Decomposition of the ozonide was accomplished in accordance with the method of Whitmore and his coworkers.[7]

The resulting aldehyde was divided into two parts, one of which was converted to the semicarbazone and the other to the 2,4-dinitrophenylhydrazone. The results follow.

(a) The semicarbazone prepared from the aldehyde produced by ozonolysis of 2-pentadecenoic acid melted at 103.5–104.5°. An authentic specimen of tridecanal semicarbazone (m. p. 105.5–106.5°), mixed with the semicarbazone produced by ozonolysis, showed a m. p. of 104.5–105.5°.

(b) The 2,4-dinitrophenylhydrazone derived from the aldehyde produced by the ozonolysis of 2-pentadecenoic acid melted at 107–108°, and showed no melting point depression when mixed with an authentic specimen of the 2,4-dinitrophenylhydrazone of tridecanal.

Anal. Calcd. for $C_{19}H_{30}O_4N_4$: C, 60.28; H, 8.00. Found: C, 60.05; H, 8.07.

(c) Likewise the semicarbazone derived from 2-heptadecenoic acid by ozonolysis was identical with an authentic specimen of the semicarbazone of pentadecanal.

(d) The 2,4-dinitrophenylhydrazone (m. p. 107.5–108°) obtained from the aldehyde produced by the ozonolysis of 2-heptadecenoic acid was analyzed.

Anal. Calcd. for $C_{21}H_{34}O_4N_4$: C, 62.04; H, 8.43. Found: C, 61.81; H, 8.54.

A mixed melting point with an authentic specimen showed no depression.

Summary

A series of reactions has been outlined by which it is possible to convert the higher saturated fatty acids into α,β-unsaturated acids with one more carbon atom. 2-Pentadecenoic and 2-heptadecenoic acids have been prepared in this way starting with myristic and palmitic acids, respectively.

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[7] Whitmore, *et al.*, THIS JOURNAL, **54**, 3710 (1932); **56**, 176 (1934).

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

Isolation of a New Phytosterol: Campesterol

BY ERHARD FERNHOLZ AND H. B. MACPHILLAMY[1]

In a previous communication from this Laboratory[2] the possibility that 22,23-dihydrobrassicasterol is present in natural phytosterol mixtures was considered. With this in mind an attempt was made to isolate this sterol from several natural oils. While we have not been able to find any 22,23-dihydrobrassicasterol, we have obtained an isomeric sterol whose properties indicate that it has not before been isolated. Since the isolation was first accomplished from rapeseed oil (*Brassica campestris* L.) we have called it campesterol. Further experiments have shown that it can also be isolated from soya bean oil and wheat germ oil, but it was not found in either cottonseed oil or tall oil.

The sterol was obtained from the acetate residues after the removal of any sterols which gave insoluble acetate tetrabromides. After debromination and hydrolysis of the soluble portion, the free sterol mixture was subjected to repeated recrystallizations from acetone, some fifteen to eighteen being necessary to obtain a substance with a constant melting point. Recrystallizations from ethanol were quite unsatisfactory. Several other methods of purification such as chromatographic adsorption, bromine–digitonin precipitation[3] and attempts at selective hydrolysis of the *p*-toluenesulfonic esters[4] effected no appreciable separation. The pure campesterol as finally obtained had a melting point of 157–158° and $[\alpha]^{23}\mathrm{D}$ −33°.

Titration with perbenzoic acid showed the presence of only one double bond and this fact together with the analytical data of the *m*-dinitrobenzoate indicate an empirical formula of $C_{28}H_{48}O$. It is therefore an isomer of 22,23-dihydrobrassicasterol but a comparison of their constants in Table I shows the difference.

TABLE I

Compound	M. p., °C.	$[\alpha]^{23}\mathrm{D}$
Campesterol	157–158	−33°
Acetate	137–138	−37
Benzoate	158–159	− 8.6
m-Dinitrobenzoate	202–203	− 6.0
22,23-Dihydrobrassicasterol[1]	158	−46
Acetate	145	−46
Benzoate	162	−19
m-Dinitrobenzoate	197.5	−17

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[1] The preparation of this study for publication was carried out by H. B. M. after the untimely death of Dr. Fernholz.

[2] Fernholz and Ruigh, THIS JOURNAL, **62**, 3346 (1940).

[3] Schöenheimer, *Z. physiol. Chem.*, **192**, 80 (1930).

[4] Stoll, *ibid.*, **207**, 147 (1932); **246**, 1 (1937).

The sterols as obtained from each of the three sources showed no difference as indicated by no depressions in a complete series of mixed melting point determinations on the free sterol and all derivatives. The constants found in all cases, as shown in the experimental part, agree quite closely considering the difficulty in purification.

Experimental

Isolation from Rapeseed Oil.—Thirteen gallons of rapeseed oil was saponified and the saponification mixture diluted with water and ether extracted. The extract was dried, concentrated and the crude sterol recrystallized twice from an ethanol–benzene mixture yielding 113.5 g. of material. This was acetylated with acetic anhydride and after two crystallizations from ethanol–benzene 112 g. of acetate was obtained, m. p. 130–134°.

A mixture of 65 g. of the above acetate, 570 cc. of ether, 800 cc. of acetic acid and 14 cc. of bromine was allowed to stand overnight. The insoluble tetrabromide was filtered and stirred several hours with ether. After filtration the combined filtrates were debrominated and on concentration and recrystallization yielded 45 g. of sterol acetate, which was used for the isolation of campesterol. The insoluble brassicasteryl acetate tetrabromide (3.25 g.) on debromination yielded 1.57 g. of acetate, m. p. 153–155°, $[\alpha]^{23}_{\mathrm{D}}$ $-69.3°$.

