-28.62, and $[\alpha]^{27}D$ -28.06, respectively, were brominated by the Schöenheimer[6] method. To the reaction mixture was added a one per cent. solution of digitonin. A small amount of digitonide precipitated, which upon decomposition with pyridine[11] yielded a sterol of somewhat lower rotation, $[\alpha]^{24}D$ -21.30. This value seemed to indicate that dihydrositosterol, if present, would be found only in very small amount.

The dibromide still in solution was precipitated with water, debrominated, and then acetylated. The acetyl derivative was recrystallized ten times in the hope of concentrating the dihydrositosterol, if present, in the top fraction. It was then brominated,[9] but since no precipitate of insoluble stigmasterol tetrabromide was formed, the presence of this sterol was eliminated. The dibromide was precipitated with water and debrominated according to the method of Anderson and Nabenhauer.[7] The regenerated acetyl derivative was dissolved in carbon tetrachloride and treated twenty times with portions of acetic anhydride and sulfuric acid. There was only a slight color reaction after these treatments. The carbon tetrachloride was evaporated but only a small amount of brown resinous mass remained from which no crystals of dihydrositosterol could be obtained.

A separate sample of 33 g. of crude sterol (m. p. 138–139°; $[\alpha]^{25}D$ -31.53) was recrystallized twenty-three times, after which it had a melting point of 137°; $[\alpha]^{24}D$ -8.41. This sample was now dissolved in alcohol, brominated by the Schöenheimer method[6] and an excess of digitonin solution added. However, no insoluble digitonide was formed.

The fact that the specific rotation could be lowered to -8.41 and that the rotation of the two top fractions was lowered by treatment with digitonin would indicate the possibility that small amounts of dihydrositosterol were present. However, these amounts were too small for isolation.

(11) R. Schöenheimer and H. Dam, *Z. physiol. Chem.*, **215**, 59 (1933).

Summary

A new doubly unsaturated sterol which is an isomer of stigmasterol has been isolated in a pure state from the most soluble sterol fraction of rye germ oil.

The presence of α_1-, β-, and γ-sitosterol has been demonstrated.

The absence of α_2-sitosterol and stigmasterol has been shown.

An analysis for dihydrositosterol failed to yield the pure compound by either the Schöenheimer[6] or Anderson–Nabenhauer[7] method.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Studies in the Sitosterol Complex. The Isolation of α_3-Sitosterol

BY SEYMOUR BERNSTEIN[1] AND EVERETT S. WALLIS

That sitosterol is in reality a mixture of several sterols was first shown by Anderson and his co-workers.[2] These investigators showed that sitosterol obtained from various sources contains dihydrositosterol and at least three other sterols which they named α-, β- and γ-sitosterol.

In 1936 Wallis and Fernholz[3] showed that the most soluble fraction of the sitosterol complex from wheat germ oil named by Anderson and his co-workers α-sitosterol was in reality a mixture of at least two new sterols α_1- and α_2-sitosterol. A detailed method of their separation and isolation was given, and certain properties of these sterols and their derivatives were listed.

In this paper we wish to report the results of certain experiments which show that a third sterol, α_3-sitosterol, is also present in the sterol complex from wheat germ oil. Our work in this connection was prompted by the fact that for continuance of our studies on the structure of α_1-sitosterol, the results of which will be reported shortly in another paper, it was necessary to obtain further quantities of this interesting material. Following the procedure described by Wallis and Fernholz,[3] we obtained from recrystallization of 1627 g. of a sitosterol from wheat germ oil, 6.5 g. of α_1-sitosteryl *m*-dinitrobenzoate and 6.0 g. of the more soluble fraction which in melting point corresponded to α_2-sitosteryl *m*-dinitrobenzoate. Hydrolysis of this latter fraction and conversion into its acetate gave a product from which not only α_2-sitosteryl acetate (m. p. 126°, $[\alpha]D$ $+17$) was isolated but also there was obtained in a pure condition 1.0 g. of a new acetate, α_3-sitosteryl acetate (m. p. 152–153° $[\alpha]^{20}D$ $+6.1$).

Several derivatives of this new sterol were prepared and their properties together with those of α_1- and α_2-sitosterol are given in Table I.

(1) Research Assistant on Special Funds from the Rockefeller Foundation.

(2) Anderson, Shriner and Burr, THIS JOURNAL, **48**, 2987 (1926). See also Anderson, *ibid.*, **46**, 1450 (1924).

(3) Wallis and Fernholz, *ibid.*, **58**, 2446 (1936).

