4. *Total and Organic Residue at 160°C., Ea 3-38*

   Replace this Official Method with a new Tentative Method, Ea 3-56, for "Total and Organic Residue at 175°C."

   The proposed tentative method yields much more precise values. The time required is shortened. The U.M.C. suggests that the words "to obtain organic residue" be added to the end of the second sentence under "Principle."

   Approved by U.M.C. Adopted.

5. *Total, Free, and Combined Glycerol, Fats, Oils, and Related Products*

   Adoption of this new, tentative method is recommended by the Glycerine Analysis Committee. The U.M.C. suggests revising the second sentence in Section D-7 to read: "Prepare two blanks exactly as described above, but with sample omitted, and from each pipette 50 ml. into a beaker containing 50 ml. of periodic acid reagent." This is to compensate for any effect of the reagents used in preparation of the sample.

   The Editor of Methods will place this new method in "E. Sampling and Analysis of Glycerine." Its applicability to fats, oils, fatty acids, soaps, etc., will be covered in the "Product-Method" and "Method-Product" lists. Some editorial changes will be necessary to bring within the standard methods format.

   Approved by U.M.C. Adopted.

Seed and Meal Analysis Committee, T. H. Hopper, chairman

The Seed and Meal Analysis Committee has made the following recommendations:

1. *Residual Lint on Cottonseed, Aa 7-55*
   Advance this tentative method to make it official.
   Approved by U.M.C. Adopted.

2. *Oil in Castor Beans, Ae 3-52*
   Work by the subcommittee on Analysis of Castor Beans and Pomace (V. B. Shelburne, chairman) has justified revision of this tentative method. The essential change is that commercial hexane will be used, instead of carbon tetrachloride, as extraction solvent. Substances other than oil are extracted by carbon tetrachloride.

   Approved by U.M.C. Adopted.

3. *Oil in Castor Pomace, Bd 3-52*
   Revision of this tentative method by a change of solvent from carbon tetrachloride to hexane is recommended.

   Approved by U.M.C. Adopted.

4. *B—Specifications for Reagents, Supplies and Apparatus*
   Specifications for commercial hexane for use as a laboratory extraction solvent are made necessary by the revisions of Ae 3-52 and Bd 3-52, if adopted. Suitable specifications are recommended.

   Approved by U.M.C. Adopted.

5. *Moisture and Volatile Matter in Castor Beans, Ae 2-52*
   In Section B-1 delete the words:

   ". . . unfilled beans (usually referred to as 'poppers') . . ."

   Approved by U.M.C. Adopted.

Soapstock Analysis Committee, K. E. Holt, chairman

1. *pH of Acidulated Soapstocks*
   Adoption of this new method, as tentative, is recommended by the Soapstock Analysis Committee for controlling the mineral acid remaining in acidulated soapstocks.
   Approved by U.M.C. Adopted.

Spectroscopy Committee, R. T. O'Connor, chairman

1. *Polyunsaturated Acids, Cd 7-48*
   Based on extensive and thorough investigation, a revision of this tentative method, so complete as to amount practically to its rewriting, is recommended by the Spectroscopy Committee. The method is greatly simplified and its range extended to include pentaenoic acids. It will require some editorial alteration to make it conform to the standard format.

   Tentative Method L 12a-55, for Polyunsaturated Acids in Commercial Fatty Acids, is based upon Cd 7-48. The references in L 12a-55 will require editing in order to make them conform to revised method Cd 7-48.

   Approved by U.M.C. Adopted.

Never in A.O.C.S. history have our technical committees shown greater activity. Progress reports, submitted to the Uniform Methods Committee by the chairmen of the various technical committees, indicate that additions to, and revisions of, our Official and Tentative Methods will continue to increase rapidly.

The Uniform Methods Committee wishes to thank the chairmen and members of our technical committees for their efforts which are making this steady progress possible.

M. M. DURKEE
J. J. GANUCHEAU
D. L. HENRY
T. H. HOPPER
R. G. HOULE
R. R. KING
J. T. R. ANDREWS, chairman

[Revised May 17, 1956]

Structural Studies on Sucrose Monolaurate

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THE PREPARATION of sucrose monoesters of fatty acids by alcoholysis has recently been reported (2, 3). Present studies concerning the structure of sucrose monolaurate involve inversion, tosylation, and periodate oxidation. The only detailed structural study of a sucrose monoester reported in the literature is that of sucrose 2-phosphate (1). This ester was inverted, and glucose phosphate was characterized by periodate oxidation. Consumption of periodate corresponded to the structure assigned. Formic acid production was only 87% of theoretical.

