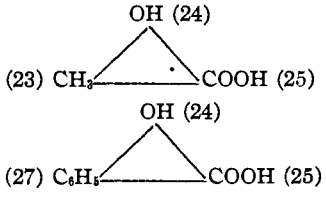
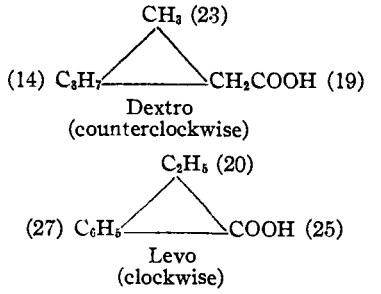
facts as determined by Levene and his collaborators.

Similarly some three hundred known configurations have been checked successfully by means of the ordinal numbers of the table. No exception has been found to the rule: *If the arrangements of the ordinal numbers in two compounds are both clockwise or both counterclockwise, the two compounds have the same configurations for the same sign of rotation.* Another expression of these relations is as follows: two or more configurationally related substances rotate in the same direction when the ordinal numbers are in the same clockwise or counterclockwise order in both, and rotate in opposite directions when the order in one is opposite to that in the other. The value of such a rule in detecting Walden inversions is obvious.



It may be proper to indulge in one speculative application of the rule. No direct chemical means have been found for relating the configurations of lactic and mandelic acids. Applying the rule, we conclude that levorotatory lactic and mandelic acids have the same configuration. Freudenberg[3] has come to the same conclusion from indirect evidence.

Similarly, all three groups may be substituted by other radicals and the configurational relationships of the compounds established as shown by the following.



The present rule is being applied to the still unsettled problem of the configurational relationships of the secondary carbinols to their halides.

Summary

An empirical table of ordinal numbers has been developed for 29 groups which occur in many optically active carbon compounds. By means of this table the configurational relations of a wide range of compounds can be predicted.

(3) Freudenberg, *Ber.*, **66**, 177 (1933).

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[CONTRIBUTION FROM THE INSTITUTE OF EXPERIMENTAL BIOLOGY, UNIVERSITY OF CALIFORNIA]

The Use of the Fractionating Column for the Separation of Fatty Acids[1]

BY SAMUEL LEPKOVSKY, GEORGE V. FESKOV AND HERBERT M. EVANS

Introduction

Although fractional distillation has long been used for the separation of fatty acids, the results have been anything but satisfactory, and in many cases have been decidedly confusing.[2-6]

It is surprising that the fractionating column has found so little use in laboratories engaged in fat analyses. Several such columns have been described. One, described from this Laboratory,[7] has been improved and will be described in detail below. It is useful only when large amounts of fatty acids are to be distilled. A more suitable apparatus for smaller amounts has been described by Bush and Schwartz.[8] Jantzen and Tiedcke[9] have also described a very efficient column for rather small amounts of material.

The fractionating column has undergone intensive development in the last few years, every part of it having received attention—the still head,[10]

(1) Aided by grants from the Rockefeller Foundation of New York City, Board of Research and the College of Agriculture of the University of California.

(2) H. J. Channon, J. C. Drummond and J. Golding, *Analyst*, **49**, 311 (1924).

(3) C. Crowther and A. Hynd, *Biochem. J.*, **11**, 139 (1917).

(4) E. B. Holland, M. E. Garvey, H. B. Pierce, A. C. Messer, J. G. Archibald and C. O. Dunbar, *J. Agri. Research*, **24**, 365 (1923).

(5) E. B. Holland and J. P. Buckley, *ibid.*, **12**, 719 (1918).

(6) E. F. Armstrong, J. Allan and C. W. Moore, *J. Soc. Chem. Ind.*, **44**, 63T (1925).

(7) H. M. Evans, R. E. Cornish, S. Lepkovsky, R. C. Archibald and G. Feskov, *Ind. Eng. Chem., Anal. Ed.*, **2**, 339 (1930).

