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Reaction mechanism in the formation of methyl esters of fatty acids

by M. Jaky

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The reaction mechanism ~~of~~ in the formation of fatty acid methyl esters 216

by Dr. M. Jáky

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Abstract

Reaction Mechanism in the Formation of Methyl Esters of Fatty Acids

The studies showed that methyl esters of fatty acids are formed exclusively in the hydrolysis of fats under definite conditions of reaction. Methyl esters are formed within a few seconds by treating triglycerides with alkaline methanol in homogeneous phase. If mineral acids are used as catalysts, the formation of esters from fatty acids and methanol is very rapid even at low temperatures. The author has reported two rapid methods for preparation of methyl esters of fatty acids which are especially suited for esterification and gas chromatographic investigation of polyenoic fatty acids.

The formation and preparation of fatty acid methyl esters has been investigated in the past by numerous workers. This subject has become currently important especially since gas-chromatographic methods for the study of fatty acids have become so widespread; in the gas-chromatographic analysis of fatty acids, it is the methyl esters of the acids that are used (1-13). The present work deals with the trans-esterification and hydrolysis mechanism of fats, as well as the preparation of fatty acid methyl esters by practical methods; in addition, esterification of sensitive polyunsaturated fatty acids can be carried out without decomposit

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Experimental Section 217

Two methods were chosen for the studies. In the first method, trans-esterification of natural fats to methyl esters was carried out by a cold process. In the second method, natural fats were first saponified by a cold process, the free fatty-acids separated, then finally the methyl esters prepared by a cold esterification process. In the first method, that of R. Henrique (14), the fats were completely saponified in a homogeneous alcoholic alkaline solution within a specific period of time even without mixing. This process takes place quantitatively with the use of a corresponding excess of alkali so that the saponification number (Köttsdorfer number) can also be determined in this way. For the experiments carried out in the present work the procedure of R. Henrique was modified. For the saponification R. Henrique used an ethanolic 1 N sodium hydroxide solution. Three to four grams of the fat under study were dissolved in 25 ml. petroleum ether and 25 ml. of an ethanolic 1 N sodium hydroxide solution added. In this method the sodium hydroxide concentration of the solution amounted to 2% and the sodium hydroxide excess was ca. 50%. This excess is sufficient for complete saponification but the saponification reaction can slow down in the final stages of saponification. In addition incomplete mixing of the petroleum ether and the 1 N sodium hydroxide solution made up in 96% alcohol according to the original procedure, can occur, so that the saponification does not go to completion.

In our own studies, the method of R. Henrique was modified so that pure fresh fat was first dissolved in peroxide free ethyl ether, then 5% potassium hydroxide in absolute methanol added. Finally the total mixture was shaken, producing a homogeneous solution. In such a solution complete saponification of the fat occurs at room temperature within a specific period of time. As described below, the saponification takes place faster if one employs 10% potassium hydroxide dissolved in absolute alcohol.

Fortini, Anderson and Brown established many years ago that in the hydrolysis of fats and alkalies in alcoholic solution, fatty-acid methyl esters and ethyl esters were always formed as intermediates depending upon which alcohol was used to dissolve the alkali. This was later also demonstrated in the experiments of Toyama, Tsuchiya and Ishikawa; the authors, however, did not reveal how rapidly the ester formation (trans-esterification) took place or whether it occurred selectively or not.

For saponification in alcoholic solution raw, fresh and filtered sun flower oil, obtained by pressing, was used. The process of hydrolysis was studied as a function of time with identical concentration and temperature conditions

Method

0.2 g. freshly pressed sunflower oil was weighed out in a test-tube, dissolved in 2 ml. peroxide-free ether and mixed with 2 ml. 5% methanolic potassium hydroxide solution. The trans-esterification or hydrolysis was interrupted at specific time intervals, when 2 ml. water were added to the homogeneous solution. The reaction mixture separated into two layers. The ether layer contained any oil which remained unaltered and the methyl esters formed, while the hydrolysed fatty-acids in the form of potassium soaps are contained in the aqueous methanolic alkaline layer. After a reaction time of 1,5 and 10 minutes, then finally 1 hr. the trans-esterification or hydrolysis was interrupted and the content of methyl esters or the fatty-acid content was determined. 2 ml. petroleum ether was then added to the reaction mixture and the two layers separated in the separatory funnel. The ether-petroleum ether layer is washed with water and used for further investigations (thin-layer and gas chromatography). The lower aqueous layer containing methanol was shaken with 5 ml. petroleum ether and the separated aqueous methanolic layer was finally heated to 50-60 °C. On addition of hydrochloric acid the fatty-acids were obtained, then taken up in 4 ml. petroleum ether and used in chromatographic studies.