Experimental and Results

Sucrose Monolaurate. Sucrose (200 g., 0.586 mole) was dissolved in 700 ml. of dimethylformamide (DMF) containing 62.7 g. (0.293 mole) of methyl laurate (saponification value, dry, 258, theoretical saponification value 262). Sodium methoxide (4 g.) was added, and the solution was heated to 90°C. for 15 hrs. with stirring in an open beaker. The solution was then concentrated to 300 ml., cooled, and extracted for 24 hrs. in a liquid-liquid continuous extractor with hexane to remove unreacted methyl laurate. The DMF solution was then diluted with 1.5 liters of acetone. The unreacted sucrose which precipitated was removed by filtration, and the filtrate was distilled to a syrup. This syrup was redissolved in 350 ml. of boiling acetone, and the small quantity of sucrose remaining undissolved was separated by a rapid hot filtration. The acetone solution was allowed to cool slowly to room temperature. A white precipitate formed. This was separated, and the acetone filtrate was chilled to 0° to yield a second crop of precipitate. These two fractions were combined and reprecipitated from 150 ml. of acetone to give 43 g. (28% yield) of sucrose laurate, softening point

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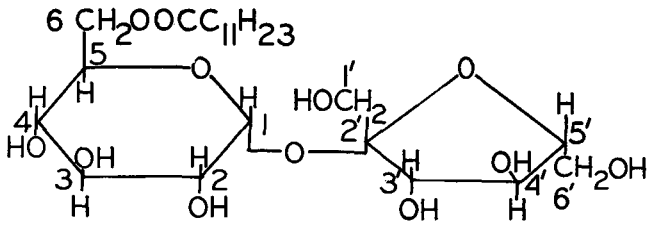

FIG. 1. Sucrose monolaurate.

90–91° C., $[\alpha]_D^{20} = +42.5°$ (0.5% concentration in chloroform), and saponification value = 103. The theoretical saponification value for pure sucrose monolaurate is 106.4.

Inversion of Sucrose Monolaurate. a) By yeast invertase (fructoinvertase). A 2% solution of sucrose laurate, corresponding to 10 g. of the ester, was inverted with 0.5 g. of yeast invertase by incubating at 30° for three days. The solution was then extracted with chloroform. This solvent removed all of the sugar esters quantitatively. The extract was dried, taken up in methanol, deacylated by the procedure of Zémplen (5), and neutralized with acetic acid. This solution, after concentrating to a small volume, was chromatographed on paper, employing ethyl acetate-acetic acid-water (3:1:3) as developing solvent. The spots were developed with a solution composed of 1 g. of aniline and 1.7 g. of phthalic acid dissolved in 100 ml. of water-saturated n-butanol. Spots corresponding to glucose and sucrose were observed. These were of the same approximate intensity. The water solution, after extraction with chloroform, was similarly chromatographed and showed spots corresponding to both glucose and fructose. Fructoinvertase, or yeast invertase, attacks only the fructose part of the sucrose molecule. Any derivative of sucrose with substitution on the fructose part of the molecule will not be affected by this enzyme.

b) By acid inversion. Sucrose laurate (5 g.) was refluxed with 300 ml. of 0.5 *N* oxalic acid. A 25-ml. aliquot of the solution was removed every 15 min. for examination until eight aliquots were collected. Each aliquot was cooled and extracted with 50 ml. of chloroform. The chloroform extracts were evaporated to dryness, taken up in 20 ml. of methanol, and treated with 0.05 g. of sodium methoxide at room temperature for 3 hrs. The methanol solutions were neutralized with acetic acid and evaporated to 5 ml. The methanol solutions were spotted on paper and chromatographed as described in a). No sugars could be found after the third aliquot. The first two aliquots, representing inversion for 15 and 30 min., contained sucrose, glucose, and fractose esters. The first two esters were present in approximately equal quantities while the fructose ester remained as a minor component. From the third aliquot only glucose and fructose esters could be isolated. The relative proportion of these two esters remained constant, with the quantity of glucose ester about four times that of the fructose ester, as estimated by visual examination of the intensity of the spots.

