7.5 g of a nonsaponifiable fraction which contained 2.3 g fucosterol (0.11% of kelp, estimated by comparison to a standard solution of sitosterol by gas liquid chromatography (GLC): 5% OV-101, 260 C).

Two kg of dry rotary filter mud, the residue left after extraction of alginic acid from the kelp with sodium carbonate solution, was extracted with chloroform-methanol as above to give 32 g of material that was hydrolyzed (500 ml 10% KOH in 95% ethanol, steam bath 4 hr) to yield 8.5 g of a nonsaponifiable fraction containing 3 g fucosterol (0.15% of dry mud).

Four liters kelp slime, the aqueous residue left after alginic acid is precipitated from the carbonate extract with acid, was acidified with 175 ml 37% HCl, heated on the steam bath 3 days to hydrolyze possible steryl glycosides, cooled, and ether extracted. Evaporation of the ether left 2.6 g of a dark tar that contained no fucosterol.

The best source of fucosterol is, therefore, the dry rotary filter mud. It not only contains more sterol than the whole kelp (0.15 vs. 0.11%), but it also contains less chlorophyll and other lipids, making workup easier. The nonsaponifiable fractions from the kelp and mud were combined (16 g), dissolved in benzene (60 ml), and chromatographed on a 200 g column of neutral alumina with the same solvent. Fractions rich in sterol [0.6 to 2.5 liters of eluate, thin layer chromatography (TLC) 60:40 cyclohexane-ethyl acetate] were pooled, concentrated to dryness, and the residue rechromatographed on 200 g of fresh alumina to yield 4.1 g crude fucosterol after crystallization from acetone, m.p. 124-5 C, contaminated with about 5% 24-methylene-cholesterol and a trace of cholesterol (GLC).

The crude material (3.5 g) was acetylated with 10 ml acetic anhydride on the steam bath overnight, dissolved in benzene (20 ml), and chromatographed on a 20% silver nitrate-silica gel column (500 g) with 10:1 hexane-benzene. Fractions were monitored by TLC (10% $AgNO_3$-silica gel plates, 50:50:1 chloroform-carbon tetrachloride-acetic acid); those containing only fucosteryl acetate were combined and the product crystallized from methanol-benzene to yield pure (GLC, TLC) fucosteryl acetate (1.2 g), m.p. 119-20 C (in vacuo, corrected), $[\alpha]_D$ -44.7 C (c5,$CHCl_3$); lit[3] m.p. 120-2 C, $[\alpha]_D$ -42.1 C. The mother liquors from the crystallization of the acetate were evaporated to dryness and the residue hydrolyzed with 10% KOH in 95% ethanol (20 ml) on the steam bath 2 hr. Addition of water to the solution formed a precipitate which was recrystallized from methanol-benzene to yield 0.8 g fucosterol m.p. 123.5-4 C (in vacuo, corrected), $[\alpha]_D$ -42.0 C (c5,$CHCl_3$); lit[3] m.p. 123-4 C, $[\alpha]_D$ -38.5 C.

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ACKNOWLEDGMENTS

We wish to express our appreciation to the I.W. Cottrell, Kelco Co., for supplying the kelp samples and the Nippon University, Tokyo, for a travel grant (T.S.). This work was supported in part by NSF Grant DEB 74-19148 A03.

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[Received September 27, 1976]

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A Simple Method for the Preparation of Cholesteryl Esters

ABSTRACT

A simple and convenient procedure for the synthesis of cholesterol esters of long chain saturated and unsaturated fatty acids is presented. Condensation is achieved with thionyl chloride as a catalyst.

INTRODUCTION

The method of using thionyl chloride as a catalyst in the esterification of amino acids (1) has been applied to the synthesis of long chain fatty acid esters of cholesterol. The synthesis was carried out by reacting cholesterol and fatty acid in benzene in the presence of thionyl

TABLE I

Melting Points and Percentage Yields of Cholesteryl Esters

Product	Melting points (C)		Percentage yields	
	Observed	Reported (5)	At room temperature	At 70 C
Cholesterol oleate	42	42.5	91[a]	96.3[a]
Cholesterol laurate	91	92	90	–
Cholesterol stearate	82	82	90	95.6

[a]The percentage yield represents cholesteryl esters of oleic and linoleic acids.

chloride. Although this reaction proceeded smoothly overnight at room temperature and resulted in high yields (90%), the reaction rate could be increased at higher temperature (70 C), which would result in an overall increase in percentage conversion (96% in 1½ hr). The present method serves as an efficient alternative to other reported methods (2,3).

MATERIALS AND METHODS

A mixture of oleic and linoleic acids and lauric acid, isolated from vegetable fats, were used. Stearic acid and cholesterol of high purity were purchased commercially. Silicic acid (column chromatography grade) was also purchased commercially.

Cholesterol (1.3 mmol, i.e. 500 mg) and a mixture of oleic and linoleic acids (1.6 mmol, i.e. 450 mg) were dissolved in dry benzene and cooled to 0 C. Thionyl chloride (0.25 ml) was added dropwise, and after the flask was flushed with dry carbon dioxide, the reaction mixture was kept overnight at room temperature (25-27 C). Alternatively, the reaction mixture was refluxed at around 72 ± 2 C for 1½ hr. The mixture was transferred to a separating funnel and washed twice with 50 ml of distilled water. The benzene layer was dried over anhydrous sodium sulfate.

The percent conversion of cholesterol to cholesterol ester was determined by thin layer chromatography (TLC). The analytical separation of unreacted cholesterol and free fatty acid from cholesterol ester was achieved in a TLC system Silica Gel G/petroleum ether:diethyl ether:acetic acid 90:10:1 v/v. The separated cholesterol and cholesteryl esters were estimated by the method of Zlatkis et al. (4).

After a mixture of unsaturated cholesteryl esters was isolated in the above system, it was subfractionated by preparative argentation TLC in the system 5% $AgNO_3$ in Silica Gel G/petroleum ether (40-60 C): diethyl ether 90:10 v/v. The separated esters were extracted with 25 ml of chloroform:methanol 1:2, for further characterization.

Cholesterol laurate and stearate were prepared and purified individually by the same procedure.

For the isolation of cholesteryl esters on a macroscale, a silicic acid column 30 cm x 2 cm was used. Silicic acid (80-120 mesh), 50 g was activated overnight at 110 C. Cholesteryl ester was eluted from the column with 1% benzene in petroleum ether (40-60 C). In 75 ml of eluent, the cholesterol ester of a saturated fatty acid was completely removed from the column, recovery (85 ± 2%) being determined either by weighing or by colorimetry. Most of the ester was eluted in the first 25 ml of eluent.

RESULTS AND DISCUSSION

The infrared spectrum of individual cholesteryl esters showed typical strong ester absorption at 1735 cm^{-1}, as reported previously (5). The melting points of cholesteryl oleate, laurate, and stearate resembled closely the values reported in the literature (Table I).

The present method gives yields similar to those reported by Phillips and Viswanathan (2) and Lentz et al. (3) and thus serves as a very useful alternative method in the preparation of cholesteryl esters. When compared to the method of Lentz et al., the present method is a one-step synthesis and hence, should be a preferred choice. The utilization of this method in the synthesis of wax-ester and mono- and diesters of diols and fatty acid derivatives of glycerol will be reported shortly in a separate communication.

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ACKNOWLEDGMENTS

The author is indebted to his teacher, Dr. C.V. Viswanathan, for his valuable suggestions and guidance, and is also thankful to the Maharashtra Associa-

tion for the Cultivation of Science, Poona, for providing laboratory facilities.

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[Received October 4, 1976]