

THE PREPARATION AND PROPERTIES OF THE EIGHT  
DIASTEREOISOMERS OF 9,10,12,13-TETRAHY-  
DROXYSTEARIC ACID

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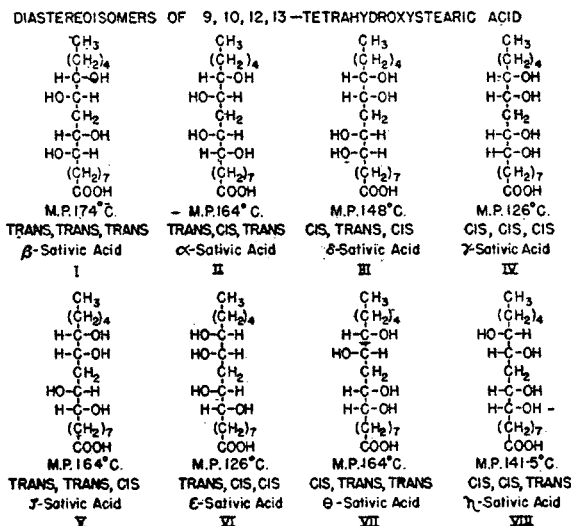
The eight diastereoisomers of 9,10,12,13-tetrahydroxystearic acid were prepared from  $\alpha$ -linoleic acid (cis-9-cis-12-octadecadienoic acid). A new method of preparation was developed which establishes the spacial relationships of the hydroxyl groups in each diastereoisomer.

Hazura (1) was the first to isolate a 9,10,12,13-tetrahydroxystearic acid (sativic acid) from the alkaline potassium permanganate oxidation of linoleic acid. Further study (2-6) of this oxidation reaction resulted in methods for improving the yield and it is now established that two diastereoisomers of the tetrahydroxystearic acid are formed in this reaction. The melting points of these acids are 173° (I) and 164° (II). The more soluble one has also been reported to melt at 157-159°, but according to Riemenschneider *et al.* (7) this is an eutectic mixture of the  $\alpha$ - and  $\beta$ -sativic<sup>1</sup> acids melting at 173° and 164°. Nicolet and Cox (8) obtained two different diastereoisomers of 9,10,12,13-tetrahydroxystearic acid by treating linoleic acid with hypochlorous and hypobromous acids to form the dichloro- and dibromo-dihydroxystearic acids. Replacement of the halogen atoms by hydroxyl groups gave two tetrahydroxystearic acids melting at 145° and 135°. Later work suggests that these acids are the same as the two diastereoisomers obtained in the peracetic acid oxidation of linoleic acid (9), or the alkaline potassium permanganate oxidation of linoleic acid (10) which are now described as melting at 146° (III) and 126° or 122° (IV). Kass and Burr (10) prepared a third set of diastereoisomers of 9,10,12,13-tetrahydroxystearic acid by the alkaline potassium permanganate oxidation of the trans-cis or cis-trans geometric isomer of linoleic acid. The melting points of these acids are given as 156-158° (V) and 126° (VI). The melting point of the former has now been raised to 164°. A seventh diastereoisomer of tetrahydroxystearic acid was re-

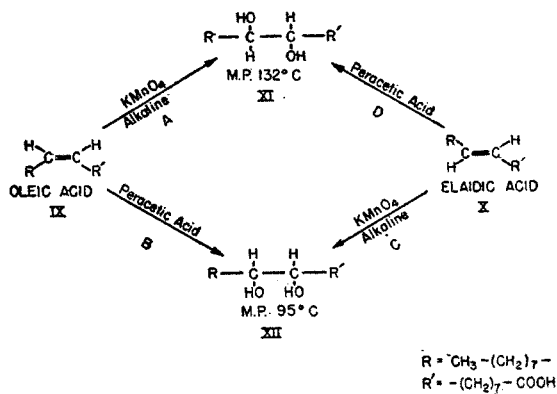
<sup>1</sup> The use of the name sativic acid for tetrahydroxystearic acid has led to a great deal of confusion in the study of the diastereoisomers of this acid. The main difficulty is that the terms  $\alpha$ ,  $\beta$ ,  $\gamma$  etc., sativic acids give no indication of the structure of these compounds. Moreover the same Greek letters have been assigned to different diastereoisomers. Thus we propose that the system of nomenclature based on the use of the terms cis and trans be used to name these diastereoisomers. This system allows no misinterpretation of data and is more convenient for the correlation of experimental studies of these acids. In this method of naming the diastereoisomers of 9,10,12,13-tetrahydroxystearic acid three combinations of cis and trans are used *e.g.*,  $\alpha$ -sativic acid becomes trans, cis, trans-9,10,12,13-tetrahydroxystearic acid. The first trans refers to the spacial relationship of the C<sub>9</sub> and C<sub>10</sub> hydroxyl groups, the cis refers to the relative configuration of the C<sub>10</sub> and C<sub>12</sub> hydroxyl groups and the final trans to the spacial interrelationship of the C<sub>12</sub> and C<sub>13</sub> hydroxyl groups. The eight diastereoisomers of 9,10,12,13-tetrahydroxystearic acid are given in formulas I-VIII, with this proposed system of nomenclature together with the sativic acid system.

ported by McKay, Jones, and Sinclair (11), which also melts at 164° (VII). In this report the eighth diastereoisomer (m.p. 141.5°) (VIII) is described.

