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

On the Constitution of Campesterol

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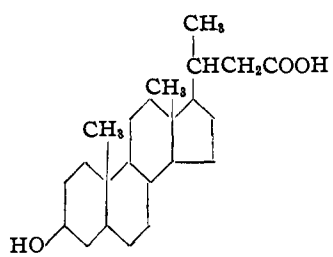
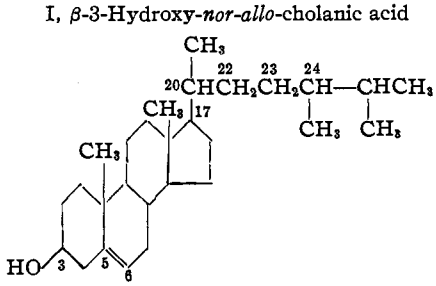
Campesterol has been isolated recently from a number of botanically unrelated plant species. Rapeseed, soybean and wheat-germ oils are readily available sources of this newly characterized sterol. Analyses of campesterol and its derivatives, particularly those of the *m*-dinitrobenzoate, are consistent with the empirical formula $C_{28}H_{48}O$. A perbenzoic acid titration of the sterol showed the presence of a single double bond.[2]

The carbon skeleton of campesterol lacking a portion of the side chain was determined by the isolation of β-3-hydroxy-*nor-allo*-cholanic acid (I)



I, β-3-Hydroxy-*nor-allo*-cholanic acid



II, Campesterol

as a product of the gentle chromic acid oxidation of the saturated campestanyl acetate. The identity of this acid was shown by mixed melting points both of the free acid and the methyl ester acetate with authentic samples of β-3-hydroxy-*nor-allo*-cholanic acid and its derivative. In addition, titration of the free acid gave the correct equivalent weight and the methyl ester acetate gave satisfactory analytical values for carbon and hydrogen. Identification of β-3-hydroxy-*nor-allo*-cholanic acid as an oxidation product also fixed the position and orientation of the hydroxyl group of campesterol on carbon atom 3.

(1) The completion of this study and its preparation for publication was carried out by W. L. R. after the untimely death of Dr. Fernholz.

(2) Fernholz and MacPhillamy, THIS JOURNAL, **63**, 1155 (1941).

The fact that campesteryl *p*-toluenesulfonate formed a characteristic low melting dextrorotatory *i*-methyl ether showed the single double bond in campesterol to be located between C-5 and C-6.[3,4]

The structure of the side chain was determined by an investigation of the volatile ketonic fragments obtained from the energetic chromic acid oxidation of campesteryl acetate. An oily, water insoluble, steam distillable ketone was isolated in the form of a dextrorotatory semicarbazone, m. p. 152–153°, $[\alpha]^{24}D$ + 11.9°, which gave a nitrogen analysis fitting the formula $C_{10}H_{21}ON_3$ corresponding to the formula $C_9H_{18}O$ for the ketone itself. A mixed melting point with a sample of the levorotatory methyl isoheptyl ketone semicarbazone, m. p. 153–154°, $[\alpha]^{24}D$ −14.4°, obtained from the oxidation of α-ergostenol showed no depression.[5] Wallach[6] gives 152–153° for the melting point of *d,l*-methyl isoheptyl ketone known as dihydrothujaketone. The ketone is probably *d*-methyl isoheptyl ketone formed by the scission of the entire side chain of campesterol in the normal manner at C-17.[7,8,9] Additional evidence supporting this assumption was the isolation of acetone in good yield as the *p*-nitrophenylhydrazone from the water-soluble volatile ketonic fraction of the same oxidation. The usual terminal isopropyl group of the sterol side chain is thus indicated. The previous isolation of β-3-hydroxy-*nor-allo*-cholanic acid fixes the position of four of the nine carbon atoms of the side chain. It is also evidence for the presence of a branched chain at C-24 and the absence of branching on C-22 and C-23. With three additional carbon atoms accounted for by the isopropyl group, only two structures, III and IV, are

(3) Wallis, *et al.*, *ibid.*, **59**, 137, 1415 (1937); **60**, 413 (1938); **61**, 3483 (1939).

(4) Fernholz and Ruigh, *ibid.*, **62**, 3346 (1940).

