neutral lipids, each representing a particular lipid class, on a TLC plate of Silica Gel G at 250 μ, by this two-step procedure. All eight classes were well resolved. As an example of the application of this method, the separation of neutral lipids extracted from tobacco leaves is shown on the same plate. Comparisons with reference standards indicated that this sample contained hydrocarbon waxes, steryl esters, fatty acids, and sterols, as well as other unidentified neutral components. Elution of bands from a preparative plate and subsequent analysis by gas chromatography have proven these identifications to be true (J.J. Ellington, P.F. Schlotzhauer, and A.I. Schepartz, unpublished results). For informational purposes the chromatographic response of a chlorophyll mixture, xanthophyll, and β-carotene are also shown. It may be noted that chlorophyll, β-carotene, and the more polar lipids remained at the origin.

Preparative plates with Silica Gel H gave similar results, except that the triglycerides and methyl esters generally ran together. Separations were otherwise good and recoveries were quantitative. Gravimetric recoveries of a separation of the standard mixture of eight neutral lipids (47 mg total in 2 ml hexane) on a preparative plate were between 97 and 103%.

Initially, we investigated the use of commercially prepared silica gel plates from a variety of sources and encountered problems with impurities and reproducibility. Hence, we elected to prepare our own plates. The use of 2000 μ layers for preparative work proved most convenient, enabling the application of samples as large as 80 mg. This may not be the upper limit, since, even at this level, there was no overloading.

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[Received August 23, 1976]

Preparation of Fucosterol from Giant Kelp

ABSTRACT

Commercial extraction of alginic acid from *Macrocystis pyrifera* leaves a residue that is a good source of fucosterol, 5,24(28)E-stigmastadien-3β-ol. The isolation and purification of this sterol is described.

INTRODUCTION

Fucosterol is the predominant sterol in brown algae (1). The giant kelp, *Macrocystis pyrifera*, is a member of this class that grows off the coast of southern California and is used for the preparation of alginic acid in the United States. Attempts are now being made to rear the plant in large marine farms (2). Dried kelp and two by-products of the commercial process, rotary filter mud and kelp slime, were obtained from the Kelco Co., San Diego, CA, for evaluation as easily accessible sources of fucosterol.

EXPERIMENTAL SECTION

Coarsely powdered dry kelp (2 kg) was extracted at room temperature three times with 4 liters 2:1 chloroform:methanol. Evaporation of the combined extracts left 82 g of a dark green residue that was hydrolyzed on the steam bath with 1.2 liters 10% KOH in 95% ethanol for 4 hr. After cooling, the solution was added to 2 liters water and ether extracted to remove

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