If one considers the simpler case of 9,10-dihydroxystearic acid, then only two diastereoisomers are theoretically possible. 9-Octadecenoic acid exists in two geometric isomers, the cis form commonly known as oleic acid (IX) and the trans



Scheme A



Scheme B

form as elaidic acid (X). The two 9,10-dihydroxystearic acids melting at 132° (XI) and 95° (XII) are formed respectively by the alkaline potassium permanganate oxidation (A) and the peracetic acid oxidation (B) of oleic acid. In antithesis to this, the alkaline potassium permanganate (C) and peracetic acid (D) oxidations of elaidic acid (X) give the dihydroxystearic acids melting at 95° and 132° respectively. Infrared studies (12), and titration with lead tetraacetate (13) show the two hydroxyl groups in the higher-melting 9,10-dihydroxystearic

acid, to be trans to each other, and in the lower-melting diastereoisomer to be cis. Thus, alkaline potassium permanganate oxidation of a double bond supporting a cis configuration gives a trans  $\alpha$ -glycol and vice versa. The opposite is true when peracetic acid is the oxidizing reagent. With these established facts in mind the eight diastereoisomers of 9,10,12,13-tetrahydroxystearic acid were prepared.

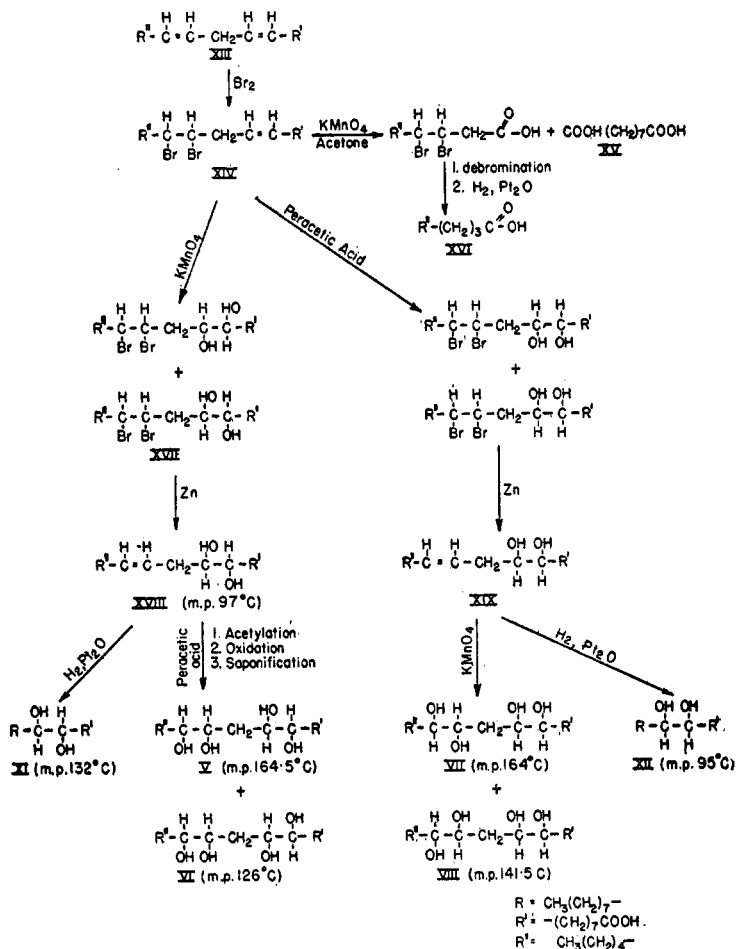
$\alpha$ -Linoleic acid (XIII) was brominated with slightly more than one molar equivalent of bromine at low temperatures ( $-15^\circ$ ). The desired dibromo derivative, 12,13-dibromo-9-octadecenoic acid (XIV) contained impurities of unchanged linoleic acid and 9,10,12,13-tetrabromostearic acid. The dibromination of linoleic acid and the oxidative degradation of the dibromo derivative have been reported by Toyama and Tutiya (14) without experimental details. In order to confirm the structure of 12,13-dibromo-9-octadecenoic acid, it was converted into its methyl ester (Iodine Value, 56.0), and then oxidized with potassium permanganate in acetone. The product, after debromination and hydrogenation gave azelaic acid (XV) in 78% yield and impure *n*-nonanoic acid (XVI) in 61% yield. These results prove that the double bond farther removed from the carboxyl group is preferentially brominated in the low temperature dibromination of  $\alpha$ -linoleic acid.

