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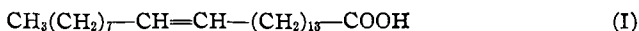
SYNTHESIS OF NERVONIC ACID

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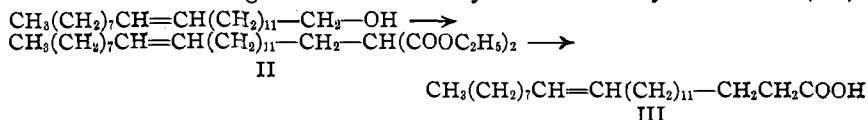
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Klenk¹ isolated from cattle and human brains a cerebroside which upon hydrolysis gave galactose, sphingosin and an unsaturated acid melting at 41°. He established its formula as C₂₄H₄₆O₂ and its exact constitution as tetracosene-13-oic acid (I) by oxidizing it to pelargonic acid and tridecymethylene dicarboxylic acid. Moreover, on catalytic reduction it yielded a saturated acid melting at 85° identical with lignoceric acid from beechwood tar and with *n*-tetracosanoic acid synthesized from behenic acid. He also reported that by melting over nitric acid, a new isomeric acid melting at 61° was obtained. Nervonic acid was shown to be identical with selachaleic acid isolated from whale oil.²



It is obvious that nervonic acid is probably the *cis* form of erucylacetic acid. By synthesis this has been shown to be the case. Ethyl erucate was reduced to erucyl alcohol (II) which was converted to the bromide and this in turn carried through a malonic ester synthesis to erucylacetic acid (III):



Several runs of product were made and only once was the yield essentially all acid melting at 39–40° (Klenk 41°). In general the product was a mixture of *cis* and *trans* isomers which could, however, be separated by crystallization, giving the low melting at 39–39.5° and the high melting at 61°. On account of the high boiling point of all the intermediates the tendency to isomerize to the stabler *trans* forms of the intermediates was probably increased.

The low-melting erucylacetic acid was readily converted to the high-melting form by melting over nitric acid and both forms were reduced to *n*-tetracosanoic acid identical with an authentic sample of this acid. Moreover, the mixed erucylacetic acids were esterified and reduced and gave a product melting at 55°, identical with that of ethyl tetracosanoate made by a different method.

Experimental

Erucyl Alcohol.—To a mixture of 200 g. of methyl erucate (0.5 mole) and 2000 cc. of anhydrous *n*-butyl alcohol in a 5-liter flask equipped with a reflux condenser was added

¹ Klenk, *Z. Physiol. Chem.*, **145**, 244 (1925); **157**, 283 (1926); **166**, 268 (1927); **166**, 287 (1927).

² Tsujimoto and Mitsumaru, *Z. deut. Öl. Fett.-Ind.*, **46**, 385 (1926); *J. Soc. Chem. Ind. Japan (Suppl.)*, **30**, 868 (1927); Hilditch and Houlbrook, *Analyst*, **53**, 246 (1928).

slowly 50 g. of sodium in large chunks. It was necessary to control the reaction occasionally with an ice jacket. When nearly all the sodium had been consumed, 80 cc. of water was added to the flask and the contents refluxed vigorously for half an hour. After partial cooling, 600 cc. more water was added, whereupon two layers separated. The lower layer was siphoned off and discarded and the upper layer was washed twice with 500-cc. portions of 20% sodium chloride solution. An aqueous solution of 25 g. of calcium chloride was added and the mixture subjected to steam distillation until removal of the butyl alcohol was complete. After cooling, the solid material was removed from the flask by hand, pressed between filter paper to remove as much water as possible and dissolved in two liters of toluene by gentle heating. On cooling with tap water, a slush of calcium erucate separated out and was removed by filtration. The filtrate was separated from the small quantity of water that had been carried along and then the toluene removed by distillation. The crude product thus obtained was purified by distillation under diminished pressure; b. p. 200–205° at 1 mm. The yields varied up to 55% of the theoretical amount. A small sample of this product after several recrystallizations from petroleum ether melted at 34–35°. Willstätter and Mayer,³ who have prepared erucyl alcohol by a somewhat similar method, record the melting point of their product as 34.5°.

Erucyl Bromide.—A solution of 129 g. of erucyl alcohol (0.4 mole) in 400 cc. of anhydrous toluene was cooled to –10° in a 2-liter flask equipped with a mechanical stirrer, thermometer and dropping funnel. To this solution 40 g. of phosphorus tribromide (0.44 mole) in 200 cc. of anhydrous toluene was added, with stirring, at such a rate that the temperature never rose above –5°. The mixture was then heated on a steam-bath for four hours and the toluene removed by distillation. The product was dissolved in 500 cc. of ether, washed with two 100-cc. portions of a solution containing 10% potassium hydroxide and 10% sodium chloride and finally with water. The ether solution was dried with anhydrous magnesium sulfate, the ether removed and the product purified by distillation under diminished pressure. The yields were from 50–60% of the theoretical amount of erucyl bromide; b. p. 203–207° at 1 mm.

