A Simple Method of Preparing Alcohol Acetates, Wax Esters, and Cholesterol Esters

THE EXCESS OF LITHIUM ALUMINUM HYDRIDE, used in the reduction of carboxylic esters to the corresponding alcohols, is decomposed either by water or ethyl acetate (1). Our experience in using ethyl acetate in these reactions indicated partial acetylation of the alcohols, probably because of in-situ formation of lithium aluminum ethylate. This assumption seemed to be confirmed when palmityl alcohol could be quantitatively acetylated with ethyl acetate in the presence of a similar basic catalyst, sodium methylate.

In the present communication the method of preparing alcohol acetates, wax esters, and cholesterol esters in high yields is described.

Five grams of oleyl alcohol were dissolved in 50 ml of ethyl acetate and mixed with 100 mg of sodium methylate. The mixture was agitated vigorously with a magnetic stirrer in a 250-ml flask. The flask was flushed with nitrogen, immersed in a water bath maintained at 70C, and evacuated with a water aspirator at a rate such that the excess of ethyl acetate and ethyl alcohol resulting from the reaction were completely removed in 15 min. The flask was cooled, and the residue was extracted with diethyl ether; the extract was washed free of alkali with water and dried over anhydrous sodium sulfate. The oleyl acetate so obtained was purified by thin-layer chromatography (TLC) (system: silica gel G/toluene) before infrared analysis and a melting-point determination (mp, –17C to –16C).

The reaction between 1.2 g of methyl linolenate and 1.0 g of palmityl alcohol in the presence of 100 mg of sodium methylate (in the absence of any solvent) was carried out as described in the case of oleyl acetate except that the reaction was continued for 1 hr. Palmityl linolenate was purified by TLC before infrared analysis, and the melting point was determined after crystallization from ethanol (mp, 11.5C to 12.5C).

The reaction between 100 mg of cholesterol, purified by the dibromide method, and 70 mg of methyl palmitoleate in the presence of 20 mg of sodium methylate in 15 ml of benzene was conducted in the same manner as de-

TABLE I
Infrared Characterization

Compounds Investigated	Source of Compound	Phase for Spectra	Typical Stretching Vibrations of R—C(=O)—O—R′ C=O	R—C—O	—O—R′
Acetates					
Palmityl acetate	Acetic anhydride-pyridine method	Liquid film	1745 (S) [a]	1242 (S)	1042 (S)
Heptadecanyl acetate	Obtained during reduction of methyl heptadecanoate with $LiAlH_4$ (purified by TLC[b] for spectra)	Liquid film	1745 (S)	1240 (S)	1040 (S)
Oleyl acetate	Present technique	Liquid film	1742 (S)	1240 (S)	1040 (S)
Palmityl acetate	Present technique	Liquid film	1745 (S)	1242 (S)	1040 (S)
Wax Esters					
Palmityl stearate	Acid chloride method	Solid film	1735 (S)	[c]	[c]
Palmityl linolenate	Present technique	Liquid film	1740 (S)	1245 (WM)	1175 (MS)
Palmityl palmitate	Present technique	Solid film	1732 (S)	[c]	[c]
Cholesteryl Esters					
Cholesteryl esters	Blood plasma	Liquid film	1735 (S)	1250 (WM)	1175 (MS)
Cholesteryl palmitoleate	Present technique	Liquid film	1737 (S)	1250 (WM)	1175 (MS)

[a] It is difficult to specify these peaks in solid spectrum. [b] Silica gel G/toluene.
[c] S — strong; WM — weak medium; MS — medium strong.

The spectra were determined with a Perkin-Elmer Model 21 double beam spectrophotometer, equipped with sodium chloride optics.

scribed for palmityl linolenate. Cholesteryl palmitoleate was purified by TLC before infrared analysis. The melting point was determined after recrystallization from acetone (mp, 49.5C to 50.5C).

By the methods described above, the following compounds were also prepared: palmityl acetate, heptadecanyl acetate, palmityl palmitate, and cholesteryl palmitate. The acetates were recovered quantitatively (>98%); the yields of wax esters and cholesteryl esters were 85-90%.

The important infrared absorption peaks characterizing the compounds are given in Table I. The comparison of the mass spectra of palmityl acetate with that of n-docosyl acetate confirmed its identity (2). Both the compounds had the following peaks: M,M-60, M-88, m/e=116, m/e=61, and m/e=43 (base peak attributable to acetyl ion). Both the spectra also showed agreement in other minor peaks.

Alcoholysis for synthesizing alcohol acetates, wax esters, and cholesteryl esters had the following advantages over other procedures: there was a saving of one step in the synthesis of wax and cholesteryl esters by starting with alcohols rather than acetates (3,4); less noxious reagents were used in the preparation of alcohol acetates.

Although a well-known reaction, alcoholysis up to the present time has been used almost entirely with short-chain alcohols, and the present application to high-molecular-weight alcohols, such as wax alcohols and cholesterol, is of considerable value.

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Extraction of Chromatographically Isolated Fatty Acids from Liquid Scintillation Fluid

THE EXAMINATION of a specific biosynthesized fatty acid is often impeded when a biological system synthesizes a variety of fatty acids and when this quantity is small. It became necessary to develop methods for the isolation and subsequent examination of a particular fatty acid when the amount available was minute. A method was devised by which an individual fatty acid, isotopically labeled, could be recovered from the scintillation fluid after it had been separated as a methyl ester by gas-liquid chromatography and trapped for radio-assay.

The 1-^{14}C-palmitic acid, 16-^{14}C-palmitic acid, and 1-^{14}C-linoleic acid (Nuclear Chicago Corporation) were used to establish the procedure. These were diluted with nonradioactive palmitic or linoleic acid and methylated with diazomethane; separate aliquots ranging from 7 to 70 μg of each acid were passed through a gas-liquid chromatograph. A series 5000 Barber-Colman apparatus was used, employing a glass U tube, 6 ft×6 mm ID, with 15% HIEFF-2BP (ethylene glycol succinate) on Chromosorb W (WA) 80/100 mesh (Applied Science Company) at 185C and nitrogen gas as carrier (flow rate 200 ml/min). A splitter divided the gas effluent so that a portion passed through the flame ionization detector; the fatty acid peak was detected and recorded. Simultaneously the majority of the effluent passed through a Pasteur pipet containing glass wool, moistened with scintillation fluid (5 g 2,5-diphenyloxazole and 0.3g *p*-bis[2-(5-phenyloxazolyl)]-benzene per liter of toluene), which served to trap the fatty acid (Meinertz and Dole, J. Lipid Res. *3*, 140, 1962). Only the peak corresponding to the methyl ester of the radioactive palmitate or linoleate was collected. The contents of the pipet were flushed into a scintillation vial with 12 ml of scintillation fluid and were then radio-assayed on a Packard Tri-Carb scintillation counter. Approximately 70% of the load applied to the column