

sample as directed in A.O.C.S. Official Method, Cc 8a or 8b-25.

11. Cold pressed refined cottonseed oil is treated by adding 0.5 g. of filtercel to the contents of the cup and agitating at 250 plus or minus 10 r.p.m. at room temperature for 2 to 3 minutes before filtering for the color reading.

It is suggested that the following be added after number 11:

Caution: It is essential that the filtered oil obtained in 10 or 11 above be absolutely clear. If not, refilter, using one of the following filter papers: Eaton & Dikeman No. 192, Whatman No. 12, Reeve-Angel No. 871 or S&S No. 596.

The present color method reads as follows:

The sample must be absolutely clear. If not, filter through a close textured paper such as Eaton & Dikeman No. 617 or Reeve-Angel No. 230 at the temperature specified for reading.

It is suggested that this be changed to read as follows:

The sample must be absolutely clear and if not, filter through a fine porosity paper such as Eaton & Dikeman No. 192, Whatman No. 12, Reeve-Angel No. 871 or S & S No. 596.

## Preparation of Hydroxy Acids by Sulfation of Oleic and Linoleic Acids

EDWARD T. ROE, BENJAMIN B. SCHAEFFER, JOSEPH A. DIXON and WALDO C. AULT

Eastern Regional Research Laboratory,\* Philadelphia 18, Pa.

ALTHOUGH the preparation of monohydroxystearic acid by sulfation of oleic acid has been the subject of numerous investigations, the poor yields and doubtful purity of the hydroxy acid obtained makes further investigation of this problem desirable. Moreover, sulfation of linoleic acid had previously been studied only to a limited extent, and since this acid is present in nearly all commercial preparations of oleic acid, an investigation was made of the products obtained by sulfation of linoleic acid.

It is generally believed that the main product obtained by sulfation and subsequent hydrolysis of oleic acid is 10-hydroxystearic acid and that the 9-hydroxystearic acid is formed only to a limited extent. Theoretically, either the 9- or 10-hydroxystearic acid can be produced, depending on whether the  $-\text{OSO}_3\text{H}$  group is added to oleic acid at the 9- or 10-carbon atom. Clutterbuck (1) isolated  $\gamma$ -stearolactone from the sulfation reaction products, and from it prepared the corresponding  $\gamma$ -hydroxystearic acid. In addition to the hydroxystearic acids and  $\gamma$ -stearolactone, other products are obtained, which have variously been described as anhydrides, lactides (2), and estolides (3).

No reference has been found in the literature for formation of hydroxy acids from linoleic acid by sulfation. It seems likely, however, that sulfation of one or both double bonds of linoleic acid would result in the formation of monohydroxyoleic acids and dihydroxystearic acids.

We have studied the effects of temperature, time, and molar ratio of sulfuric acid to oleic acid to determine the optimum conditions for sulfation of oleic acid. In this study the extent of sulfation was determined by the iodine numbers of the acid hydrolysis products. Ester numbers of the hydrolysis products

The Uniform Methods Committee approved this for adoption.

### Refining Committee:

The Refining Committee recommends that the present two tentative methods for refining soybean oil (one for extracted oil and one for degummed oil) be made official after removing the ambiguity on cooling of the degummed oil by requiring that the latter be held after refining at 12-15°C. for a minimum of 12 hours. The Uniform Methods Committee recommends that these two methods be made official.

On motion of the chairman of the Uniform Methods Committee, properly seconded from the floor, each of the above recommendations were approved for adoption by the Society.

J. T. R. ANDREWS    T. H. HOPPER  
M. M. DURKEE        T. C. LAW  
J. J. GANUCHEAU    L. B. PARSONS  
J. J. VOLLERTSEN, chairman

were used as a measure of the formation of lactones and ester-type polymeric materials.

The effects of reaction conditions on the iodine number and ester number of the acid hydrolysis products are shown in Table I. Most complete sulfation was accomplished with a three-to-one molar ratio of sulfuric acid to oleic acid at 10° for one hour. Increasing the time or temperature of the reaction did not decrease the iodine number further but increased the ester number. The minimum iodine number which could be obtained varied with the linoleic acid content of the starting material. When as little as 3% linoleic acid was present, the minimum iodine number obtained was 10; when 14% was present the minimum iodine number obtained was 20.

