Equivalent weights were determined by heating an accurately weighed sample of the methyl acylpyruvate, about 0.5 g., under reflux with a small excess of 0.25 *N* sodium hydroxide for four to six hours. The excess of base was then back titrated with standard hydrochloric acid.

Summary

Five aliphatic methyl ketones have been condensed with diethyl oxalate by means of sodium methoxide in absolute methanol to give methyl acylpyruvates in consistently good yields. This modification of the Claisen reaction is superior to the usual procedure in that good yields of acylpyruvates may be obtained with less care.

ATLANTA, GEORGIA RECEIVED JUNE 5, 1945

[CONTRIBUTION NO. 553 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

Determination of Linoleic Acid in Cholesteryl Linoleate[1]

BY JACQUELINE S. FRONT AND B. F. DAUBERT

In a study of methods for the quantitative determination of linoleic acid in small volumes of blood, we have had occasion to prepare the linoleic acid ester of cholesterol in a high degree of purity. The ester was prepared by a slight modification of the method of Page and Rudy[2] and also by direct esterification of cholesterol with linoleyl chloride in quinoline–chloroform solution.

It was anticipated that if cholesteryl linoleate could be directly isomerized in alkaline ethylene glycol solution and the absorption spectra measured on the isomerized product according to the method of Mitchell, Kraybill and Zscheile,[3] an additional and perhaps a more satisfactory method of ascertaining its purity would result. The assumption that the ester could be directly isomerized was based upon the fact that natural fats,[3] synthetic glycerides[4] and potassium linoleate are directly isomerizable, and the linoleic acid content determined from subsequent spectral absorption data on the isomerized products.

It has been determined experimentally in this investigation, however, that it is not possible to isomerize cholesteryl linoleate directly in alkaline ethylene glycol or glycerol solution, precisely according to the method of Mitchell, *et al.*[3] Cholesteryl linoleate is not soluble in ethylene glycol or glycerol. Consequently, when the mixture of alkaline glycol or glycerol is heated with the cholesteryl linoleate at 180° for thirty minutes in an oil-bath, the ester fuses and rises to the surface. Partial hydrolysis of the ester does occur under these conditions as may be observed from the data in Table I. The average $E^{1\%}_{1\,cm.}$ value of 46 from six isomerizations of cholesteryl linoleate in alkaline ethylene glycol definitely indicates that although partial hydrolysis occurred, it represents only 12.3% of the theoretical $E^{1\%}_{1\,cm.}$ value of 374. This value is based on an experimentally determined $E^{1\%}_{1\,cm.}$ of 867 for linoleic acid. The bulk of the ester was recovered unhydrolyzed. Mechanical agitation of the mixture during the heating did not affect materially the magnitude of the hydrolysis.

The apparent failure of the ester to saponify completely when subjected to the above conditions seems to indicate that isomerization did not occur for one or both of two reasons. First, because of the insolubility of the ester in the solvent, and, second, because saponification was inhibited under the almost anhydrous conditions of heating. If the latter reasoning is correct, then saponification of the ester seems to be necessary before isomerization of the linoleic acid can occur.

Preliminary efforts to find a material suitable as a solvent for both potassium hydroxide and cholesteryl linoleate in order to test the hypothesis that saponification must be preliminary to isomerization have not been successful. Therefore, a modification of the procedure of Mitchell, *et al.*, has been used to determine the linoleic acid in cholesteryl linoleate, to thus serve as a method for the determination of the purity of the ester.

Experimental

Cholesteryl Linoleate.—The ester was prepared from cholesterol (m.p. 148°) and linoleyl chloride according to the method of Page and Rudy[2] with the following modification: after reaction, the cooled liquid ester was dissolved in 300 ml. of ethyl ether and the solution washed with 5% potassium carbonate and water. The ether was removed from the dried and filtered liquid under nitrogen and the liquid residue dissolved in small volume of ether. Sufficient ethyl alcohol was then added to impart slight turbidity to the solution. Crystallization of the ester occurred on cooling overnight at 0 to 5°. Recrystallization several times from the same mixture of solvents yielded a product melting sharply at 42.5° (Page and Rudy, 42.0°).

The ester was also prepared by the following method. Cholesterol (5 g.) was dissolved in a mixture of chloroform (20 ml.) and quinoline (5 g.) and to this solution there was added linoleyl chloride (3.9 g.). The mixture was refluxed on a steam-bath for three hours and after cooling was dissolved in ethyl ether (300 ml.). The ether solution was transferred to a separatory funnel and washed successively with 30-ml. portions of 0.5 *N* sulfuric acid, 5% potassium carbonate solution and water. After drying over anhydrous sodium sulfate, the solution was treated in the same manner as above. The melting point of the crystallized product is 42.0–42.5°; mol. wt.,[5] 465 (calcd.

(1) The generous financial assistance of the Buhl Foundation and Nutrition Foundation, Inc., is gratefully acknowledged.

(2) Page and Rudy, *Biochem. Z.*, **220**, 304 (1930).

(3) Mitchell, Kraybill and Zscheile, *Ind. Eng. Chem., Anal. Ed.*, **15**, 1 (1943).

(4) Baldwin and Daubert, *Oil & Soap*, **22**, 180 (1945).

(5) Hanson and Bowman, *Ind. Eng. Chem., Anal. Ed.*, **11**, 440 (1939).

