• *Letter to the Editor*

A Note on the Preparation of Cholesteryl Esters of Long-Chain Fatty Acids

THE PHYSIOLOGICAL ROLE of cholesteryl esters of long-chain fatty acids has received considerable attention since Alfin-Slater and co-workers (1) suggested that they may be involved in essential fatty acid transport and metabolism. In our studies of the metabolism of cholesterol and its esters in relation to atherosclerosis, it became necessary to prepare the esters in moderately large quantities.

Various workers have prepared the cholesteryl esters of saturated fatty acids by reacting cholesterol with fatty acids, the acid anhydrides, or acid chlorides (2, 3). The preparation of the cholesteryl esters of unsaturated fatty acids has been mainly confined to the C_{18} series, *viz.*, oleic, linoleic, and linolenic. The esterification is effected usually by reacting cholesterol with the appropriate acid chloride (2,3,4). The reaction involving cholesterol and free fatty acids is not always feasible; it requires relatively high temperature, causing the production of some undesirable side-products that are difficult to remove. The methods using acid anhydrides or acid chlorides also suffer from many disadvantages, *viz.*, they are not readily available or are laborious and expensive to prepare.

It is the purpose of this note to report, for the first time, a simple and convenient procedure for the synthesis of cholesteryl palmitate and stearate by the well-known interesterification reaction. By reacting cholesteryl acetate and the methyl esters of the appropriate fatty acids in the presence of a suitable catalyst, such as sodium ethylate, chromatographically pure cholesteryl esters have been prepared in good yield.

A typical reaction was conducted in a 200-ml. round-bottom flask as follows: 0.05–0.1 g. of sodium ethylate was added to a mixture of 4.3 g. (0.01 mole) of cholesteryl acetate, obtained by acetylation of cholesterol purified by bromination-debromination procedure, and 3.0 g. (0.01 mole) of methyl palmitate (free from contaminants, as determined by gas-liquid chromatography). The flask was heated under vacuum (20–30 mm.) at 80–90°C. for 1 hr. under a slow stream of pure nitrogen. The reaction mixture was cooled and washed with petroleum ether (30–60°) and filtered to remove insoluble material. The filtrate was evaporated to dryness under vacuum, and the cholesteryl palmitate was crystallized twice from acetone. Yield 4.6 g., m.p. 77–78°C., $[\alpha]_D - 24.8°$ in chloroform (c 5.0).

Cholesteryl stearate (m.p. 81.5–82.5°C., $[\alpha]_D -$ 23.7° in chloroform [c 5.0]) also was obtained in good yield.

Paper chromatography of these esters by the method of Labarrere *et al.* (5) yielded single spots and showed that they were free from cholesteryl acetate or cholesterol.

The preparation and properties of the cholesteryl esters of the C_{18} unsaturated acids as well as arachidonic and other longer-chain fatty acids and their separation by thin-layer chromatography are in progress. Details will be published later.

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ABSTRACTS . . . . R. A. REINERS, Editor

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• Fats and Oils

COLOR REVERSION OF REFINED AND DEODORIZED SOYBEAN OIL. I. EFFECTS OF TEMPERATURE, AIR, AND LIGHT ON THE COLOR REVERSION OF REFINED SOYBEAN OIL, AND A METHOD OF MEASURING COLOR REVERSION. Ichiro Harada, Yutaka Saratani, and Masao Ishikawa (Hohnen Oil Co., Ltd., Shimizu, Shizuoka-ken). *Nippon Nôgei-kagaku Kaishi* **34**, 545–51 (1960). The darkening of refined vegetable oils (especially soybean oil) during keeping is called "color reversion." When deodorized soybean oil was kept at 20, 50, 75, or 100°, color reversion occurred earlier at higher temperatures, and then promptly the color faded; however, the maximum color (expressed in red value by the Lovibond colorimeter) was similar at 50–100°. The change of velocity of color reversion according to the temperature was very similar to the change of initial velocity of autoxidation of oil as expressed by peroxide value or oxygen uptake. The color of decolorized (but not deodorized) oil was stable at 50°, and faded at 75 and 100° (more rapidly at 100°). When the decolorized oil was heated at 180–270°, the color faded more rapidly at higher temperatures under reduced pressure, but it faded only slightly in the air. Experiments about the influence of the air showed that the velocity of color reversion of deodorized oil depended on partial pressure of oxygen. Solar irradiation of decolorized and deodorized soybean oils produced irreversible color fading. The color reversion in the dark was specific to soybean oil.

II. GENERAL ASPECTS OF COLOR REVERSION AND THE RELATION BETWEEN COLOR REVERSION AND TOCOPHEROL CONTENTS. *Ibid.* 551–8 (1960). Comparative experiments with some oils showed that cottonseed oil reverted in color, but sunflower oil, rapeseed oil, and safflower oil (very similar to soybean oil in fatty acid composition) were very stable with respect to color. Thus the substrate for color reversion was not in fatty acids but probably in unsaponifiable matter. Soybean oil was examined as to the refining stages: deacidified oil showed stable color, decolorized oil showed a little reversion, but only deodorized oil showed appreciable color reversion. Thus heat treatment during deodorization had some relation with color reversion. The substrate for color reversion was resistant to alkali, ad-