TABLE I

Derivative	α_1-Sitosterol M. p., °C.	α_1-Sitosterol $[\alpha]_D$	α_2-Sitosterol M. p., °C.	α_2-Sitosterol $[\alpha]_D$	α_3-Sitosterol M. p., °C.	α_3-Sitosterol $[\alpha]_D$
Sterol	164–166	− 1.7	156	+ 3.5	142–143	+ 5.2
Acetate	137	+29	124–126	+17	152–153	+ 6.1
Benzoate	168–172	+42	164–166	+27	173–175	+12.0
m-Dinitrobenzoate	222	+37	206	+26	210–211.5	+12.2

We also wish to report certain other important observations. α_3-Sitosterol in all probability is an isomer of stigmasterol, $C_{29}H_{48}O$, and α_1-sitosterol, $C_{29}H_{48}O$. Titration with perbenzoic acid shows that two double bonds are present. That the two double bonds are not conjugated is indicated by an absorption spectrum study. No maxima were found in the ultraviolet region of the spectrum. α_3-Sitosterol gives a positive Liebermann color reaction. The final color is a dark blue with a reddish tint. The Salkowski reaction for α_3-sitosterol is similar to that of ergosterol, α_1- and α_2-sitosterol; the sulfuric acid layer becomes colored, while the chloroform stays colorless. The opposite is true for cholesterol, γ-sitosterol, and stigmasterol. α_3-Sitosterol is precipitated by digitonin.

Experimental Part

Sixteen hundred and twenty-seven grams of a crude sitosterol (source, wheat germ oil) was worked up according to the method of Wallis and Fernholz,[3] and yielded 6.5 g. of α_1-sitosteryl *m*-dinitrobenzoate and 6.0 g. of a material which in melting point (206°) corresponded with that of α_2-sitosteryl *m*-dinitrobenzoate previously described by Wallis and Fernholz.[3]

Isolation of α_3-Sitosteryl Acetate.—The above described material (m. p. 206°) was hydrolyzed by refluxing it with a 5% alcoholic potash solution. The free sterol, so obtained, was then converted into the acetate by refluxing with acetic anhydride. Crystallization of the crude acetate from ethyl alcohol, gave two distinct fractions. The more insoluble fraction, α_3-sitosteryl acetate (plates, wt. 1.0 g.), melted at 152–153°, $[\alpha]^{20}D$ +6.1 (0.0264 g. in 2 cc. of chloroform solution gave $\alpha^{20}D$ +0.08). The more soluble portion was found to be the α_2-sitosteryl acetate (m. p. 124–126°, $[\alpha]^{25}D$ +17) already described by Wallis and Fernholz. The melting point of the sterol acetate (152–153°) is depressed by α_2-sitosteryl acetate.

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.8; H, 11.3.

α_3-Sitosterol.—A portion of the above described α_3-sitosteryl acetate was hydrolyzed with a 5% alcoholic potash solution by heating on the water-bath for one hour. The free sterol was worked up in ether. Crystallization from alcohol gave platelets which melted at 142–143°, $[\alpha]^{20}D$ +5.2° (0.0482 g. in 5 cc. of chloroform solution). α_3-Sitosterol is precipitated by digitonin.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.73. Found: C, 84.44; H, 11.83.

An absorption spectrum study of α_2-sitosterol was carried out by Dr. T. J. Webb of the Research Laboratories of Merck and Company, Inc. No maxima were found in the ultraviolet region of the spectrum.

α_3-Sitosteryl Benzoate.—One-tenth of a gram of α_3-sitosterol dissolved in pyridine was heated for one hour on the water-bath with benzoyl chloride. The benzoate was worked up in ether. Crystallization from benzene and alcohol gave plates of m. p. 173–175°, $[\alpha]^{20}D$ +12.0° (0.0266 g. in 2 cc. of chloroform).

Anal. Calcd. for $C_{36}H_{52}O_2$: C, 83.66; H, 10.14. Found: C, 83.4, 83.5; H, 10.18, 10.00.

α_3-Sitosteryl *m*-Dinitrobenzoate.—A sample of α_3-sitosterol, dissolved in pyridine, was heated for one hour on the water-bath with *m*-dinitrobenzoyl chloride. The product was extracted with ether and the ether solution was decolorized with animal charcoal and worked up in the usual manner. Recrystallization from ethyl acetate and alcohol gave needles which were white with a very slight yellow luster; m. p. 210–211.5°; $[\alpha]^{20}D$ +12.2° (0.0312 g. in 2 cc. of chloroform solution).

Anal. Calcd. for $C_{36}H_{50}N_2O_6$: C, 71.25; H, 8.31; N, 4.62. Found: C, 71.40, 71.13; H, 8.36, 8.37; N, 4.80.

Titration with Perbenzoic Acid.—Thirty-three and three-tenths milligrams of α_3-sitosteryl acetate took up 2.12 mg. of oxygen on standing for three days at −2°, with an excess of a chloroform solution of perbenzoic acid. The theoretical amount for two atoms of oxygen is 2.34 mg.

We wish to take this opportunity to express our thanks to the Rockefeller Foundation for a grant-in-aid for this work, to Merck and Company, Inc., of Rahway, New Jersey, for the analyses and absorption data published in this paper, and to Dr. O. H. Emerson of the University of California for kindly furnishing part of the crude sterol from wheat germ oil used in this work.

Summary

1. A new doubly unsaturated sterol, α_3-sitosterol, has been isolated from the most soluble fraction of the sitosterol complex obtained from wheat germ oil.

2. The acetate, benzoate and *m*-dinitrobenzoate of the new sterol have been prepared and characterized.

3. An absorption spectrum study indicates that the two double bonds in the new sterol are not conjugated.

4. The new sterol is precipitated by digitonin.

5. α_3-Sitosterol is probably an isomer of stigmasterol, and α_1-sitosterol.

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