(8) M. T. Bush and A. M. Schwartz, *ibid.*, **4**, 142 (1932).

(9) E. Jantzen and C. Tiedcke, *J. prakt. Chem.*, **235**, 277 (1930).

(10) E. C. Wagner and J. K. Simons, *Ind. Eng. Chem., Anal. Ed.*, **5**, 183 (1933).

the packing[11,12] and other parts.[12] Though the column has been developed largely for purposes other than the distillation of fatty acids, it can be adapted excellently for fatty acid distillation.

Of fortunate circumstance for precision analysis of fatty acids is the activity shown in the development of microfractionating columns.[13,14] Weston[14] described a microfractionating column that will handle as little as 10 cc. of liquid. These microfractionating columns should lend themselves to fatty acid analysis.

Of importance in fractional distillation is the phenomenon of association, the combination of the carboxyl group of one fatty acid with that of another. This increases the difficulty of separating fatty acids from each other regardless of whether the method used be distillation,[15] crystallization,[16] or distribution between immiscible solvents.[17] Association evidently can be prevented by protecting the carboxyl group by esterification.[15,18]

The use of esters (methyl or ethyl) in distillation has three distinct advantages over the use of fatty acids: (1) lower boiling point; (2) greater stability, especially when unsaturated fatty acids are being distilled; and (3) prevention of association. The difference between fractionating a mixture of methyl esters and the corresponding fatty acids from coconut oil will be described in detail.

Experimental

Preparation of Fatty Acids and Methyl Esters from Coconut Oil.—Coconut oil[19] was subjected to an aqueous saponification with potassium hydroxide, neutralized with sulfuric acid, dried, and given a preliminary distillation *in vacuo*. The methyl esters were prepared[6] by using redistilled methanol. They were dried and also given a preliminary distillation *in vacuo*. The product contained 0.1–0.3% free fatty acids calculated as oleic acid.

The Fractionating Column.—The prototype of this still has been already described[7] but since then a larger unit was constructed. The general arrangement of the apparatus is shown in Fig. 1.

The retort (I) is a 12-liter flask sealed to a 1.8 meter (6 ft.) column made from drawn tubing, 63.5 mm. (2.5 in.) inside diameter and 3.2 mm. (1/8 in.) wall. The column is filled with cut glass tubing, 5–8 mm. long, 3–4 mm. inside diameter and about 1 mm. wall. The retort is charged through the tube, which is closed during distillation by means of a ground-glass stopper (II). A thermometer used to register temperature of ascending vapors is suspended from a platinum wire sealed inside of the stopper. The use of a mercury seal is avoided to prevent contamination of the product.

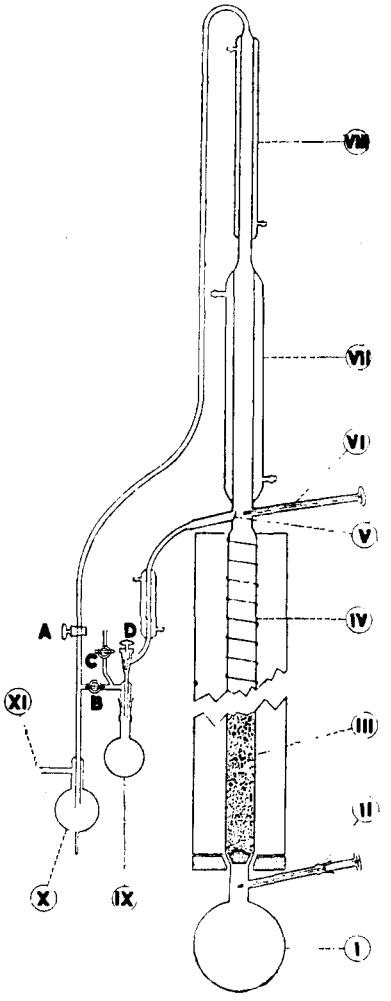

Fig. 1.—The 1.8 meter fractionating column, capacity of retort 12 liters.