The effect of changing the molar ratio of sulfuric acid to oleic acid on the yield of hydroxy acids is shown in Table II. In order to obtain the maximum

TABLE I  
Effects of Reaction Conditions on the Iodine Number and Ester Number of the Acid Hydrolysis Product

Starting Material			Reaction Conditions			Acid Hydrolysis Product	
Composition			Moles $\text{H}_2\text{SO}_4$ per mole Oleic Acid	Temp. °C.	Time hrs.	Iodine Number	Ester Number
Oleic Acid, %	Linoleic Acid, %	Iodine Number					
88.4	2.7	83.3	1	10	1	47	5
			2	10	1	15	26
			3	10	1	10	25
			3	10	4	13	68
88.4	2.7	83.3	1.5	10	4	27	23
			1.5	20	4	21	42
			1.5	40	4	21	61
72.2	14.2	90.7	1.5	10	1	35	23
			1.5	20	1	40	23
			1.5	24	1	31	22
72.2	14.2	90.7	4	10	1	20	47
			4	20	1	21	64

\* One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE II  
Effect of Variations in the Molar Ratio of Sulfuric Acid to Oleic Acid on Yield and Characteristics of Hydroxy Acids

Starting Material	Moles H <sub>2</sub> SO <sub>4</sub> per mole Oleic Acid	Hydrolysis	Once-Crystallized Product		No. of Recryst.	Final Product				
			Yield <sup>a</sup> %	M.P. °C.		M.P. °C.	Acid Number	Sapon. Number	OH %	Iodine Number
72% Oleic Acid.....	1.5	Acid	.....	77 -78.5	1	77.5-79	186.1	186.3	6.08	3.1
72% Oleic Acid.....	2	Acid	35.7	69.5-72.5	1	71 -74	187.7	188.2	6.03	3.4
72% Oleic Acid.....	3	Acid	43.4	66 -71	1	73 -75	187.7	187.8	.....	3.9
72% Oleic Acid.....	2	Acid-Alkaline	65.1	67 -72	1	72 -74	188.0	188.2	5.95	4.0
72% Oleic Acid.....	3	Acid-Alkaline	72.0	.....	4	73 -75	187.5	187.5	5.68	2.8
97% Oleic Acid.....	3	Acid	29.0 <sup>b</sup>	57 -62	3	68 -72	188.8	188.8	5.69	1.4
97% Oleic Acid.....	3	Acid-Alkaline	67.3	.....	1	57 -66	.....	.....	.....	.....
97% Oleic Acid <sup>d</sup> .....	3	Acid-Alkaline	86.4	57 -62	1	63 -69	.....	.....	.....	.....
Theoretical Values.....							186.7	186.7	5.66	0

<sup>a</sup> Yields are based on the total theoretical amount of monohydroxystearic acid and dihydroxystearic acid obtainable from oleic and linoleic acids, respectively, plus the saturated acids present in the starting material.

<sup>b</sup> Twice crystallized.

<sup>c</sup> Melting point of 10-hydroxystearic acid, 81-82° (5).

<sup>d</sup> With diluent.

yield of hydroxy acids, it was necessary to hydrolyze the ester-type polymer formed during sulfation. This was accomplished by refluxing the product obtained after acid hydrolysis with alcoholic potassium hydroxide (4). The higher the ester number, the more difficult it was to hydrolyze completely the polymeric by-product. It was found that prolonging hydrolysis or increasing the amounts of potassium hydroxide darkened the hydroxy acids obtained and that difficulty was experienced in removing the color by crystallization.

The yield of hydroxy acids is based on the weight of material obtained by crystallization of the acid or acid-alkaline hydrolysis product from commercial normal hexane at -25°. The best yields of hydroxy acids were 72% from commercial oleic acid and 86% from purified oleic acid. The latter yield was obtained by using commercial normal hexane as a diluent during sulfation. In the absence of a diluent, the yields with 97% oleic acid were comparatively low, presumably because the fatty acid solidified at the temperature of the reaction and the reactants could not be thoroughly mixed. The last two runs listed in Table II show the effectiveness of a diluent in increasing the yield.

