tetracosenoic acid (I) and 2-hydroxytetracosanoic acid. Both acids were easily separated due to the low solubility of the hydroxy acid in petroleum ether. Thus, the crude unsaturated acid was obtained in 27% yield based on the bromo acid. Other methods which were also examined proved less satisfactory.

The presence of the double bond in the acid which could not be purified to give the correct elemental analysis, was established by catalytic hydrogenation.

The infrared spectrum showed peaks at 975 and 1651 cm^{-1} (Myers⁶ gives 1653 cm^{-1} for the C_{18} -acid), indicating the *trans*-ethylenic structure.

The acid I was hydroxylated with alkaline potassium permanganate (*cis*-addition) and the 2,3-dihydroxytetracosanoic acid, m. p. 123° , was isolated in 12% yield. Hydroxylation of I with peracetic acid (*trans*-addition) gave the isomeric 2,3-dihydroxytetracosanoic acid melting at 103 – 105° (analytical sample). Somewhat impure acid, m. p. 100 – 102° , was obtained in about 10% yield. The low yields can be explained by the fact that the nucleophilic cha-

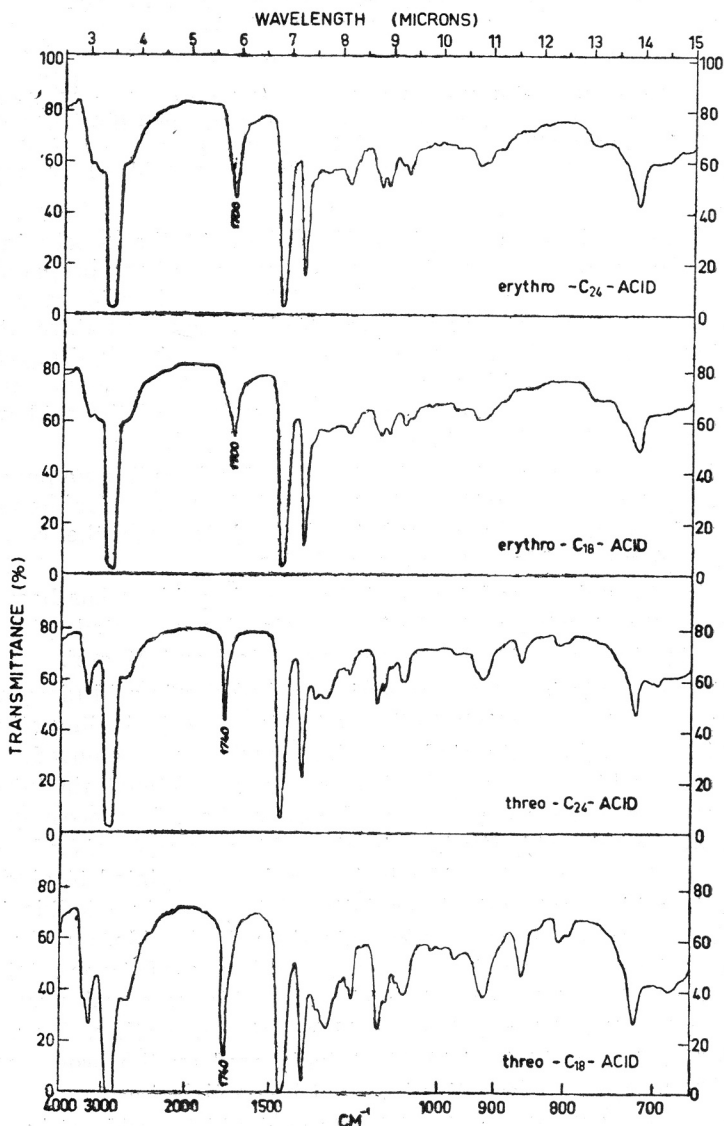


Fig. 1. Infrared spectra in nujol of *erythro* and *threo*-2,3-dihydroxyoctadecanoic acids and *erythro* and *threo*-2,3-dihydroxytetracosanoic acids.

acid was filtered off and the extraction with boiling ether repeated three times. Lower and unsaturated acids which are more soluble in ether remained in the filtrate. Docosanoic acid thus obtained melted at 79°.

Ethyl Docosanoate

Esterification of docosanoic acid (85 g., m. p. 79°) with ethanol in the presence of concentrated sulphuric acid gave 87.6 g. (92%) of a product melting at 48—48.5°.