The glass column is wrapped with a layer of asbestos paper 3.2 mm. thick, butted together (not lapped). It is wound over the entire length of the column with No. 24 B. S. gage Chromel A wire with 1.2 cm. spacing. The wire totals 256 turns and is 21.5 meters long. The resistance of the wire is 115 ohms. The rheostat allows regulation of the heating of the column. The heating is necessary only while distilling fatty acids having high boiling points. Commercial 85% magnesia pipe covering 10.2 cm. (4 in.) thick, is used for insulating the column with its coil (IV). A glass pocket (V) covering about one-fifth the

(11) C. D. Wilson, G. T. Parker and K. C. Laughlin, THIS JOURNAL, **55**, 2795 (1933).
(12) W. J. Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **3**, 177 (1931).
(13) C. M. Cooper and E. V. Fasce, *Ind. Eng. Chem.*, **20**, 420 (1928).
(14) P. E. Weston, *Ind. Eng. Chem., Anal. Ed.*, **5**, 179 (1933).
(15) H. H. Escher, *Helv. Chim. Acta*, **12**, 27 (1928).
(16) A. Grün, "Analyse der Fette und Wachse," Vol. I, Julius Springer, Berlin, 1925, p. 226.
(17) A. E. Smith and J. W. Norton, THIS JOURNAL, **54**, 3811 (1932).
(18) A. Haller, *Compt. rend.*, **143**, 657, 803 (1906).
(19) The coconut oil was supplied by the Durkee Famous Foods, Berkeley, Calif., to whom we wish to express our appreciation.

circumference of the inside diameter is located at the bottom of the vertical reflux condenser and removes about one-fifth of the condensate, four-fifths running back into the column. The efficiency of the fractionation indicates that the descending liquid is fairly well distributed over the packing.

The temperature of the vapors just before entering the condenser is read from a thermometer inserted opposite the outlet. This thermometer is permanently sealed inside of a glass leg (VI). The reflux condenser consists of two parts. The lower part (VII) is the condenser while the upper part (VIII) serves mostly as a trap. Caught by the glass pocket, the condensate runs down through the cooler and is collected in the receiver. The receiver (IX) is connected to the delivery tube by means of an interchangeable No. 20 ground joint. A series of flasks of different capacities is provided with female parts which fit the male part of the delivery tube. Proper manipulation of the stopcocks enables the separation of fractions without appreciably disturbing the vacuum in the column.

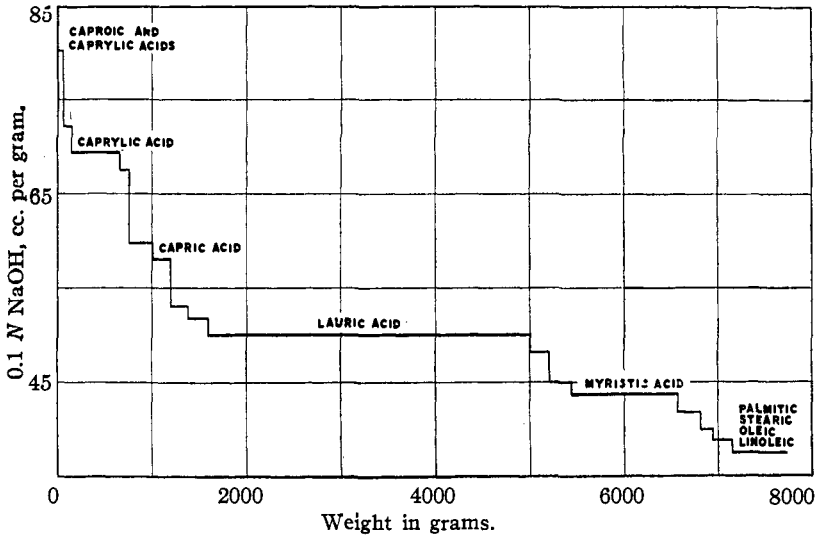

Fig. 2.—Distillation of fatty acids.