Erucyl Malonic Ester.—To a solution containing 4 g. of sodium dissolved in 104 cc. of absolute alcohol was added 33 g. of diethyl malonate (6.2 moles). Then 68 g. of erucyl bromide (0.175 mole) was run in slowly and the mixture refluxed with stirring for thirty-six hours, after which time it was only faintly alkaline to moist litmus paper. The alcohol was distilled off and 400 cc. of water containing about 3 cc. of hydrochloric acid was added. The product appeared on the surface as a brown oil which was separated from the rest of the mixture. This was dried by distilling from it 50 cc. of benzene. The yield of crude product thus obtained was 95 g.

It was found that the ester boiled above 270° at 1-mm. pressure so a high vacuum distillation system was arranged. This apparatus consisted of a high vacuum pump in connection with a mercury vapor pump which in turn was connected through all glass seals to a distilling flask and receiver. A liquid-air trap between the mercury vapor pump and the distilling flask prevented any fouling of the vacuum-producing system with volatile organic material. The pressure was recorded by means of a McLeod gage sealed in between the mercury vapor pump and the liquid-air trap. Bumping was prevented by inserting a small piece of clay plate into the liquid in the distilling flask before sealing it off. All low-boiling compounds were removed before placing the erucyl malonic ester in this system by heating to 220° first on a water pump and finally at 1 mm.-pressure on an oil-pump as long as any volatile material distilled. In this manner the product was found to boil at 203–207° at 0.001 mm. (It should be recorded that the pressure measured in this system was that existing between the mercury vapor pump

³ Willstätter and Mayer, *Ber.*, 41, 1478 (1928).

and the cold trap. The pressure on the ester itself may have been as high as 0.01 mm.) The yield of erucyl malonic ester was 62.5 g. (78%).

Anal. Subs., 0.2113: CO₂, 0.5400; H₂O, 0.1971. Calcd. for C₁₉H₃₄O₄: C, 74.68; H, 11.69. Found: C, 74.68; H, 11.99.

Erucylacetic Acid or Nervonic Acid.—The crude erucyl malonic ester obtained above was saponified without further purification.

A mixture of 95 g. of crude erucyl malonic ester, 56 g. of potassium hydroxide and 350 cc. of 60% alcohol was refluxed for forty-eight hours. The condenser was changed for downward distillation and after most of the alcohol had been removed, 500 cc. of water containing 60 g. of sulfuric acid was added. The resulting precipitate was extracted with several portions of ether, dried with anhydrous magnesium sulfate and filtered. After removal of the ether the product was heated at 175° for one hour to remove carbon dioxide, then distilled under diminished pressure. This distillation was carried out as rapidly as possible to minimize decomposition, and 40 g. of a product, b. p. 235–250° at 1 mm., was obtained. This was a mixture melting at 25–43° but was separated into three pure components by a scheme of fractional recrystallization as follows.

The entire 40 g. was dissolved in 300 cc. of absolute alcohol and cooled very slowly to –10°. The precipitate of fine white crystals was filtered and the mother liquor again cooled to –10°, when a second crop appeared which was filtered and added to the first. The combined product was then recrystallized twice more from 100-cc. portions of alcohol and a 10-g. yield of pure presumably *trans*-erucylacetic acid, m. p. 61°, was obtained. This corresponds to the *trans* form of nervonic acid, m. p. 61°, obtained by Klenk on melting the *cis* form over dilute nitric acid.

The original mother liquor was now evaporated on the steam-bath to about 75 cc. and cooled until a small precipitate of white crystals appeared, which were filtered without further cooling. A melting point indicated that this was a mixture of the *cis* and *trans* forms of the acid. On further cooling the mother liquor to –10° a generous crop of crystals separated which after drying melted at 36–38°. Two subsequent recrystallizations yielded 3 g. of a product, m. p. 39–39.5°; the melting point did not change after a third recrystallization. This corresponds to the nervonic acid of Klenk, m. p. 40–41°.

Anal. Subs., 0.1668: CO₂, 0.4795; H₂O, 0.1886. Calcd. for C₂₄H₄₆O₂: C, 78.60; H, 12.64. Found: C, 78.39; H, 12.65.