Oxidation of 12,13-dibromo-9-octadecenoic acid with aqueous alkaline potassium permanganate solution gave a mixture of 9,10-dihydroxy-12,13-dibromostearic acids (XVII). Trans-9,10-dihydroxy-12-octadecenoic acid (m.p.  $97^\circ$ ; I.V., 80.6) (XVIII) was obtained from this product on debromination. Another set of reactions, in which 12,13-dibromo-9-octadecenoic acid was oxidized with peracetic acid and the product debrominated, saponified, and then distilled, gave an oil identified as cis-9,10-dihydroxy-12-octadecenoic acid (I.V., 80.2) (XIX). The structures of both the trans and cis-9,10-dihydroxy-12-octadecenoic acids were substantiated by reduction of these compounds to the corresponding 9,10-dihydroxystearic acids melting at  $132^\circ$  (XI) and  $95^\circ$  (XII) respectively. Nearly theoretical yields were obtained in each case.

The trans-9,10-dihydroxy-12-octadecenoic acid (XVIII) was acetylated and then oxidized with peracetic acid solution. After saponification of the product and acidification of the resulting alkaline solution, the free acids were obtained. These acids, on partition by solvents, gave two racemates of 9,10,12,13-tetrahydroxystearic acid in which the  $C_9$  and  $C_{10}$  hydroxyl groups were trans and the  $C_{12}$  and  $C_{13}$  hydroxyl groups were cis. These diastereoisomers melted at  $164^\circ$  (V) and  $126^\circ$  (VI). The isomer having the  $C_{10}$  and  $C_{12}$  hydroxyl groups cis to each other was assigned the lower melting point. It is well known that in a set of cis, trans isomers the lower-melting, more soluble member has the cis configuration.

The fact that the spacial arrangement about the double bond was still cis in the trans-9,10-dihydroxy-12-octadecenoic acid was established on alkaline permanganate oxidation, when the two diastereoisomers of 9,10,12,13-tetrahydroxystearic acids melting at  $174^\circ$  (I) and  $164^\circ$  (II) were obtained. These are the acids obtained from linoleic acid on oxidation with this reagent.

Still another set of diastereoisomers of tetrahydroxystearic acid was prepared by the alkaline permanganate oxidation of *cis*-9,10-dihydroxy-12-octadecenoic acid (XIX). These acids melted at 164° (VII) and 141.5° (VIII). In these acids, the C<sub>9</sub> and C<sub>10</sub> hydroxyl groups are known to be *cis* to each other, and the C<sub>12</sub> and C<sub>13</sub> hydroxyl groups are known to be *trans* because of the method of prep-



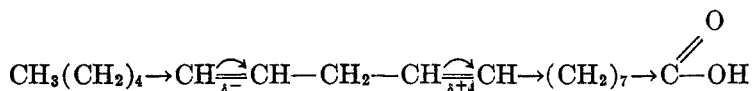
Scheme C

aration. If *cis*-9,10-dihydroxy-12-octadecenoic acid is acetylated and then oxidized with peracetic acid solution, the product on saponification gives the two diastereoisomers of 9,10,12,13-tetrahydroxystearic acid melting at 148° (III) and 126° (IV). These are identical with the two diastereoisomers prepared from linoleic acid by peracetic acid oxidation. Therefore the previous treatment of *cis*-9,10-dihydroxy-12-octadecenoic acid did not alter the configuration about the remaining double bond.

Riemenschneider *et al.* (7) concluded that bromination of *cis*-9-*cis*-12-octade-

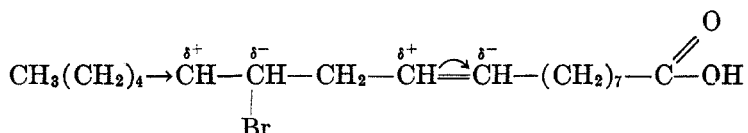
cadienoic acid would give two racemates of 9,10,12,13-tetrabromostearic acid. This conclusion was supported by previous observations that bromine adds to a double bond exclusively by *cis*-addition or by *trans*-addition. Applying the same arguments to the oxidation of  $\alpha$ -linoleic acid, they concluded that alkaline potassium permanganate oxidation or peracetic acid oxidation would give only two racemates of 9,10,12,13-tetrahydroxystearic acid. The present work and the earlier studies of Toyama and Tutiya (14) clearly establish that bromine adds first to the  $C_{12}$ -double bond of linoleic acid, by the isolation and identification of 12,13-dibromo-9-octadecenoic acid from the products of its partial bromination. The stepwise addition of bromine to *cis*-9-*cis*-12-octadecadienoic acid becomes clear when considered in the light of the fundamental postulates of the English school regarding rates of reactions of olefins (15). Olefins are nucleophilic in character, and any substituent which increases the electron density of the ethylenic group increases its rate of reaction with electrophilic reagents. An electron