When distilling low boiling substances, the trap (X) is surrounded with a freezing mixture. It can be emptied at the termination of distillation by breaking off the tip of the tube at the bottom.

The still is made of Pyrex glass but the glass beads and block supporting them are of common glass. The retort is immersed in an oil-bath which is heated either by an electric hot plate or gas burner. The column is supported in a wooden framework, the supports making contact with only the magnesia covering. As shown in Fig. 1, the column and magnesia covering are set on a wooden block cushioned with asbestos. This serves as the main support of the column, thus allowing unhampered expansion and contraction.

The assembly is connected through trap (X) to a mercury diffusion pump operated in series with a Cenco-Megavac pump, obtained from the Central Scientific Company, Chicago.

The vacuum can be measured with a McLeod gage near the mercury pump. Such vacuum measurements are useful indicators of the general vacuum of the system, but they do not show the vacuum at the point where the fatty acid vapors are ascending into the condenser.

Results

Distillation of Fatty Acids.—About 8.5 kilos of coconut oil fatty acids constitute a charge, fractions being taken according to the temperature of vapors entering the condenser. The total time of the distillation was twenty-four hours. In all 24 fractions were collected. The titration value, saponification value, and melting point were used to determine the purity of the fatty acids. The pure fatty acids and all intermediate fractions were plotted in their proper order, giving a complete picture of the result of the fractionation. It is shown in Fig. 2.

It is evident that there is present in coconut oil a fatty acid of lower molecular weight than caprylic acid. Pure fatty acids were obtained through myristic acid, but no pure palmitic acid was obtained, although as much as 9% is present in coconut oil. The temperature of the vapors entering the condenser varied from 79 to 176°; that in the retort, 180 to 260°. The highest temperature of the oil-bath was 300°. The oil in the bath was Standard Aircraft Engine oil obtained from the Standard Oil Company of California.

Six hundred and ten grams of fatty acids of dark red color were left in the retort. Attempts to separate these fatty acids into their components by crystallization or redistillation led to perplexing mixtures with such low iodine and saponification numbers as to suggest extensive decomposition.

Distillation of Methyl Esters.—The fractionation of the methyl esters (Fig. 3) of coconut oil runs very much the same course as that of the fatty acids through methyl myristate when a notable difference occurs. Pure methyl palmitate was obtained in good yield with only a small intermediate fraction between methyl myristate and methyl palmitate. A small intermediate fraction was also obtained between the methyl palmitate and the methyl esters of the C_{18} fatty acids—stearic, oleic and linoleic acids. The residue remaining in the retort (210 g.) had a light yellow color, indicating less decomposition than was found with fatty acids.

The temperatures of the vapors entering the condenser varied from 45 to 155°. The temperatures in the retort varied from 135 to 225° while the highest temperature in the oil-bath was 287°.

The Isolation of Caproic Acid.—The first distillate collected in the distillation of the methyl esters (Fig. 2) should contain the caproic acid if it is present in the coconut oil. By *fractional* crystallization with barium chloride,[16] caproic acid was isolated and identified by its titration value—85.5 cc. of 0.1 *N* sodium hydroxide per gram—and by its saponification number, 482. The theoretical values