Tosylated Sucrose Laurate. The sucrose laurate preparation was tosylated with p-toluenesulfonyl chloride in anhydrous pyridine to give an amorphous precipitate. It could not be made to crystallize from acetone-water and ethanol-water mixtures. The tosylated sucrose laurate melted at 55–57°C., had a specific rotation of $[\alpha]_D^{21} = +31.7°$ (1.0% concentration in chloroform), a sulfur content of 10.09%, and a molecular weight of 1,000. The sulfur analysis indicated the presence of 3.15 tosyl groups per mole. The molecular weight determined by the ebullioscope method in benzene corresponded to 2.93 tosyl groups.

Treatment of the tosylated sucrose laurate dissolved in acetone with sodium iodide, by heating in a sealed tube at 105°C. for 2.5 hrs. and 16 hrs., resulted in recoveries of sodium p-toluenesulfonate in 71.5 and 81% yields, respectively, based on two tosyl groups being capable of removal with sodium iodide.

Periodate Oxidation of Sucrose Laurate. Sucrose laurate (3.82 millimoles) was dissolved in 50 ml. of water, 35 ml. of 0.4522 *M* sodium periodate (15.82 millimoles) were added, and the entire solution was made up to 100 ml. The solution was allowed to react at room temperature for 24 hrs. after which the solution was assayed.

This solution consumed 8.41 millimoles of sodium periodate, or 2.20 millimoles of sodium periodate per millimole of sucrose laurate. An analysis at the end of 48 hrs. showed no change. At the end of 24 hrs. 2.00 millimoles of formic acid were produced, which are equivalent to 0.524 millimole of formic acid produced per millimole of sucrose laurate. Replicate oxidations for 24-hr. periods resulted in the consumption of 2.28 and 2.32 molar equivalents of periodic acid and the formation of 0.544 and 0.642 molar equivalents of formic acid.

In the above oxidations of the sucrose laurate in water solution a voluminous precipitate formed after 16 hrs. of oxidation. It was thought that this represented underoxidized aldehydic products. The combined precipitate from three separate reactions with sodium periodate was separated and crystallized from ethanol. A molecular weight determination by the ebullioscopic method in benzene gave a value of 490. A 2.0137-g. (4.12 millimoles) sample of this precipitate was dissolved in 75 ml. of methanol and combined with 25 ml. of 0.4522 *M* sodium periodate (11.3 millimoles). This was allowed to stand at room temperature for 24 hrs. This solution consumed 2.7 millimoles of periodate and formed 0.462 millimole of formic acid. These figures correspond to 0.655 millimole of periodate consumed and 0.112 millimole of formic acid produced per mole of material being oxidized. A study of the three periodate oxidations of sucrose laurate in which water alone was employed as solvent shows an average 2.26 millimoles periodate consumed. Further periodate oxidation in methanol of the precipitate which formed during the oxidation, produced an added 0.655 millimole periodate consumption, for a total of 2.915 millimoles periodate consumed. Similarly the total formic acid produced was raised to 0.682 millimole per millimole of sucrose laurate.

Discussion

Inversion experiments establish that the acyl group of a sucrose monolaurate preparation is primarily on

TABLE I

Theoretical Values for Periodate Consumption and Formic Acid Production

Position of lauroyl radical	Molar equivalents of periodate consumed	Molar equivalents of formic acid produced
6, 1′, 6′	3	1
2, 4	2	0
3	1	0
3′, 4′	2	1

the glucose moiety. The ratio of attachment on glucose and fructose moieties is of the order of 4 to 1. The tosylation method for determining whether the lauroyl group was attached exclusively to a primary alcohol of sucrose proved inadequate. With sucrose monolaurate, recoveries of 61.5 and 81.0% were obtained, assuming two free primary groups. These low recoveries are not uncommon (4). These results suggest, but do not establish that one primary alcohol is bound by the lauroyl radical.