The melting points of the products shown in Table II varied widely. Pure 10-hydroxystearic acid is generally reported to have a melting point ranging from 80 to 85° (5). Once-crystallized hydroxy acids obtained by sulfation with a 1.5 to 1 molar ratio of sulfuric acid to oleic acid had a higher melting point than those obtained by sulfating with larger quantities of sulfuric acid. Repeated crystallization of the low melting acids from various solvents was necessary to raise the melting point, and large losses were in-

curred with each crystallization. The same variations in melting points were found when either commercial or purified oleic acids were used as starting materials. Fractional crystallization resulted in the isolation of small amounts of low melting solids, which had the correct analytical values for monohydroxystearic acid. In addition to this, low-melting crystalline material having an ester number was isolated. The fact that  $\gamma$ -stearolactone is a product of sulfation (6) seems to indicate that a shift of the substituent group takes place, with the result that hydroxy acids other than the 9- and 10-hydroxystearic acids may be formed. Experimental evidence confirming this observation has been presented in another paper (7).

The difference in the melting points of the several products obtained may be explained by the fact that different amounts of these isomeric hydroxy acids are produced under different conditions of sulfation. This work has also demonstrated that hydroxy acids are formed from the linoleic acid in the starting material. Such compounds would also affect the melting points of the products.

In an attempt to purify the hydrolysis products of sulfated 97% oleic acid, their methyl esters were prepared and fractionally distilled under high vacuum. Analyses of the various fractions obtained from this distillation are presented in Table III. Fractions 2 and 3 contained most of the methyl monohydroxystearate, as shown by percent hydroxyl and saponification equivalent. The analytical constants of Fraction 5 and the residue indicate the possible existence of dimeric and trimeric ester-type polymeric material formed by elimination of water. The formation of these polymers during distillation was a disadvan-

TABLE III  
Distillation of Methyl Esters of the Hydrolysis Products of Sulfated Oleic Acid (97%)

	Distillation		Weight, Grams	Saponification Equivalent	Iodine Number	Hydroxyl %	Remarks
	Temp. °C.	mm.					
Fraction 1.....	141-155	0.20-0.23	71.4	297.5	63.5	1.04	First 4 fractions distilled through Vigreux-column
Fraction 2.....	155-167	0.20-0.22	19.1	307.0	9.95	4.39	M.P., 27-28°
Fraction 3.....	167-170	0.22-0.23	94.0	312.5	2.34	4.88	M.P., 29-31°
Fraction 4.....	170-174	0.24	3.4	.....	.....	.....	Solid at room temperature
Fraction 5.....	125-204	0.04-0.08	19.2	293.5	47.7	0.00	Changed to Claisen head
Residue.....	.....	.....	20.6	289.5	34.8	0.04	Cryoscopic mol. wt., 828
Theoretical Values				Saponification Equivalent	Iodine Number	Molecular Weight	
Dimer minus H <sub>2</sub> O.....				289.9	43.8	578.9	
Trimer minus H <sub>2</sub> O.....				286.8	29.4	861.4	

tage of this method of purification because of the lowered yield of methyl monohydroxystearate.

The hydroxy acids obtained from commercial oleic acid had a higher percent hydroxyl than the theoretical value for monohydroxystearic acid. The high percent hydroxyl can be accounted for by the presence of dihydroxystearic acid derived from the linoleic acid. In order to investigate the role played by linoleic acid, a sample of 87% linoleic acid was sulfated by two procedures. In the first, the same technique was employed as in the case of oleic acid in which approximately a six-to-one molar ratio of sulfuric acid to linoleic acid was used without a diluent. No solid material was recovered in the distilled fractions, and more than 60% of the starting material was converted to polymer. These preliminary results were apparently in agreement with the work of Grün (8), who was unable to isolate any solid hydroxy acids from the product obtained by the action of sulfuric acid on linoleic acid.