(5) Professor Everett S. Wallis of Princeton University, noting the coincidence that the active forms of the semicarbazone of methyl isoheptyl ketone had the same melting point as that of the *d,l*-mixture, suggested that a study of the phase diagram would be of interest. It was found that the melting points of approximately 25–75 and 50–50 mixtures of the *d* and *l* forms did not significantly differ from either component. Thus we are dealing in this case with a solid solution or mixed crystals of two isomorphous optical antipodes which exist only in a single phase.

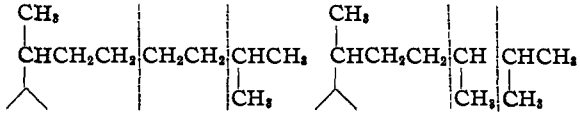
(6) Wallach, *Ann.*, **381**, 51 (1911).

(7) Windaus and Resau, *Ber.*, **46**, 1246 (1913).

(8) Nakayima, *Ann.*, **494**, 119 (1932).

(9) Bengtsson, *Z. physiol. Chem.*, **215**, 179 (1933).

possible for the side chain. Structure III having the two unplaced carbon atoms in a straight chain, is ruled out due to the observed optical activity of the ketone. Structure IV is the only alternative, and the ketone from the oxidation has thus been shown to be *d*-methyl isoheptyl ketone.



III IV

Identification of this ketone confirms the empirical formula $C_{28}H_{48}O$ for campesterol. The structure of campesterol is therefore as shown in formula (II). It is isomeric with 22,23-dihydrobrassicasterol[4] and differs from it only in the optical configuration on C-24.

The melting points and rotations of campesterol and 22,23-dihydrobrassicasterol and their derivatives are compared with the corresponding completely saturated derivatives in Table I.

TABLE I

	Alcohol M. p., °C.	Alcohol $[\alpha]_D$	Acetate M. p., °C.	Acetate $[\alpha]_D$	*m*-Dinitrobenzoate M. p., °C.	*m*-Dinitrobenzoate $[\alpha]_D$
Campesterol[2]	158	−33	138	−37	203	−7
Campestanol	147	31	147	18	198	22
Algebraic diff.		64		55		29
22,23-Dihydrobrassicasterol[4]	158	−46	145	−46	197.5	−17
Brassicastanol[10]	143	16	145	7	202	13
Algebraic diff.		62		53		30

It is interesting to note that, within the experimental error, the algebraic difference of the rotation of campesterol or its derivatives with campestanol or its derivatives is equal to the corresponding difference in the 22,23-dihydrobrassicasterol series. Evidently the hydrogenation of the 5,6 double bond results in an increase of rotation in the positive direction of 63° in the case of the sterol and 54° for the acetate. The corresponding differences for β-sitosterol–β-sitostanol and their acetates are 61 and 55 and for γ-sitosterol–γ-sitostanol and their acetates are 59 and 55.[11] A similar relationship for certain steroid derivatives was first pointed out by Callow and Young.[12]

Although 22,23-dihydrobrassicasterol has not been directly isolated, it is reasonable to postulate its existence just as one must now admit the possible presence in nature of two series of isomeric sterols differing in their configuration on C-24. Evidence to this effect is found in the case of brassicasterol,[10] in which the low rotation of the semicarbazone of methylisopropylacetaldehyde obtained by ozonolysis of brassicasterol was presumably due to an admixture of an isomer differing from brassicasterol only in the configuration on C-24. This as yet unisolated-isomer of brassicasterol would hence be 22,23-dehydrocampesterol.

(10) Fernholz and Stavely, THIS JOURNAL, **62**, 428, 1875 (1940).

(11) Anderson and Shriner, *ibid.*, **48**, 2976 (1926).

(12) Callow and Young, *Proc. Roy. Soc.* (London), **A157**, 194 (1936).

Experimental

Hydrogenation of Campesteryl Acetate to Campestanyl Acetate.—A mixture of 4.22 g. of campesteryl acetate from rapeseed oil in 50 cc. of acetic acid with 0.5 g. platinum oxide was shaken with hydrogen overnight. The product worked up in the usual manner and crystallized from benzene–alcohol, weighed 3.5 g. and gave a strong Liebermann reaction; m. p. 139–141°; $[\alpha]_D$ +14. The crude product was dissolved in 50 cc. of ether and a mixture of 10 cc. of acetic anhydride and 5 cc. of concentrated sulfuric acid added while cooling. After standing overnight, more ether was added and then drop by drop cold water. The ether layer was washed with water and sodium carbonate solution. The ether residue crystallized from benzene and alcohol reached constancy of m. p. and rotation after the second crystallization, m. p. 143–144°, $[\alpha]^{23}_D$ +18.3° (29.5 mg. in 2 cc. of chloroform gave α_D +0.27°; l = 1 dm.).