Theoretical values for periodate consumption and formic acid production for various positions of the lauroyl radical on sucrose are given in Table I. The experimental value of 2.9 millimoles of periodate consumed per millimole of ester provides strong evidence to the effect that the lauroyl radical is attached almost entirely to a primary alcohol. Some uncertainty is introduced as to the proportion of bound primary alcohol groups by the fact that only 0.68 molar equivalents of formic acid were produced.

Conclusion

A product corresponding to sucrose monolaurate was prepared by the alcoholysis of methyl laurate with sucrose. This product appears to be a mixture, with the principal component having the lauroyl radical at the 6-position of the glucose portion.

Acknowledgment

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Determination of Tocopherol in Autoxidizing Methyl Esters of Fatty Acids[1,2]

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MUCH WORK has been done on the estimation of tocopherol in fats and oils (7). The chief problem has been interference by oxidizing and reducing materials, but, in addition, the fats themselves may affect the values obtained in colorimetric (4, 6) and spectrophotometric (2) procedures. This paper deals with modifications of these procedures developed for use in a study of the stability of tocopherol in autoxidizing methyl esters of fatty acids. Removal of interfering substances was attempted by treatment with sulfuric acid (8), or by saponification. Oxidation of tocopherol to the quinone form (2) was studied as a means of increasing the sensitivity of tocopherol estimation.

Experimental

Treatment with Sulfuric Acid. Pure methyl stearate, oleate, linoleate, or linolenate (obtained from the Hormel Foundation) did not affect the bipyridine colorimetric reaction (8) for *d-α* tocopherol,[3] but oxidized methyl esters reduced the tocopherol values obtained in recovery experiments. Treatment of petroleum ether solutions of tocopherol and oxidized methyl esters with 80% sulfuric acid (80 parts by weight of concentrated acid, sp. gr. 1.84, to 20 parts of water) allowed the colorimetric reaction to develop fully.

Pure methyl esters did not interfere with the characteristic ultraviolet absorption peak of tocopherol at 2980 Å, but oxidized methyl esters obscured the peak, even after treatment with sulfuric acid. A more complete separation of tocopherol from oxidized fat appeared necessary to make spectrophotometry applicable. Treatment with 90 or 95% sulfuric acid, to which tocopherol alone was stable, removed most of the fat but resulted in large losses of tocopherol (Table I), presumably by adsorption on the charred fat (1). Subsequent dilution of the acid phase gave increased recoveries of tocopherol, but appreciable fat was reintroduced into the ether phase (Table I).

It was concluded that sulfuric acid treatment was a useful preliminary to colorimetric determination of tocopherol but could not be used to effect a complete separation of tocopherol from oxidized methyl esters.

Saponification. Saponification in the presence of protective agents was tried at room temperature since earlier applications of this procedure at higher temperatures have not been uniformly successful (4, 11, 12, 13).

At room temperature, potassium hydroxide in water or aqueous alcohol did not completely saponify methyl esters in petroleum ether solution and

TABLE I

Effect of Sulfuric Acid Treatments on Recovery of Tocopherol

Treatment[a]	Tocopherol recovery, % of original amount added[b]	Residual fat, % of original amount
A. 0.01% tocopherol in petroleum ether.		
None	100	—
80% sulfuric acid	100	—
90% sulfuric acid	98.8	—
95% sulfuric acid	97.1	—
Conc. sulfuric acid	23.2	—
B. 0.01% tocopherol and 4% pure methyl oleate in petroleum ether.		
None	100	—
80% sulfuric acid	99.3	99.8
C. 0.01% tocopherol added to 4% oxidizing[c] methyl oleate-tocopherol in petroleum ether.		
None	64.4	—
80% sulfuric acid	98.9	91.8
90% sulfuric acid	86.7	25.9
95% sulfuric acid	31.5	7.7
95% sulfuric acid diluted, then 80% sulfuric acid	97.0	50.9

[a] The acid treatment in each test was followed by washing with 1% potassium hydroxide saturated with sodium sulfate.
[b] Determined colorimetrically by the bipyridine procedure.
[c] Peroxide value about 40 ml. of 0.002 *N* thiosulfate per g. Similar results were obtained with other fatty acid esters.

[1] N. R. C. No. 4053.
[2] Presented in part at meeting of American Oil Chemists' Society, Minneapolis, Minn., Oct. 11–13, 1954.
[3] Similar results were obtained throughout with *dl-α* tocopherol.