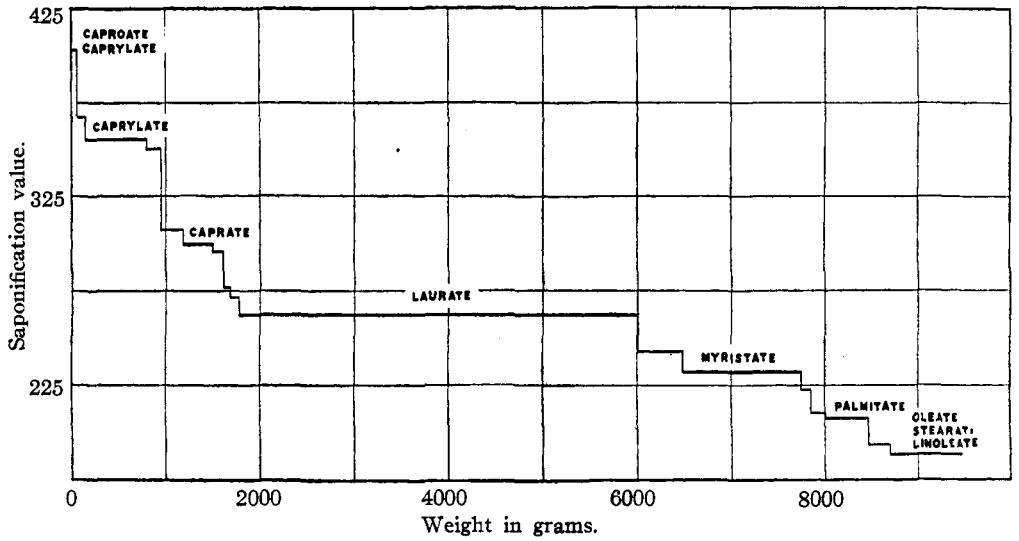

Fig. 3.—Distillation of methyl esters.

are 86.0 cc. of 0.1 *N* sodium hydroxide per gram and a saponification number 483.

The Isolation of Stearic Acid and Partial Separation of the Unsaturated Fatty Acids.—The mixtures containing the methyl esters of oleic, linoleic and stearic acids could not be separated by distillation because their boiling points are too close together. Pure methyl stearate was separated by successive crystallization from methyl alcohol. Crystallization occurred readily at 0°. Partial separation of the oleic acid from the linoleic acid was made by crystallizing their barium soaps from a mixture of 1:1 benzene and 95% ethyl alcohol.[20]

The Composition of Coconut Oil.[21]—The intermediate fractions of the lower fatty acids consisted of two known fatty acids and each could accordingly be calculated. In this way the average fatty acid composition of coconut oil was established and is given in Table I. A total of about 250 kilos of coconut oil was used in this investigation.

TABLE I

THE FATTY ACID COMPOSITION OF COCONUT OIL

Acid	%	Observed m. p., °C.
Caproic	0.5	−3−−4
Caprylic	9.0	15–16
Capric	6.8	30–31
Lauric	46.4	43–44
Myristic	18.0	53–54
Palmitic	9.0	62–63
Stearic	1.0	69–70
Oleic	7.6	...
Linoleic	1.6	...

The Isolation of Erucic Acid from Rape Seed Oil.—The methyl esters of rape seed oil were distilled, largely for the purpose of preparing pure erucic acid.

The methyl esters distilling at 165° were identified as those of erucic acid. They had an iodine number of 71.8. They were decomposed and the erucic acid had an iodine number of 75.5 (calcd. 75.8) and a melting point of 31–32°. The titration value obtained was 29.6 cc. of 0.1 *N* sodium hydroxide per gram of erucic acid. The yield of erucic acid was approximately 40%.

Summary

1. Caprylic, capric, lauric, myristic, palmitic and erucic acids have been obtained in pure state in large amounts by the use of the fractionating column described.

2. The constitution of coconut oil has been determined. From it a fatty acid of lower molecular weight than caprylic acid—caproic acid—has been isolated and identified, and a relatively large amount of pure stearic acid has been prepared.

3. When isolating caprylic, capric and lauric acids, the fatty acids serve as well as the methyl esters. For fatty acids of higher molecular weight, the use of the methyl esters is preferable and sometimes necessary.

4. An indication of association of the higher fatty acids at high temperature has been found in the difficulty of separating by distillation palmitic acid from a mixture consisting largely of oleic acid with some linoleic and stearic acids, but their complete separation from palmitic acid was possible by distillation of the methyl esters.

BERKELEY, CALIF. RECEIVED OCTOBER 4, 1935

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(21) E. R. Taylor and H. T. Clarke, THIS JOURNAL, **49**, 2829 (1927).