1-Docosanol

The substance was obtained by reducing ethyl docosanoate (85 g., 0.23 mole) with lithium aluminium hydride (40 g., 0.27 mole) in ether. Yield 76 g. (99%), m. p. 69°.

1-Bromodocosane

Refluxing of 1-docosanol (75 g.), 48% hydrobromic acid (120 ml.) and concentrated sulphuric acid (30 ml.) gave 74 g. of the bromo compound, m. p. 43—46°.

Diethyl n-Docosylmalonate

To a solution of sodium ethoxide prepared from 4.4 g. (0.19 mole) of sodium and 100 ml. of absolute ethanol, 30 g. (0.19 mole) of diethyl malonate and 70 g. (0.18 mole) of 1-bromodocosane were added. After refluxing for 3 hrs. most of ethanol was distilled off on the water-bath and the reaction mixture poured into 300 ml. of ice-cold sulphuric acid. Extraction with ether and evaporation of the solvent gave 77 g. (92%) of the dicarboxylic acid ester melting at 47.5—48°.

n-Docosylmalonic Acid

A mixture of the foregoing ester (77 g., 0.165 mole) and a solution of potassium hydroxide (33 g., 0.59 mole) in water (70 ml.) was evaporated to dryness in a porcelain dish on the water-bath. The residue was treated with 5*N* sulphuric acid at 100° for 15 min. The crude dicarboxylic acid was filtered off by suction and extracted with a large quantity of ether. From the concentrated ether solution 65 g. (96%) colourless, crystalline product, m. p. 117—119°, was obtained. The alternative method is the recrystallization of the crude acid from glacial acetic acid. For analysis the substance was recrystallized twice from acetone; m. p. 123—124°.

Anal. 7.945 mg. subst.: 21.22 mg. CO₂, 8.47 mg. H₂O
 C₂₅H₄₈O₄ (412.63) calc'd: C 72.76; H 11.73%
 found: C 72.61; H 11.89%

n-Docosylbromomalonic Acid

To a suspension of *n*-docosylmalonic acid (55.5 g., 0.135 mole) in boiling ether (500 ml.) bromine (22.4 g., 0.14 mole) was added dropwise over a period of 1 hr. The initially slow reaction soon became vigorous, as seen by the rapid absorption of bromine, and all acid was finally dissolved. The ether solution was washed with water to remove hydrogen bromide and dried with sodium sulphate. The concentrated ether solution yielded colourless crystals (56 g., 85%, m. p. 96.5—100°) upon diluting with petroleum ether. Recrystallization from carbon tetrachloride gave the analytical sample melting at 103°.

Anal. 7.955 mg. subst.: 17.88 mg. CO₂, 7.08 mg. H₂O
 C₂₅H₄₇O₄Br (491.57) calc'd: C 61.09; H 9.64%
 found: C 61.34; H 9.96%

2-Bromotetracosanoic Acid

The crude *n*-docosylbromomalonic acid (56 g.) was decarboxylated by heating it on the oil-bath at 150—170° for 2 hrs. The brown-coloured melt was recrystallized from acetone to yield 47.2 g. (92%) of a slightly coloured product, m. p. 70—71°. For the analysis it was recrystallized from petroleum ether; m. p. 74°.

Anal. 8.440 mg. subst.: 20.07 mg. CO₂, 8.01 mg. H₂O
 C₂₄H₄₇O₂Br (447.55) calc'd: C 64.41; H 10.59%
 found: C 64.90; H 10.62%

trans-2-Tetracosenoic Acid

A mixture of 2-bromotetracosanoic acid (44.8 g., 1.0 mole), finely powdered potassium iodide (25 g., 0.15 mole) and 95% ethanol (150 ml.) was refluxed for 5 hrs. Potassium hydroxide (25 g., 0.45 mole) was added to the brown-coloured reaction mixture and refluxed for additional 2 hrs. Ethanol was distilled off from the water-bath and the flask content poured into 2*N* sulphuric acid. The precipitate was filtered off by suction and dissolved in ether. The ether solution was washed with sodium sulphite and water and dried with sodium sulphate. After standing overnight in the refrigerator 10.5 g. of crude 2-hydroxytetracosanoic acid, m. p. 84—87°, separated from the ether solution. A second crop of the hydroxy acid (5.1 g.) was added to the alkaline solution. The flask was immersed into an ice-bath and solution. The final filtrate was then evaporated to dryness, the residue dissolved in petroleum ether (200 ml., b.p. 40—65°) and cooled in the refrigerator overnight. The pale yellow crystals of the crude unsaturated acid (10.1 g., 27%) melted at 56—57°. It was used in the subsequent preparations without further purification.