In Figures 1 to 3 the experimental results are given.
From Fig. 1 it can be seen that the trans-esterification
in homogeneous phase begins within a few seconds at room
temperature. Figs. 2 and 3 show that the trans-esterification
of sunflower oil takes place selectively and the sapon- 218
ification of the methyl ester begins in the first 5
minutes after termination of the trans-esterification.
Fig. 4 shows the progress of the trans-esterification and
hydrolysis. The process of hydrolysis does not take place
completely linearly. For the experimental conditions
chosen, 50% of the fatty-acids are present after ca. 7
hours hydrolysis; a complete saponification occurs between
the 40th and 41st hours.

In a further series of experiments a 5% alkali concentration was used (Fig. 5). Fig. 5 shows that the curve is steeper. The formation of the methyl ester required approximately the same time as for an alkali concentration of 2.5% ; complete saponification occurred in this case however, after ca. 21 hours. 50% fatty-acids had formed after ca. 3 hours.

A further study was made in order to ascertain whether and how much the ratio of individual components in the fatty-acid composition of the methyl esters, which remained behind with progressive saponification, altered during the saponification. These studies were carried out with the DDR-gas chromatograph Giede f (Chromosorb W, 60 mesh, impregnated with 15% diethyleneglycol succinate; flame ionization detector, with argon as carrier gas; temperature: 180 °C ; gas flow velocity : 40 ml./min.; amount of substance : 0.2 μl.). For the gas chromatography experiments we used the same sunflower oil esters having various degrees of saponification, which gave the results given in Figs. 1 to 3. The results obtained by gas chromatography are given in Table 1.

On the basis of the data given in Table 1 it

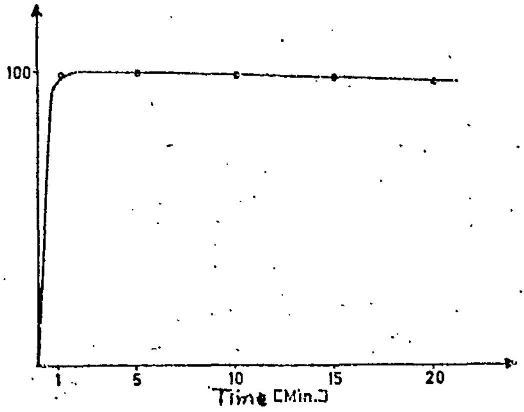

Fig. 1. Trans-esterification of sunflower oil with methanol in homogeneous solution at 20°C; alkali concentration: 2.5%

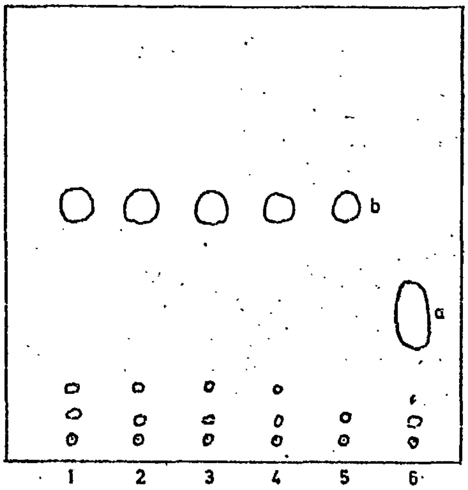

Fig. 2. Thin-layer chromatogram of sunflower oil trans-esterified with methanol in homogeneous solution at 20°C (alkali concentration: 2.5%) ; a: triglycerides; b: fatty-acid methyl ester; Carrier phase: silica gel G by Stahl's method, 0.3 mm.; mobile phase: petroleum ether + ether + glacial acetic acid = 90:10:1 ; stained with iodine vapour; amount applied: 500 γ

1. reaction time: 1 min.; 2. reaction time: 5 min.; 3. reaction time: 10 min.; 4. reaction time: 15 min.; 5. reaction time: 20 min.; 6. sunflower oil.