In the second method, approximately a two-to-one molar ratio of sulfuric acid to linoleic acid was used, with commercial normal hexane as diluent. Under these milder conditions of sulfation only about 20% of the starting material was converted to polymer. The results of this experiment indicate that sulfuric acid reacts with linoleic acid to form isomeric monohydroxyoleic acids and dihydroxystearic acids.

Analytical data for the distilled methyl esters of the hydrolysis products of sulfated linoleic acid are summarized in Table IV. Fractions 2 and 3 evidently consisted of methyl hydroxystearate (from oleic acid in the starting material) and methyl hydroxyoleate, judging from the percent hydroxyl, the iodine number and the saponification equivalent. Fraction 4 undoubtedly contained a considerable quantity of methyl dihydroxystearate, as shown by its hydroxyl content of 8.13%.

When Fraction 4 was further purified by crystallization, the percent hydroxyl of the first and second crystallized fractions was 9.92 and 8.65, respectively, as shown in Table VI.

The results of the crystallization of combined distilled methyl ester fractions 2 and 3 are given in Table V. Methyl monohydroxyoleate appeared to be concentrated in the second fraction and filtrate of this crystallization, as indicated by the high iodine numbers.

Further separation of the methyl monohydroxyoleate and methyl dihydroxystearate fractions into pure components was not successful. The failure to isolate pure components is thought to be due to the presence of isomeric compounds, as it was in the case of oleic acid (7).

Ester-type polymeric materials are apparently formed during the sulfation of linoleic acid. These are converted to hydroxy acids by alkaline hydrolysis and reformed upon distillation. In addition to the ester-type polymers, other polymeric substances are formed during sulfation which cannot be hydrolyzed to monomers with alkali. The data indicate that these unsaponifiable polymers are oxygen linked and not of the double bond type. It is indicated furthermore that these unsaponifiable polymers are not formed during distillation. The formation of such polymers during sulfation of linoleic acid accounts in part for the lower yield of hydroxy acids obtained from commercial oleic acid, as compared with that of purified oleic acid.

TABLE IV  
Distillation of Methyl Esters of the Hydrolysis Products of Sulfated Linoleic Acid

Distilland 195 grams						
	Distillation Temp., °C. at 0.2-0.4 mm.	Weight Grams	Saponification Equivalent	Iodine Number	Hydroxyl %	Remarks
Fraction 1...	162-167	54.3	305.6	72.5	2.41	Liquid
Fraction 2...	167-170	23.5	307.1	58.9	4.65	Liquid
Fraction 3...	170-176	24.1	309.6	56.0	5.49	Liquid
Fraction 4...	180-199	32.8	316.3	19.7	8.13	M. P., 48-52° Cryoscopic mol. wt., 634
Residue*.....	.....	58.2	298.1	89.9	1.56	
Theoretical Values					Hydroxyl %	Mol. Wt.
Methyl Monohydroxystearate.....					5.41	314.5
Methyl Monohydroxyoleate.....					5.44	312.5
Methyl Dihydroxystearate.....					10.29	330.5

\* Anal. C, 74.31; H, 11.36; O (by difference), 14.33.

### Experimental

**Materials.** Oleic acid of three different grades of purity was used. The first was a high grade of commercial red oil containing 72% oleic acid, 14% linoleic acid, and 14% saturated acids. The second grade of oleic acid (88%) was prepared by fractional distillation and crystallization of commercial oleic acid (9). Its composition was 88.4% oleic acid, 2.7% linoleic acid, and 8.9% saturated acids.

The third grade of oleic acid (97%) was prepared by fractional distillation and crystallization of commercial oleic acid (10). The iodine number of this material was 88.2. Its melting point was 11.9-12.4°. Spectrophotometric analysis showed 0.2% linoleic acid, 0.1% linolenic acid, 0.005% arachidonic acid, 0.3% diene conjugated acids, and 0.006% triene conjugated acids. Calculations based on these data indicate that the product was approximately 97% oleic acid.

Linoleic acid was prepared by low temperature crystallization and fractional distillation of corn oil fatty acids (11). The percentage composition was 87.0% linoleic acid, 10.3% oleic acid, and 2.7% saturated acids.