Further working of the mother liquor yielded only an oil which, although unidentified, was probably ethyl erucyl ether formed during the malonic ester condensation.

Conversion of the *Cis* Form of Erucylacetic Acid to the *Trans* Form.—A small sample of the *cis* nervonic acid, m. p. 39–39.5°, was melted over 15 cc. of dilute nitric acid, to which a pinch of sodium nitrite had been added. The product was separated by cooling and after two recrystallizations from absolute alcohol melted at 62–63°. A sample of this mixed with a sample of the *trans* form obtained before melted at 61–62°.

Anal. Calcd. for C₂₄H₄₆O₂: C, 78.60; H, 12.64. Found: C, 78.35; H, 12.59.

Reduction of *Cis* and *Trans* Forms of Nervonic Acid to *n*-Tetracosanoic Acid.—A sample of 0.2 g. of *cis* nervonic acid, m. p. 39–39.5°, was dissolved in 25 cc. of absolute alcohol, and the solution shaken with hydrogen under a pressure of 3 atm. for one hour, in the presence of 0.1 g. of platinum oxide catalyst. The reduced acid separated as fine white crystals as the reduction proceeded. The product thus obtained after removal of the platinum was recrystallized three times from absolute alcohol, and three times from acetone. It then melted at 83–84°.

A sample of *trans* nervonic acid was reduced and the product purified according to the same procedure. This product also melted 83–84°, and a mixed melting point of the two acids showed no depression.

Anal. Calcd. for $C_{24}H_{46}O_2$: C, 78.17; H, 13.13. Found: C, 78.54; H, 13.29.

Various values have been recorded in the literature for the melting point of *n*-tetracosanoic acid. Levene and Taylor,⁴ who have synthetically prepared all the normal acids of this series, report 85–86° as the melting point of this member. Meyer, Brod and Soyka⁵ obtained 85.5–86°, Brigl⁶ 85°, and Levene, West, Allen and von der Scheer⁷ 87.5–88°. Shriner, Nabenhauer and Anderson⁸ obtained *n*-tetracosanoic acid from corn wax, and report as its melting point 85–86°. The last authors made mixed melting-point determinations with a sample of the same acid prepared by Taylor and Levene.⁴ The acid prepared in this investigation melted at 83–84° and a mixed melting-point determination with a sample of Shriner's naturally occurring acid was made. It was found under the melting point conditions used that the melting point of the *n*-tetracosanoic acid, from reduction of erucylacetic acid, the natural *n*-tetracosanoic acid of Shriner, Nabenhauer and Anderson and a mixture of the two was exactly the same, 83–84°. Recrystallization from various solvents failed to alter the melting point of either acid.

Ethyl Erucylacetate.—The ethyl ester of erucylacetic acid was prepared by refluxing 110 g. of the acid (0.3 mole) (which was presumably a mixture of the *cis* and *trans* isomers) with 1 liter of absolute alcohol to which 2 cc. of concentrated sulfuric acid had been added, overnight. The product boiled at 233–234° at 1 mm., and the yield was 117 g.

Anal. Subs., 0.1553: CO_2 , 0.4489; H_2O , 0.1774. Calcd. for $C_{26}H_{50}O_2$: C, 79.11; H, 12.77. Found: C, 78.83; H, 12.79.

Ethyl *n*-Tetracosanoate.—A solution of 5 g. of ethyl erucylacetate in 100 cc. of 95% alcohol was shaken with hydrogen under 2.5–3 atmospheres' pressure, in the presence of 0.2 g. of platinum oxide catalyst. The reduction required only a few minutes and the product crystallized out as it was formed. The pressure was released and the alcohol heated to boiling to take the ester back into solution. The hot solution was filtered free from platinum and cooled again. The white plates which crystallized out were filtered and recrystallized three times from ethyl alcohol and four times from acetone; m. p. 55–56° (corr.). Levene and Taylor⁴ record the melting point of the same compound prepared by esterifying the ester as 55.5–56.5°.

Summary

Erucylacetic acid has been prepared from erucyl alcohol through the bromide and malonic ester synthesis and has been shown to be identical with nervonic acid obtained from hydrolysis of the cerebroside isolated from human brains.

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⁴ Levene and Taylor, *J. Biol. Chem.*, 59, 905 (1924).

⁵ Meyer, Brod and Soyka, *Monatsh.*, 34, 113 (1913).

⁶ Brigl, *Z. physik. Chem.*, 95, 161 (1925).

⁷ Levene, West, Allen and von der Scheer, *J. Biol. Chem.*, 23, 71 (1915).

⁸ Shriner, Nabenhauer and Anderson, *THIS JOURNAL*, 49, 1290 (1927).