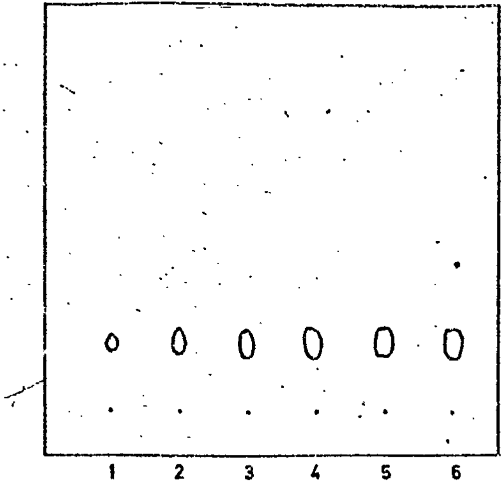

Fig. 3. Thin-layer chromatogram of the hydrolysed methyl ester. 1. reaction time: 1 min.; 2. reaction time: 5 min.; 3. reaction time: 10min.; 4. reaction time: 15 min.; 5. reaction time: 20 min.; 6. fatty-acids from sunflower oil.

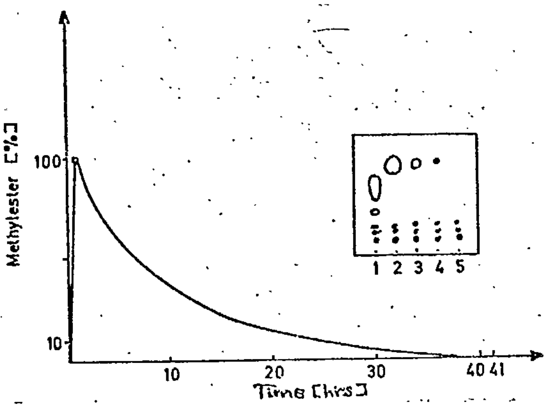

Fig. 4. Trans-esterification and saponification of sunflower oil at an alkali concentration of 2.5%. 1. sunflower oil 2. reaction time: 5min.; 3, reaction time: 20 hours ; 4. reaction time: 40 hours ; 5. reaction time: 41 hours

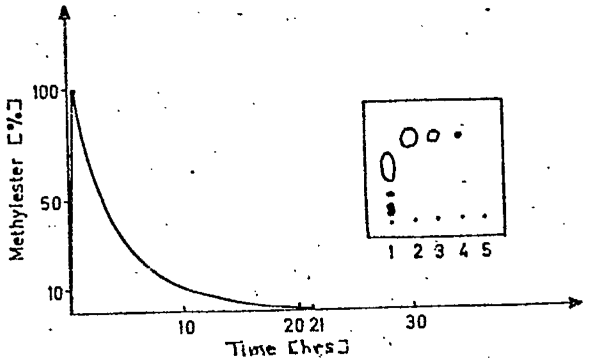

Fig. 5. Trans-esterification and saponification of sunflower oil at an alkali concentration of 5.0%

1. sunflower oil
2. reaction time 5 min.
3. reaction time 10 hrs.
4. reaction time 20 hrs.
5. reaction time 21 hrs.

Table 1. Fatty-acid composition of the methyl esters of sunflower oil after different periods of saponification.

Sample	Duration of Saponification	Fatty-acid Composition (%)			
		Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
1	1 min.	4.9	3.9	19.3	71.9
3	30 min.	5.3	3.9	19.3	72.2
6	2 hrs.	5.0	4.0	19.6	71.4
9	10 hrs.	4.7	4.4	19.7	71.2
10	20 hrs.	4.7	4.0	19.4	71.9

appears that with progressive saponification, the fatty-acid components of the methyl esters are saponified corresponding to their percent content; that is, for the hydrolysis of fatty-acids of various chain lengths and differing degrees of saturation no selectivity was observed.

In additional experiments we were concerned with the trans-esterification to methyl esters of the free fatty-acids, obtained by the method of Henrique.