attractive group *e.g.*,  $\overset{\text{O}}{\parallel}\text{C}-\text{OH}$ ,  $-\overset{\text{O}}{\parallel}\text{C}-\text{OR}$ ,  $\text{OH}$ ,  $-\overset{\text{H}}{\text{C}}=\text{O}$  etc., will have the reverse effect. Thus, in  $\alpha$ -linoleic acid there are present two opposing forces, the electron repulsive alkyl group attached to  $C_{13}$  and the electron attractive carboxyl group. The inductive effect of these groups may be represented as follows:



The electron attractive carboxyl group will have a greater effect on reducing the electron density of the 9-double bond than of the 12-double bond while the electron repulsive alkyl group will have a greater effect in increasing the electron density of the 12-double bond than of the 9-double bond. Both of these effects operate to increase the difference of the electron densities of the two double bonds giving the 12-double bond the stronger nucleophilic character. The  $-\text{CH}_2-$  group between the two double bonds would aid in maintaining a potential difference. We have represented this by assigning the fractional charges  $\delta^-$  and  $\delta^+$  to the ethylenic linkages as a whole rather than representing each ethylenic

linkage as a polar entity as is general procedure, *e.g.*,  $\overset{\delta^+}{\text{C}}\text{H}=\overset{\delta^-}{\text{C}}\text{H}_2$ . Thus the attack of the electrophilic reagent  $:\text{Br}:\overset{+}{\text{}}$  will be preferentially directed towards the 12-double bond. Once addition of this electrophilic reagent has occurred then the intermediate may be represented as:



and the polarity between the  $C_{12}$  and  $C_{13}$  positions must be greater than the polarity between the  $C_9$  and  $C_{10}$  positions. This would then favor the addition

of the nucleophilic  $\text{:Br:}^-$  ion to  $\text{C}_{13}$ . The over-all effect of these electron shifts causing an increased reaction rate for the  $\text{C}_{12}$ -double bond over that of the  $\text{C}_9$ -double bond. Besides the inductive effect of the carboxyl group one would expect a field effect to be operative. However, it is not possible to distinguish between the field and inductive effects.

The oxidation of linoleic acid may be explained also by use of these electronic mechanisms. Swern (16) has summarized the present knowledge of the per-acid oxidation of olefins, and electronically interpreted the effect of substituents on their rates of reactions. This interpretation may be applied equally well to linoleic acid oxidation with per-acids.

The stepwise method of preparation of the tetrahydroxystearic acids through the intermediate 12,13-dibromo-9-octadecenoic acid, has permitted the assignment of definite structures to the eight possible diastereoisomers. On the basis of these structures it is possible to predict the two diastereoisomers of 9,10,12,13-tetrahydroxystearic acid that would be obtained on alkaline potassium permanganate or peracetic acid oxidations of the geometric isomers of linoleic acid. Thus, Kass and Burr (10) must have obtained *cis*-9-*trans*-12-octadecadienoic acid on the partial elaidinization of  $\alpha$ -linoleic acid rather than the *trans*-9-*cis*-12-octadecadienoic acid suggested, because only alkaline oxidation of the former would give the two diastereoisomers of tetrahydroxystearic acid melting at 156–158° (164°) and 126°.

The tetrahydroxystearic acids described in this paper were obtained as well-formed crystals with the exception of the two lowest-melting (126°) members of the series. The possibility of the existence of eutectic mixtures is realized and we are continuing with the studies of these acids.

#### EXPERIMENTAL

All melting points and boiling points are uncorrected.

*Linoleic acid (XIII)*.  $\alpha$ -Linoleic acid was prepared from corn oil through the tetrabromide by the method of McCutcheon (18). After debromination, the linoleic acid distilled in a vacuum (1 mm.) at 198–200°. The yield from the tetrabromostearic acid (m.p. 115°) was 88.9%, iodine value 180.8;<sup>2</sup> calc'd 181.0. Ultraviolet absorption analysis gave a specific  $\alpha$  at 2340 Å of 87.2, while that reported by Mitchell and co-workers (19) for pure linoleic acid is 87.1.

*12,13-Dibromo-9-octadecenoic acid (XIV)*. To a vigorously stirred solution of 31.2 g. (0.11 mole) of pure linoleic acid in 2700 cc. of petroleum ether cooled to 15°, a solution of 17.5 g. (0.11 mole) of bromine in 300 cc. of petroleum ether was added dropwise over a period of 2.5 hours. The reaction temperature was not allowed to go above –10°. At the end of the reaction, the solvent was removed *in vacuo* at ca. 35°. The last traces of solvent were removed by heating to 100° at a pressure of 1 mm. mercury. A viscous yellow oil (48.6 g.) remained.