The brassicasterol free acetates were saponified with alcoholic alkali and the free sterols thus obtained recrystallized from ethanol–benzene yielding 27 g., m. p. 133–138°. The campesterol was isolated from this mixture by repeated crystallization. It was found advantageous to use hexane for the first two crystallizations and then continue with acetone until a product with constant melting point was reached. In this way, and by similarly working up the mother liquors, 1.6 g. of material was obtained, m. p. 157–158°, $[\alpha]^{23}_{\mathrm{D}}$ $-33°$ [22.5 mg. in 2 cc. chloroform, 1-dm. tube, α_{D} $-0.38°$]. The yield is approximately 3% of the original sterol mixture.

Anal. Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.08. Found: C, 84.34, 84.29; H, 12.21, 12.30.

Titration with Perbenzoic Acid.—A mixture of 25.9 mg. of campesterol in 2 cc. of chloroform and 2 cc. of a perbenzoic acid solution on standing for three days at 0° consumed 1.125 mg. O = 1.09 atoms.

Campesteryl Acetate.—Two hundred milligrams of campesterol was acetylated by boiling for one-half hour with acetic anhydride. On cooling the crystalline acetate was filtered and recrystallized from ethanol, m. p. 137–138°, $[\alpha]^{23}_{\mathrm{D}}$ $-35°$ [28.8 mg. in 1 cc. of chloroform, 1-dm. tube, α_{D} $-1.0°$].

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.05, 81.13; H, 11.30, 11.26.

Campesteryl Benzoate.—A solution of 100 mg. of sterol in 2 cc. of dry pyridine was allowed to stand overnight with 0.2 cc. of benzoyl chloride. The product was worked up and recrystallized from ethanol–benzene, m. p. 158–160°, $[\alpha]^{23}_{\mathrm{D}}$ $-8.6°$ [16.3 mg. in 2 cc. of chloroform, 1-dm. tube, α_{D} $-0.07°$].

Anal. Calcd. for $C_{35}H_{52}O_2$: C, 83.28; H, 10.39. Found: C, 83.30, 83.23; H, 10.29, 10.35.

Campesteryl *m*-Dinitrobenzoate.—A mixture of 200 mg. of sterol, 1 g. of *m*-dinitrobenzoyl chloride and 15 cc. of dry pyridine was heated on the steam-bath for one-half hour. It was then cooled, diluted with several volumes of water, filtered and the brown precipitate washed thoroughly with water and finally once with alcohol. The dry product was heated in benzene solution with Darco and filtered through supercell. After evaporating the excess benzene, hot alcohol was added. At first the substance comes out in cotton-like masses which are converted to needles on standing. After three recrystallizations from ethanol–benzene the substance comes out immediately in long needles, m. p. 202–203°, $[\alpha]^{23}_{\mathrm{D}}$ $-6.0°$ [10.1 mg. in 1.01 cc. chloroform, 1-dm. tube, α_{D} $-0.06°$].

Anal. Calcd. for $C_{35}H_{50}O_6N_2$: C, 70.67; H, 8.47. Calcd. for $C_{36}H_{52}O_6N_2$: C, 71.02; H, 8.61. Found: C, 70.69, 70.63; H, 8.61, 8.77.

Isolation from Soya Bean Oil.—An accumulation of crude sterol acetates after the separation of stigmasteryl acetate (through the tetrabromide) was saponified with alcoholic alkali yielding 4.7 kg. of sterol, m. p. 136–138°. Repeated crystallization first from hexane and then from acetone as described for the rapeseed material yielded 75 g. of sterol, m. p. 157–158°, $[\alpha]^{23}_{\mathrm{D}}$ $-32°$. Acetate, m. p. 137–138°, $[\alpha]^{23}_{\mathrm{D}}$ $-35°$; benzoate, m. p. 158–160°, $[\alpha]^{23}_{\mathrm{D}}$ $-9.1°$; *m*-dinitrobenzoate, m. p. 202–203°, $[\alpha]^{23}_{\mathrm{D}}$ $-7.1°$.

Isolation from Wheat Germ Oil.—Two hundred grams of sterol obtained directly from the saponification of wheat germ oil was recrystallized from ethanol–benzene yielding 129 g., m. p. 134–137°. After repeated recrystallizations from hexane and acetone as described above 3.2 g. of campesterol, m. p. 157–158°, $[\alpha]^{23}_{\mathrm{D}}$ $-34.5°$ were obtained. Acetate, m. p. 139–140°, $[\alpha]^{23}_{\mathrm{D}}$ $-36°$; benzoate, m. p. 158–160°, $[\alpha]^{23}_{\mathrm{D}}$ $-13.0°$; *m*-dinitrobenzoate, m. p. 201–203°, $[\alpha]^{23}_{\mathrm{D}}$ $-8.6°$.

A fractionation of the sterols from cottonseed and tall oil was carried out in a similar manner but no indication of the presence of any campesterol could be observed.

Summary

A new phytosterol, campesterol, has been isolated from rapeseed oil, soya bean oil and wheat germ oil. The analyses indicate that it has an empirical formula of $C_{28}H_{48}O$. It has not been found in cottonseed oil or in tall oil.

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