As already mentioned, the cold saponification in alcoholic medium takes place quantitatively within several hours. If the alcoholic-ether solution of the saponified fat is diluted with water (for this purpose, in the ratio 1:1), then the ether phase separates from the soap-containing aqueous methanolic layer. The ether phase contains the unsaponifiable part of the natural fat such as sterols, vitamins, lipochromes hydrocarbons etc. By further extraction of the aqueous methanol phase with sufficient petroleum ether, one obtains in the usual way fatty-acids of high purity from the soap solution. It is possible to separate the fatty-acids by a cold method. In this case as well, freshly-pressed or extracted sunflower oil serves as starting material. On the basis of earlier observations the fatty-acids, prepared by us by a careful method, were esterified by a cold method to methyl esters and the mechanism of esterification studied. We established earlier that the pure fatty-acids dissolve easily 219
in methanol and ethanol and with the addition of a small amount of hydrochloric acid or sulfuric acid, ester formation takes place even at room temperature. With the addition of a greater amount of hydrochloric acid, the esterification occurs more rapidly.

For the solution of the pure fatty-acids we generally used absolute methanol. To vary the hydrochloric acid concentration, absolute methanol was saturated with hydrogen chloride gas. In aliquots of a solution of known standardized concentration, the decrease in the amount of free fatty- acid was monitored by titration with 0.1 N methanolic potassium hydroxide at specific time intervals; when applicable, the amount of fatty-acid methyl ester formed was calculated. The experimental results are given in Figs. 6 and 7. It is seen from Fig. 6 that 5% of the free fatty-acid is already converted into methyl ester after 2 days, and 15%, after 7 days. Accordingly mere traces of hydrochloric acid cause esterification. On the basis of the curves in Fig. 7, it can be established that the rate of ester formation increases suddenly with increase in hydrochloric acid concentration. While traces of hydroochloric acid result in only about 10 to 15% ester formation, the reaction proceeds almost quantitatively in 1 1/2 hours at a concentration of 0.2% hydrochloric acid. At a hydrochloric acid concentration of 0.42% quantitative ester formation was observed after 1/2 hour ; at a hydrochloric acid concentration of 2.3%, even after 20 min. The progress of reaction is also clearly visible in Figs. 8 to 10.

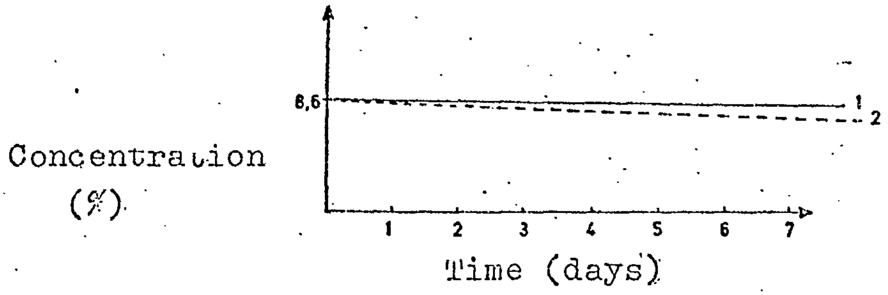

Fig. 6. Conversion of sunflower oil fatty acids to the corresponding methyl esters in methanolic solution; fatty-acid concentration 8.6% (vol./g.*). 1.Solution without HCl; 2. Solution with traces of HCl.

Discussion of Results

On the basis of the experimental results the following conclusions about the mechanism of hydrolysis

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*Translator's note: this is more likely g./vol.

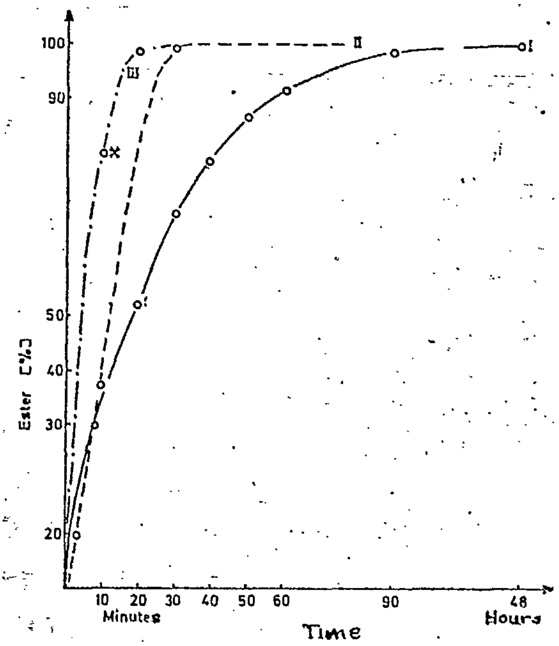

Fig. 7. Conversion of fatty-acids from sunflower oil to the corresponding methyl esters in methanolic solution at various concentrations