*Anal.* Calc'd for  $\text{C}_{18}\text{H}_{32}\text{Br}_2\text{O}_2$ : Br, 36.32; Iodine Value, 57.60.

Found: Br, 37.92, 37.65; Iodine Value, 56.65.

*Methyl ester of 12,13-dibromo-9-octadecenoic acid.* The impure 12,13-dibromo-9-octa-

<sup>2</sup> The iodine values reported here were determined by the Rosenmund-Kuhnemann method (17).

decanoic acid (39.0 g.; 0.088 mole) was dissolved in 250 cc. of methanol and 10 cc. of 4 *N* methanolic HCl was added. This solution was refluxed for two hours with the addition of 5-cc. portions of the methanolic HCl at half-hour intervals. The solution was then poured into two volumes of saturated brine. The organic layer was extracted with ether (3 × 150 cc.), the ethereal solution was washed with saturated brine (4 × 200 cc.), dried over sodium sulfate, and evaporated to dryness. The residue, an amber colored oil, was obtained in quantitative yield. This oil was distilled at a pressure of 0.5 mm.; the main fraction (b.p. 225–227°) was redistilled, and the intermediate fraction (b.p. 225°) taken for analysis.

*Anal.* Calc'd for  $C_{19}H_{34}Br_2O_2$ : Br, 35.3; Iodine Value, 55.8.

Found: Br, 34.92; Iodine Value, 56.0.

*Oxidation of the methyl ester of 12,13-dibromo-9-octadecenoic acid with potassium permanganate in acetone.* The following modification of Hilditch's (20) method was used for establishing the position of the double bond in the dibrominated linoleic acid. A suspension of sodium carbonate (2 g.) in 150 cc. of acetone containing 9.3 g. (0.02 mole) of methyl 12,13-dibromo-9-octadecenoate (I.V., 56.0) was stirred at  $-5^\circ$  to  $0^\circ$  during the gradual addition of finely powdered  $KMnO_4$ . Twenty-seven grams of  $KMnO_4$  was added before a permanent pink color was obtained. The acetone was removed by distillation and the residue extracted with hot methanol (3 × 100 cc.). After addition of zinc dust (10 g.) to the methanol solution, it was refluxed for one hour. The unreacted zinc and zinc bromide were filtered off and the combined filtrate and washings evaporated. The oily residue was saponified in 100 cc. of 3 *N* aqueous KOH by heating on the steam-bath for two hours. The clear soap solution was then poured into excess dilute hydrochloric acid and extracted with ether (3 × 100 cc.). This ethereal solution, after washing with saturated brine (4 × 100 cc.) and drying over sodium sulfate, was evaporated. A residual oil was obtained, which on solution in 50 cc. of ether deposited 3.01 g. (78.0% yield) of impure azelaic acid. Two crystallizations from water gave 2.1 g. of crystals melting at 104.5–105.5° alone and on admixture with an authentic sample of azelaic acid.

Evaporation of the ether from the original filtrate gave an oil which was dissolved in glacial acetic acid (50 cc.). This solution was treated with hydrogen in the presence of Adams platinum oxide catalyst (15 mg.) until the uptake of hydrogen ceased. The catalyst was filtered off and the filtrate was diluted with 6 volumes of water. The oil (1.98 g., 61.0% yield) could not be crystallized. Part (1 g.) of this impure *n*-nonanoic acid was converted to 2-*n*-octylbenzimidazole (m.p. 138.5–139.5°) by refluxing with *o*-phenylenediamine, as outlined by Pool, Harwood, and Ralston (21). They report the melting point 139.5–140.5°.

*trans-9,10-Dihydroxy-12-octadecenoic acid (XVIII).* 12,13-Dibromo-9-octadecenoic acid (39.0 g.; 0.088 mole) was oxidized in dilute alkaline solution with potassium permanganate by the method of Riemenschneider *et al.* (7). The product was recovered by extracting the decolorized and acidified permanganate solution with ether (3 × 400 cc.). The clear ether solution was washed with saturated brine solution until the aqueous solutions were neutral to Congo Red paper and dried over sodium sulfate. Evaporation of the ether left 38.6 g. of a light yellow oil consisting of a mixture of oxidation products, along with *trans*-9,10-dihydroxy-12,13-dibromooctadecanoic acid.