**Preparation of Hydroxy Acids From Commercial Oleic Acid (72%).** Eight hundred grams of oleic acid was weighed into a 3-liter, 3-neck flask and cooled to 10° in an ice-salt bath, and 648 grams of 95.5% sulfuric acid was added during a period of one hour. In order to obtain efficient agitation a Hershberg stirrer (12) with a blade made of B. & S. Number 16 Nichrome wire was used. Since external cooling with an ice-salt bath was not sufficient to maintain the temperature at 10°, powdered Dry Ice in small portions was added directly to the reaction mixture. After all the sulfuric acid had been added, the mixture was allowed to stand for one-half hour at 5° with occasional stirring.

Ice water was added rapidly to the reaction mass to bring the total volume to about 4 liters, and the mixture was boiled, with stirring, for one hour, while a current of carbon dioxide was passed through it to maintain even boiling. The aqueous layer was siphoned off and the hydrolyzed material was washed with boiling water. For further hydrolysis, 1696 cc. of 2 N alcoholic potassium hydroxide (20% excess) was added, and the mixture was refluxed for six hours. After removal of the alcohol by steam distillation the product was neutralized at 60 to 65° with

dilute sulfuric acid, taken up in warm commercial normal hexane (boiling range 63-70°) and washed with warm water until sulfate free. The crude hydroxy acids were crystallized overnight at -25°, filtered, and washed with cold normal hexane. The yield was 617 grams of slightly colored crystalline material.

Attempts were made to purify the monohydroxystearic acid further by crystallization from various solvents. The first recrystallization was from a mixture of 80% normal hexane and 20% acetone. Subsequent crystallizations were made from 70% ethyl alcohol and ethyl ether, and finally from the normal hexane-acetone mixture. The melting point of the products was not raised appreciably by repeated crystallizations from these solvents. The color of the products was improved by ethyl alcohol, although there was a corresponding lowering of yields.

*Preparation of Hydroxy Acids From Purified Oleic Acid (97%).* Five hundred grams of purified oleic acid was sulfated with 535 g. of 95.5% sulfuric acid in the same manner as described for commercial oleic acid. After the alkaline hydrolysis product was neutralized, it was washed with boiling water until sulfate free. The ester number of the hydrolysis product was zero. A portion of this material (125 g.) was crystallized as previously described. Another portion (249 g.) was converted to methyl esters by a standard esterification method in which a large excess of anhydrous methanol containing a small quantity of concentrated sulfuric acid was used. The dried methyl esters (232 g.) were distilled at a pressure of 0.22 mm. through a twelve-inch Vigreux column. Four fractions were taken. The Vigreux column was then removed and a fifth fraction was obtained by distilling through a Claisen head (Table III).

*Preparation of Hydroxy Acids From Purified Oleic Acid (97%) With the Use of a Diluent.* A mixture consisting of 100 g. of purified oleic acid and 50 cc. of commercial normal hexane was sulfated with 107 g. of 95.5% sulfuric acid for one hour at 10°. After addition of water to the sulfation mass, the normal hexane was removed by steam distillation. Hydrolysis and recovery of the hydroxy acids were carried out as described for commercial oleic acid.

*Preparation of Hydroxy Acids From Linoleic Acid (87.0%).* To a mixture of 200 g. of linoleic acid and 670 cc. of commercial normal hexane 135 g. of 95.5% sulfuric acid was added over a period of one hour and forty minutes. During this time the temperature of the reaction varied between 0 and 7°. Ice water was added to the reaction mixture, after which the normal hexane was removed by steam distillation. Acid and alkaline hydrolyses were carried out as previously described. The ester number was reduced from 28.2 to 0 by alkaline hydrolysis. The hydrolyzed material was esterified with a large excess of methanol, sulfuric acid being used as a catalyst. The methyl esters (cryoscopic molecular weight 381) were fractionally distilled through a six-inch Vigreux column at 0.2- to 0.4-mm. pressure (Table IV). Portions of distilled fractions 2 (15.0 g.) and 3 (13.8 g.) were combined and fractionally crystallized from 1440 cc. of acetone. Solid fractions were removed at -50 and -64°. In the same manner 19 g. of distilled fraction 4 (m.p. 48-52°) was crystallized from 950 cc. of acetone. Solid fractions were removed at -30 and -50°. The results of these crystallizations are summarized in Tables V and VI.