I. Fatty-acid concentration 3.9%
HCl concentration 0.2%
II. Fatty-acid concentration 2.8%
HCl concentration 0.4%
III. Fatty-acid concentration 10.0%
HCl concentration 2.3%

x - separation of methyl esters

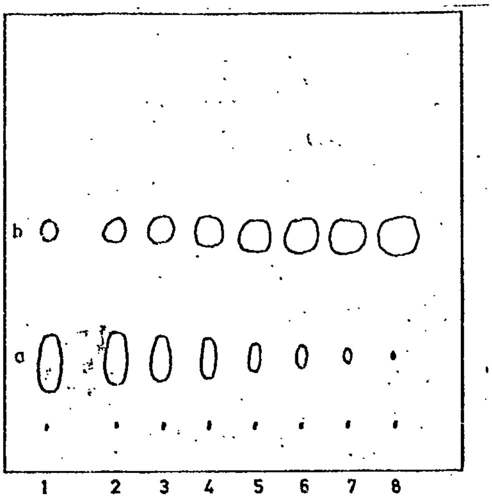

Fig. 8. Thin-layer chromatogram of reaction products obtained on conversion of sunflower oil fatty-acids to the corresponding methyl esters in methanolic solution after specific time intervals. (fatty-acid concentration 3.9%, HCl concentration 0.2%);
a: free fatty-acids ; b: fatty-acid methyl ester
1. reaction time 10 min.
2. reaction time 20 min.
3. reaction time 30 min.
4. reaction time 40 min.
5. reaction time 50 min.
6. reaction time 60 min.
7. reaction time 90 min.
8. reaction time 24 hrs.

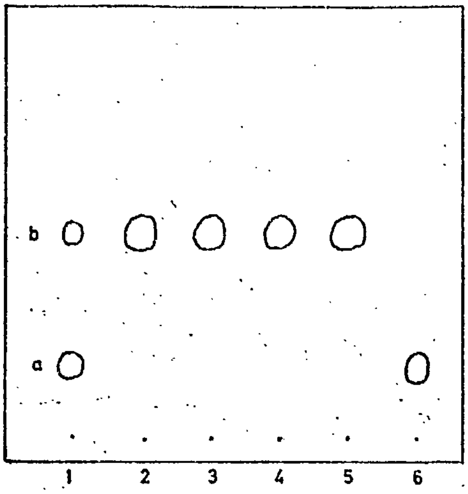

Fig. 9. Thin-layer chromatogram of reaction products obtained on conversion of sunflower oil fatty-acids to the corresponding methyl esters in methanolic solution after specific time intervals (fatty-acid concentration 2.8%; HCl concentration 0.4%). a: free fatty-acids ; b: fatty-acid methyl ester.

1. reaction time 10 min.
2. reaction time 30 min.
3. reaction time 90 min.
4. reaction time 180 min.
5. reaction time 24 hrs.
6. fatty-acid methyl ester

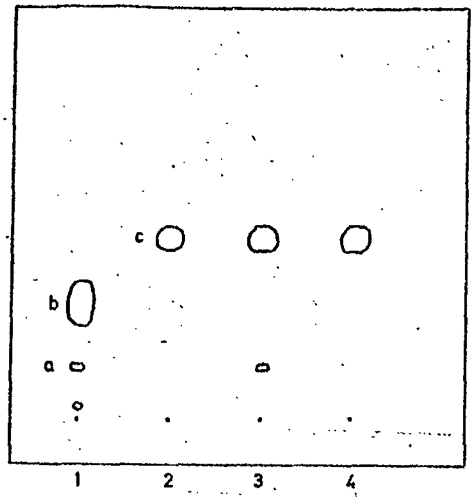

Fig. 10. Thin-layer chromatogram of the reaction products obtained on conversion of sunflower oil fatty-acids to the corresponding methyl esters in methanolic solution after specific time intervals (fatty-acid concentration 10.0% ; HCl concentration 2.3%). a: free fatty-acids ; b: triglycerides ; c: fatty-acid methyl ester.