The crude oil (38.6 g.) was dissolved in methanol (500 cc.) and the solution cooled to  $0^\circ$ . Zinc dust (30 g.) was added slowly because at first the reaction was vigorous and exothermic. After the reaction had become quiescent, the reaction mixture was refluxed on a steam-bath for two hours. On cooling, the solid (zinc and zinc bromide) was removed by filtration and the methanolic filtrate was diluted with water (500 cc.) and a white waxy, lighter-than-water solid separated. This was filtered off, washed with petroleum ether and triturated with dilute HCl solution. The remaining white solid was washed with water and petroleum ether and dried. This solid proved to be impure *trans*-9,10-dihydroxy-12-octadecenoic acid (m.p. 86–92°), yield 4.0 g. or 14.8%. After two recrystallizations from 50% aqueous ethanol 2.1 g. of 9,10-*trans*-dihydroxy-12-octadecenoic acid was obtained as shiny plates melting sharply at 97°.

*Anal.* Calc'd for  $C_{18}H_{34}O_4$ : C, 68.75; H, 10.96; I. V., 80.6.

Found: C, 68.99; H, 11.25; I. V., 80.6.

*trans-9,10-Dihydroxystearic acid from trans-9,10-dihydroxy-12-octadecenoic acid.* The *trans-9,10-dihydroxy-12-octadecenoic acid* (100 mg., 0.0003 mole) was dissolved in glacial acetic acid (30 cc.) and hydrogenated in the presence of Adams platinum oxide catalyst (15 mg.). The product, recovered in the usual manner, melted at 125–128°, yield 98 mg. (97.4%). This was crystallized twice from 50% aqueous ethanol to yield 71 mg. of crystals melting at 132° alone and on admixture with a known sample of *trans-9,10-dihydroxystearic acid* (m. p. 132°).

*Alkaline potassium permanganate oxidation of trans-9,10-dihydroxy-12-octadecenoic acid.* Eighty-nine milligrams (0.00027 mole) of *trans-9,10-dihydroxy-12-octadecenoic acid* (m. p. 97°) was oxidized with aqueous alkaline permanganate solution using the method of Riemenschneider *et al.* (7). The crude product after filtration from the acidified and decolorized potassium permanganate solution melted at 138–149°, yield 55 mg., or 55.8%. This solid was extracted with acetone (3 × 1 cc.). The acetone-insoluble fraction, after one crystallization from 50% aqueous ethanol yielded 21 mg. of crystals melting at 173° (I). This melting point was not depressed by the diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 174°) obtained from the alkaline potassium permanganate oxidation of linoleic acid. The acetone extract, after removal of the acetone, was crystallized twice from 50% aqueous ethanol to give crystals melting sharply at 164° (II), yield 14 mg. The melting point of this compound was not depressed by the low-melting diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 164°) obtained from the alkaline potassium permanganate oxidation of linoleic acid.

*The acetylation and peracetic acid oxidation of trans-9,10-dihydroxy-12-octadecenoic acid.* *Trans-9,10-dihydroxy-12-octadecenoic acid* (1.98 g. 0.006 mole) was dissolved in pyridine (5 cc.) and 1.08 g. (0.01 mole) of acetyl chloride was added dropwise to the pyridine solution cooled in an ice-water bath. After the exothermic reaction had subsided, the solution was allowed to stand at room temperature for twenty-four hours. It was poured into water (10 vols.) and extracted with ether (3 × 30 cc.). The ethereal extract was washed with dilute HCl to remove the pyridine, and with 10% brine until the aqueous washings were neutral to Congo Red. Evaporation of the ether left a light yellow oil.

This light yellow oil was further hydroxylated with peracetic acid solution using the conditions described by Swern *et al.* (22) for the hydroxylation of elaidic acid with peracetic acid; the only change was the use of three times the relative amount of  $H_2SO_4$ . This was found to hasten the reaction considerably, and less time was required for the theoretical uptake of oxygen. The uptake of peroxide was followed by titration (23). At the end of the reaction period, the acetic acid solution was poured into water (10 vols.), and the oil which separated was recovered. This oil was saponified by heating with 3 *N* aqueous KOH solution (50 cc.) for one hour on a steam-bath. On pouring the cooled soap solution into ice-cold dilute HCl solution, a yellow precipitate (m. p. 90–97°) formed, yield 870 mg. This solid was extracted with boiling ethyl acetate (100 cc.). The unextracted material (92 mg., yield 8.5%) melted at 158–163°. Crystallization from 50% aqueous ethanol yielded rod-shaped crystals (65 mg.) melting at 164–164.5°.

*Anal.* Calc'd for  $C_{18}H_{34}O_4$ : C, 62.07; H, 10.34.

Found: C, 61.80; H, 10.32.

A mixed melting point determination with the known diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 164°) from the alkaline potassium permanganate oxidation of linoleic acid was depressed to 151–157°.

The ethyl acetate extract was cooled to 0°, when white crystals separated. These crystals melted at 141–145°, yield 22 mg. The ethyl acetate filtrate was evaporated to dryness and the remaining oil was dissolved in ether (20 cc.) and stored at –35° for three days. The ether solution deposited white crystals (50 mg.) which were filtered off, washed with ether, and dried, m. p. 75–103°. Fractional crystallization from ethyl acetate gave a crop



of crystals melting at 126° (3 mg.). This experiment was repeated to obtain more of these crystals melting at 126°.