Hydrogenation of a small sample of the unsaturated acid at atmospheric pressure and at room temperature in the presence of Adams' platinum catalyst yielded tetracosanoic acid, m.p. 83°.

threo-2,3-Dihydroxytetracosanoic Acid

A sample of *trans-2-tetracosenoic acid* (2.5 g., 7 mM) was dissolved in a warm solution (60°) of potassium hydroxide (2.5 g.) in water (200 ml.). Crushed ice (300 g.) was added to the alkaline solution. The flask was immersed into an ice-bath and a solution of potassium permanganate (0.8 g., 5 mM) in water (50 ml.) was added over a period of 10 min. with stirring, which was continued at 0—5° for 1 hr. The reaction mixture was acidified with diluted sulphuric acid, decolourized with a solution of sodium sulphite and allowed to settle overnight. The filtered solid precipitate was extracted with ether, the ether solution washed with water and dried with sodium sulphate. After evaporation of the solvent to dryness the solid residue was treated with warm petroleum ether (b.p. 40—65°). The insoluble, crude dihydroxy acid melted at 119—120°. Recrystallization from 95% ethanol yielded 320 mg. (12%) of colourless crystals, m.p. 123°.

Anal. 7.505 mg. subst.: 19.88 mg. CO₂, 8.16 mg. H₂O
 C₂₄H₄₈O₄ (400.64) calc'd.: C 71.95; H 12.08%
 found: C 72.29; H 12.17%

erythro-2,3-Dihydroxytetracosanoic Acid

In a two-necked flask fitted with mechanical stirrer and air condenser are placed 1.65 g. (4.5 mM) of *trans-2-tetracosenoic acid*, 10 ml. of glacial acetic acid and 10 drops of concentrated sulphuric acid. The solution was heated to 100—120° and 5 ml. of 30% hydrogen peroxide were added in small portions through the condenser over a period of 5 hrs. The reaction mixture was left to stand at room temperature overnight. After dilution with water, the solid precipitate was filtered off and heated with 15 ml. of 30% aqueous potassium hydroxide on the water-bath for 1 hr. The mixture was acidified with 10% sulphuric acid, extracted with ether, the solution dried with sodium sulphate and evaporated to dryness. The residue was treated with boiling petroleum ether and the undissolved, crude acid (m.p. 100—102°) filtered off. Recrystallization from 95% ethanol yielded 70 mg. (4%) of colourless crystals, m.p. 103—105°.

Anal. 9.170 mg. subst.: 24.23 mg. CO₂, 9.92 mg. H₂O
 C₂₄H₄₈O₄ (400.64) calc'd.: C 71.95; H 12.08%
 found: C 72.09; H 12.11%

Acknowledgment. We are indebted to Mrs. M. Munk-Weinert (Department of Chemistry, Medical Faculty, University of Zagreb) for the microanalyses and to Mr. T. Magjer for the infrared spectra.

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IZVOD

O eritro i treo-2,3-dihidroksi-heksakozan kiselinama

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eritro-2,3-Dihidroksi-heksakozan kiselina, t.t. 103—105°, dobivena je hidroksilacijom *trans*-2-tetrakozen kiseline (I) s perocetenom kiselinom (*trans*-adicija). *Treo*-izomer, t.t. 123°, pripremljen je hidroksilacijom nezasićene kiseline I s alkalnim permanganatom (*cis*-adicija). Međusobni prostorni odnos hidroksilnih skupina na C₂ i C₃ određen je uspoređivanjem tališta i infracrvenih spektara s talištima i spektrima *eritro* i *treo*-2,3-dihidroksi-oktadekan kiselina. Opisana je sinteza *trans*-2-tetrakozen kiselina (I).

BIOKEMIJSKI ODJEL
INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

Primljeno 24. srpnja 1960,
u prerađenom obliku 23. prosinca 1960.