1. sunflower oil
2. oily layer appearing after 10 min.
3. clear methanol solution (after separation of oily layer).
4. fatty-acid methyl ester.

of sunflower oil may be drawn. The observation by Fortini, Anderson and Brown, that fatty-acid ethyl esters and methyl esters always arise as intermediates in the hydrolysis of fats carried out with alkali in alcoholic solution is actually confirmed. From our own studies it was shown that the hydrolysis of fats proceeds exclusively via fatty-acid ester formation; that is , that the triglyceride is quantitatively trans-esterified within seconds to the fatty-acid methyl ester or ethyl ester (Fig. 1) in alcoholic alkaline solution and in homogeneous phase; these derivatives are saponified by the action of alkali. On the basis of these facts, the old observations that saponification proceeds much more rapidly in alcoholic solution than in aqueous solution, can also be accounted for. The fatty-acid esters are saponified considerably more quickly than trigycerides (at boiling temperature even soda solution causes saponification). The hydrolysis of fats under the given conditions proceeds accordingly in two steps:

1. $$\text{Trigyceride} + \text{alcohol} + \text{KOH} \xrightarrow[\text{homogeneous phase}]{20^\circ\text{C}} \text{Fatty-acid ester} + \text{glycerine}$$

2. $$\text{Alcoholic fatty-acid ester} + \text{KOH} \xrightarrow[\text{homogeneous phase}]{20^\circ\text{C}} \text{Potassium salt of fatty-acid} + \text{alcohol}$$

In the first reaction step the base acts accordingly only as a catalyst, and the trigyceride is trans-esterified within seconds; in fact, in this way the di- and mono-glyceride are never found present as intermediates. In the second reaction step, the hydrolysis of the alcolholic ester of the fatty-acid takes place with the excess base. This second reaction step lasts a longer time in homogeneous phase at 20°C (Figs. 4 and 5). The time-dependent process of trans-esterification of sunflower oil was catalysed in the same way by the two base concentrations used in the experiments; the transformation into methyl esters occurred within seconds in both cases. On the basis of this knowledge it is possible to prepare methyl esters from natural fats by a mild process using a cold method within minutes. These can then be used for gas-chromatographic studies or for preparative purposes.

The new technique described in the experimental part was also suited to provide an answer to the question of whether the saponification of palmitic-, stearic-, oleic-, and linoleic-methyl esters proceeds selectively or corresponding to the percent content of the fatty-acids present. The data given in Table 1 show clearly that no significant difference is observed between the fatty-acid composition of the sample taken at the beginning of the ester formation and the fatty-acid composition of the sample taken after specific reaction periods. Accordingly, the saponification of the methyl esters of the fatty-acids arising from sunflower oil proceeds unselectively in alcoholic solution and homogeneous phase at room temperature; that is, the original fatty-acid composition is maintained until hydrolysis is complete. In the sample investigated the proportion of the four different fatty-acids did not alter during the hydrolysis.

In the second part of the present work, the mechanism of esterification with methanol of sunflower oil fatty-acids, prepared in high purity by the cold method, was studied. It was observed that methanol is a good solvent for fatty-acids. If it contains only traces of mineral acid (hydrochloric acid, sulfuric acid, etc.) ester formation occurs between methanol and the free fatty-acids.

The rate and degree of completion of the reaction can be substantially increased by increasing the hydrochloric acid content of the methanol. While traces of hydrochloric acid catalysed the methyl ester formation only to 15-20% even after a period of days, the reaction proceeded almost quantitatively with a hydrochloric acid content of 0.42% even within 1/2 hour. Methanol solutions which contained 10% fatty-acid and 2.3% hydrochloric acid became cloudy after a few minutes, and a separate oily phase could be seen which was shown to be pure fatty-acid methyl ester (Fig. 10).

In addition, we studied the fatty-acid composition of the methyl esters prepared by esterification of free fatty-acids using a cold method. The same results were obtained as with the trans-esterification or partial hydrolysis of methyl esters. Accordingly, the ester formation did not proceed selectively, but in proportion to the original fatty-acid composition.

This knowledge is important also from a practical viewpoint. One need not wait for the completion of the ester formation in methanol; the esterification can, for example with a high hydrochloric acid concentration (1-2% hydrochloric acid, g./vol.) be interrupted even after 1/2 hour and the fatty-acid methyl esters formed then be used for gas-chromatographic purposes. The esterification by a cold method is especially useful if the lipoids studied contain a large proportion of unsaponifiable products such as cholesterol, lipochromes, hydrocarbons etc. If the unsaponifiable part of the fat under study amounts to about 1 to 5%, then the alkaline esterification method discussed in the first part of the work is more advantageous.

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