*Anal.* Calc'd for  $C_{18}H_{36}O_6$ : C, 62.07; H, 10.34.

Found: C, 62.21; H, 10.40.

The melting point of this compound was depressed to 110–115° on admixture with the low-melting diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 126°) obtained by the peracetic acid oxidation of linoleic acid. The ether filtrate from the crystals melting at 75–103° was evaporated to dryness, and the remaining oil taken up in 3 *N* aqueous KOH (10 cc.) and poured into dilute HCl solution. To the milky suspension petroleum ether (10 cc.) was added and, the material left standing overnight in the refrigerator. The white crystals (22 mg.) which had formed at the interface were filtered off and dried. These crystals (m. p. 85–86°) are under investigation. A similar melting compound was found in the product from the peracetic acid oxidation of linoleic acid (12).

*Peracetic acid oxidation of 12,13-dibromo-9-octadecenoic acid.* Thirty-one grams (0.07 mole) of 12,13-dibromo-9-octadecenoic acid was hydroxylated with peracetic by the method already described. The oxidation was considered complete after two and a half hours. The reaction mixture was then poured into a saturated brine (8 vols.) to which 0.1 *N* sodium thiosulfate solution (3 cc.) had been added to remove the excess peroxide. The aqueous mixture was extracted with ether and the ether washed with saturated brine solution. Finally the ethereal extract was dried over anhydrous sodium sulfate and the ether evaporated. A light yellow oil remained, yield 31.4 g. This oil contained 9(10)-hydroxy-10(9)-acetoxy-12,13-dibromooctadecenoic acid, as well as other impurities. Several runs were made and 123.1 g. of yellow oil was collected. This oil was debrominated without further purification. The debromination was conducted as previously described and the product worked up in the same manner. The light yellow oil (81.6 g.) consisted of a mixture of 9(10)-hydroxy-10(9)-acetoxy-12-octadecenoic acid, linoleic acid, and lower oxidation products. This oil (I. V., 82.5, calc'd 80.6) was extracted with petroleum ether (6 × 100 cc.) to remove the linoleic acid, and the remaining clear oil (62.0 g.) was dissolved in acetone (350 cc.). The acetone was almost completely removed by distillation and the remaining solution was treated with petroleum ether when a white solid (0.3 g.) collected at the interface. It melted at 114–121° and after three crystallizations from 30% aqueous ethanol the melting point was raised to 124–126°. This melting point was not depressed when a sample was mixed with the low-melting diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 126°) obtained from the peracetic acid oxidation of linoleic acid. The acid isolated here was formed by action of peracetic acid on the linoleic present as an impurity in the original 12,13-dibromo-9-octadecenoic acid. The petroleum ether was separated from the oily layer by decantation and the oil was extracted again with petroleum ether (12 × 50 cc.). The remaining oil had the iodine value 51.55, whereas the calculated iodine value for 9,10-dihydroxy-12-octadecenoic acid is 80.6. This oil was purified by converting it to the methyl ester as previously described and distillation. The methyl ester (37.8 g.; I. V., 48.2) was distilled at a pressure of ca. 0.5 mm. and a colorless fraction was collected at a vapor temperature of 186–188°. This fraction had the iodine value 73.2, calc'd 77.3. One more distillation, in which large first and last fractions were discarded, gave 8.8 g. of colorless oil, I. V., 76.5. This methyl ester of *cis*-9,10-dihydroxy-12-octadecenoic acid was then saponified in the usual manner to give a quantitative yield of *cis*-9,10-dihydroxy-12-octadecenoic acid, I. V., 80.2, calc'd 80.6.

*cis*-9,10-Dihydroxystearic acid from *cis*-9,10-dihydroxy-12-octadecenoic acid. The *cis*-9,10-dihydroxy-12-octadecenoic acid (0.276 g.; 0.0008 mole; I. V., 80.2) was dissolved in glacial acetic acid (30 cc.) and hydrogenated in the presence of Adams platinum oxide catalyst (15 mg.). After the uptake of hydrogen had ceased, the catalyst was removed by filtration and the acetic acid solution diluted with water (7 vols.). A white, flocculent precipitate (0.25 g., 93% yield) formed. This was filtered off, washed with water, and dried. The melting point 78–86° was raised to 94° by three crystallizations from ethanol, yield 184

mg. A sample of this compound on admixture with an authentic sample of *cis*-9,10-dihydroxystearic acid (m. p. 95°) gave no depression in melting point.