TABLE V  
Fractional Crystallization of Combined Distilled Methyl Ester  
Fractions 2 and 3 (Table IV)—28.8 g. Crystallized

	Crystallization Temp., °C.	Weight, Grams	Saponification Equivalent	Iodine Number	Hydroxyl %	M.P. °C.
Fraction 1.....	-50	9.9	314.1	17.5	5.72	37-38
Fraction 2.....	-64	5.8	314.5	64.9	6.05	Liquid
Filtrate.....	.....	12.2	310.3	92.4	4.28	Liquid

TABLE VI  
Fractional Crystallization of Distilled Methyl Ester  
Fraction 4 (Table IV)—19 g. Crystallized

	Crystallization Temp., °C.	Weight, Grams	Saponification Equivalent	Iodine Number	Hydroxyl %	M.P. °C.
Fraction 1.....	-30	5.5	330.4	8.1	9.92	66.5-73.5
Fraction 2.....	-50	5.0	325.6	8.7	8.65	38-40
Filtrate.....	.....	6.9	313.3	50.0	8.00	Liquid

Ten grams of the distillation residue (cryoscopic mol. wt. 634) was refluxed for 24 hours with 4 grams of 87.7% potassium hydroxide in 60 cc. of n-butyl alcohol. After removal of the n-butyl alcohol, the material was neutralized with dilute sulfuric acid, washed, dried, and converted to methyl esters. The cryoscopic molecular weight was reduced to 533.

### Summary

Monohydroxystearic acids were prepared by sulfation and subsequent hydrolysis of oleic acid, and the effect of reaction conditions on yield was studied. Monohydroxystearic acids were obtained from commercial oleic acid in 72% yield and from pure oleic acid in 86% yield. Conditions for the best yields apparently gave increased amounts of isomeric hydroxy acids.

Hydroxy acids prepared by the sulfation and subsequent hydrolysis of linoleic acid were converted to methyl esters, and purified by fractional distillation and low temperature crystallization. Experimental evidence indicates that sulfuric acid reacts with one double bond of linoleic acid to form isomeric monohydroxyoleic acids and with both double bonds to form dihydroxystearic acids. The by-products formed by the sulfation of linoleic acid include both ester-type polymers and additional polymeric material which cannot be converted to monomers by alkaline saponification.

When commercial oleic acid is sulfated and subsequently hydrolyzed, the monohydroxystearic acid thus obtained presumably contains monohydroxyoleic acids and dihydroxystearic acids resulting from the linoleic acid present in the starting material. The formation of an unsaponifiable polymer during the sulfation of linoleic acid accounts in part for the lower yield of hydroxy acids obtained from commercial oleic acid, as compared with that of purified oleic acid.

### REFERENCES

1. Clutterbuck, J. Chem. Soc. 125, 2330 (1924).
2. Saytseff, J. Prakt. Chem. [2] 35, 369 (1887).
3. Riess, Collegium 1931, 557.
4. Price and Griffith, U. S. Patent 2,367,050.
5. Tomecko and Adams, J. Am. Chem. Soc. 49, 525 (1927).
6. Shukoff and Schestakoff, J. Russ. Phys.-Chem. Soc., 35, 1 (1903); J. Russ. Phys.-Chem. Soc., 40, 830 (1908).
7. Schaeffer, Roe, Dixon, and Ault, J. Am. Chem. Soc. 66, 1924 (1944).
8. Grün, Über die Konstitution der Fette; Habilitationsschrift (Universität Zurich, June 1907) Inaug. Diss.
9. Swern, Knight, and Findley, Oil & Soap 21, 133 (1944).
10. Brown and Shinowara, J. Am. Chem. Soc. 59, 6 (1937).
11. Frankel and Brown, J. Am. Chem. Soc. 63, 1483 (1941).
12. Hershberg, Ind. Eng. Chem., Anal. Ed. 8, 313 (1936).