468.6); I. V., Rosenmund-Kuhnhenn 117.7, three hours (300% excess reagent) (calcd. 117.4).

Spectrophotometric Analysis of Cholesteryl Linoleate for Preformed Conjugation.—A Beckmann spectrophotometer was used in all spectral absorption measurements.

Absorption measurements of a solution of cholesteryl linoleate in iso-octane solution showed a total diene conjugation of less than 0.1% and the triene conjugation was negligible. Corrections for preformed conjugation were therefore not made in the final $E^{1\%}_{1\,cm.}$ values given in Table I.

TABLE I

$E^{1\%}_{1\,cm.}$ VALUES FOR ALKALI ISOMERIZED CHOLESTERYL LINOLEATE

$E^{1\%}_{1\,cm.}$ 234 mμ	
Direct isomerization, Mitchell, *et al.*[1]	Modified method
29	363
49	369
59	388
63	373
43	
33	
Av. 46	Av. 373

Alkali Isomerization of Cholesteryl Linoleate.—Since direct isomerization of cholesteryl linoleate in alkaline glycol solution was not feasible, the following modification of the method developed by Mitchell, *et al.*, was used.

The alkaline glycol used in the isomerization was prepared by dissolving in redistilled ethylene glycol 7.5 g. of potassium hydroxide in 100 ml. of glycol. The solution was heated before use to 190°, cooled to room temperature, and made up to 100 ml. with ethylene glycol.

Approximately 0.1 g. of cholesteryl linoleate was weighed into the bottom of a 1″ × 6″ Pyrex test-tube. One ml. of alcoholic potassium hydroxide (55 g. to 100 ml. of 99% ethyl alcohol) was added to the sample and to a blank from a pipet. The tubes were warmed in a water-bath at 55–60° for ninety minutes. Ten ml. of alkaline ethylene glycol was added by pipet to the sample and blank and the tubes were placed in a constant temperature bath at 180° for thirty minutes under nitrogen. At three successive one-minute intervals, the tubes were removed and shaken thoroughly for proper mixture of ester and reagent. The slight foaming which occurred during the heating process did not interfere with the isomerization. After exactly thirty minutes of total heating time the tubes were removed from the bath and immediately cooled in water. The isomerized soaps and excess reagent were transferred with 99% ethyl alcohol to volumetric flasks and further diluted to optical densities suitable for measurement in the spectrophotometer. Typical results are shown in Table I.

The $E^{1\%}_{1\,cm.}$ value for linoleic acid from which the cholesteryl linoleate was prepared was 867. Comparison of the calculated theoretical $E^{1\%}_{1\,cm.}$ of 374 for cholesteryl linoleate with the average experimental value of 373 indicates a purity of the compound of 99.7%, within the limits of experimental error.

The purity of cholesteryl linoleate in terms of the linoleic acid content determined by this method is more reliable than that based on iodine value. The method should be equally applicable for the determination of the purity of cholesteryl linolenate.

Summary

A modified spectrophotometric procedure for the determination of the linoleic acid in cholesteryl linoleate is reported.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

The Acylation of Ketones with Esters to Form β-Diketones by the Sodium Amide Method[1]

BY ROBERT LEVINE, JOSEPH A. CONROY, JOE T. ADAMS AND CHARLES R. HAUSER

It was shown recently[2] that, in the acylation of ketones with esters by means of sodium amide, it is advantageous to use two equivalents of the base to one of the ketone and two of the ester. This method produced much better yields of certain β-diketones than were obtained with sodium or sodium ethoxide. The scope of the sodium amide method has now been extended considerably. Various acylations have been effected using the proportions of reactants given above, designated Method A, or the proportions of two equivalents each of sodium amide and ketone to one of ester, designated Method B. The reactions were carried out by first converting the ketone to its sodium derivative by means of sodium amide in ether suspension and, after five to ten minutes,[3] adding the ester and refluxing the mixture two hours.[2] The β-diketone was generally isolated as its copper salt from which the β-diketone was regenerated. The new results are summarized in Table I.

From these results and those reported previously,[2] it may be concluded that sodium amide is satisfactory[4] for the acylation of various methyl

(1) Paper XXX on "Condensations"; paper XXIX, THIS JOURNAL, **67**, 284 (1945).

(2) Adams and Hauser, *ibid.*, **66**, 1220 (1944).

(3) Evidently no appreciable amount of self-condensation of the ketone occurs within five or ten minutes. It has been found that with two equivalents of sodium amide to one of acetone less than 5% of the self-condensation product is formed even after one hour at the reflux temperature of ether. However, with equivalents of sodium amide and acetone, these conditions produced mesityl oxide (11%) and other products.

(4) Although Method A, in which the yield is based on the ketone, has been used most frequently, Method B, in which the yield is based on the ester, is to be preferred when the ester is relatively expensive or when the β-diketone fails to form a copper salt. When both the ketone and ester are expensive, it may be advantageous to use two equivalents of sodium amide to one of ketone and one (or slightly more) of ester, since these proportions have produced only an 8% lower yield than that obtained by Method A. The use of equivalents of all three reactants is not advantageous from the standpoint of either the ketone or ester. Although only an equivalent of sodium amide is required to convert the ketone to its sodium derivative, half of the ketone is regenerated during the acylation leaving half of the ester.