*Acetylation of the cis-9,10-dihydroxy-12-octadecenoic acid and peracetic acid oxidation of the 9,10-diacetoxy-12-octadecenoic acid.* *Cis*-9,10-dihydroxy-12-octadecenoic acid (1.95 g.; 0.0062 mole; I. V., 80.2) was acetylated by the method described for *trans*-9,10-dihydroxy-12-octadecenoic acid. The resulting oil was oxidized with peracetic acid in the usual manner. The oxidation product was a yellow oil which was saponified by heating on the steam-bath with 3 *N* aqueous KOH solution (100 cc.) for two hours. The soap was cooled, poured into ice-cold dilute HCl, and the waxy, yellow solid (1.47 g., 68.1% yield) filtered off. Trituration with ether (50 cc.) left white crystals (346 mg.), which were extracted with boiling acetone (6 × 10 cc.). The residual solid (91 mg.) after two crystallizations from 50% aqueous ethanol melted at 147–148° (III). A mixed melting point determination with a known sample of high-melting diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 148°) obtained from the peracetic acid oxidation of linoleic acid was not depressed.

The combined acetone extracts on cooling gave 12 mg. of crystals which melted at 130–139°. The acetone filtrate was evaporated to dryness and again extracted with acetone (10 cc.). The acetone was evaporated off and the residue was crystallized twice from 50% aqueous ethanol to give 9 mg. of white amorphous solid (m. p. 125–126°) (IV). A mixed melting point determination with an authentic sample of the low-melting diastereoisomer of 9,10,12,13-tetrahydroxystearic acid from the peracetic acid oxidation of linoleic acid was not depressed.

*Alkaline potassium permanganate oxidation of cis-9,10-dihydroxy-12-octadecenoic acid.* Three grams (0.009 mole) of *cis*-9,10-dihydroxy-12-octadecenoic acid (I. V. 80.2) was dissolved in 1.5% aqueous potassium hydroxide (300 cc.) and to the well-stirred solution 1% aqueous potassium permanganate (300 cc.) solution was added rapidly. After two minutes the reaction mixture was decolorized with sulfur dioxide, concentrated HCl (20 cc.) was added and the white waxy precipitate (2.10 g., 63.2% yield) was recovered by filtration. This solid was extracted with ether (180 cc.) which left white crystals (1.136 g.) melting at 131–142°. Extraction of the solid with boiling acetone (130 cc.) gave white crystals (276 mg.) which after two crystallizations from 50% aqueous ethanol melted sharply at 164°. This is the *cis*, *trans*, *trans*-9,10,12,13-tetrahydroxystearic acid (VII) and it depresses the mixed melting point to 150–158° on admixture with the *trans*, *cis*, *trans*-9,10,12,13-tetrahydroxystearic acid (II) from the alkaline potassium permanganate oxidation of linoleic acid. A mixed melting point determination between the *cis*, *trans*, *trans*-9,10,12,13-tetrahydroxystearic acid (m. p. 164°) (VII) and the *trans*, *trans*-*cis*-diastereoisomer (m. p. 165°) (V) gave on depression. However crystallographic studies show these two diastereoisomers to have different crystalline properties. The crystallographic data and infrared data will be published at a later date.

A sample of this new *cis*, *trans*, *trans*-9,10,12,13-tetrahydroxystearic acid (m. p. 164°) (VII) was submitted for analysis.

*Anal.* Calc'd for  $C_{18}H_{36}O_8$ : C, 62.07; H, 10.34.

Found: C, 61.93, 61.89; H, 10.64, 10.69.

The hot acetone extract from above was cooled to 0° and the crystals (25 mg.) which formed were filtered off and dried, m. p. 151–157°. Evaporation of the acetone left an oil which after trituration with ether deposited crystals (393 mg.) melting at 136–141°. Two crystallizations from 50% aqueous ethanol yielded 248 mg. of crystals melting at 144–148°. This material was extracted with boiling acetone (30 cc.), the acetone evaporated to dryness, and the remaining white amorphous solid crystallized twice from 50% aqueous ethanol. The resulting white shiny crystals (52 mg.) melted sharply at 141.5°. This compound (VIII) on admixture with the high-melting diastereoisomer of 9,10,12,13-tetrahydroxystearic acid (m. p. 148°) (III) from the peracetic acid oxidation of linoleic acid melted at 135–138°.

*Anal.* Calc'd for  $C_{18}H_{36}O_8$ : C, 62.07; H, 10.34.

Found: C, 61.65; H, 10.54.

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## SUMMARY

1. A method is described for the preparation of the eight diastereoisomers of 9,10,12,13-tetrahydroxystearic acid from  $\alpha$ -linoleic acid. The special arrangements of the hydroxyl groups in each diastereoisomer are deduced from this method of preparation.

2. Two new fatty acids cis- and trans-9,10-dihydroxy-12-octadecenoic acid are also described.

3. The present system of nomenclature applied to the tetrahydroxystearic acids is discussed.

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