Anal. Calcd. for $C_{31}H_{54}O$: C, 81.16; H, 11.86. Found: C, 80.89; H, 11.89.

Chromic Acid Oxidation of Campesteryl Acetate. (a) Isolation of β-3-Hydroxy-*nor-allo*-cholanic Acid.—Two grams of campestanyl acetate was dissolved in 100 cc. of acetic acid and the solution warmed to 95° on the steam-bath during the dropwise addition with stirring of a solution of 4 g. chromic anhydride in 20 cc. of water and 80 cc. of acetic acid. After five hours of heating the mixture was diluted with water and extracted with ether. The ether was washed ten times with water and then shaken with 2 *N* sodium hydroxide. The suspension of the sodium salt was heated on the steam-bath for two hours, cooled and centrifuged. The salt was decomposed with 2 *N* hydrochloric acid and the free acid extracted with ether. The solid residue from the ether crystallized from acetone yielded 59 mg. of crude product, m. p. 216–218°. The material was extracted with ether from a thimble and after a recrystallization weighed 40 mg.; m. p. 221°. The mixed m. p. 223° with an authentic sample of β-3-hydroxy-*nor-allo*-cholanic acid,[13] m. p. 224–226° showed no depression. The equivalent weight by titration in alcohol of a 33.1-mg. sample was found to be 367, the calculated value for $C_{23}H_{38}O_3$ is 362.

The titrated solution of β-3-hydroxy-*nor-allo*-cholanic acid was acidified and the precipitated acid centrifuged out. It was washed with water, dried, and then refluxed for two hours with 5 cc. of methanol with a drop of sulfuric acid added. The solution was diluted with water, centrifuged,

(13) Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).

and the precipitate washed with water, sodium carbonate and water, and dried. The precipitate was refluxed with 1 cc. of acetic anhydride for several minutes and filtered on cooling. The product crystallized from methanol melted at 156–158° and on recrystallization melted at 158–158.5°. The mixture with an authentic specimen of β-3-acetoxy-*nor-allo*-cholanic acid methyl ester, m. p. 156–158°, was unchanged.

Anal. Calcd. for $C_{26}H_{42}O_4$: C, 74.59; H, 10.12. Found: C, 74.77; H, 10.25.

(b) **Isolation of Side Chain Fragments.**—Twenty grams of campesteryl acetate derived from soybean oil was dissolved in 160 cc. of boiling glacial acetic acid. A solution of 60 g. of chromic anhydride in 90 cc. of water and 90 cc. of glacial acetic acid was added drop by drop during one hour while distilling off the acetic acid at approximately the same rate. An additional 300 cc. of acetic acid was added in portions while distilling off the acid. The distillate was neutralized with a 50% solution of potassium hydroxide, using brom thymol blue indicator, and then distilled through an efficient packed column. The first 50 cc. of distillate with its suspended drops of oily ketone was taken up with 500 cc. of ether. The solutions were separated and the ether extract washed with seven portions of water until the nitroprusside test for acetone was practically negative. The combined aqueous extracts were extracted once with ether which portion was discarded and the aqueous solution distilled in a column. The fraction boiling from 40–100° was treated with a dilute acetic acid solution of *p*-nitrophenylhydrazine. The crude hydrazone weighed 186 mg. and was crystallized twice from aqueous ethanol m. p. 146.5–148°. The mixed melting point with an authentic sample of acetone *p*-nitrophenylhydrazone was unchanged.

Anal. Calcd. for $C_9H_{11}O_2N_3$: N, 21.75. Found: N, 21.97.

The original ether extract was concentrated under a column and the oily residue which had a strong ethereal odor was treated with 0.5 g. of semicarbazide hydrochloride and 2 g. of sodium acetate in 10 cc. of water. The mixture was shaken in a flask for eight hours and then allowed to stand several days before filtering off the crude semicarbazone weighing 153 mg. The product after high vacuum sublimation was crystallized four times from hexane yielding 11 mg. of white leaflets, m. p. 152–153°; $[\alpha]^{24}D$ +11.9° (7.8 mg. in 1.03 cc. of chloroform gave $\alpha^{24}D$ +0.09, l = 1 dm.).

Anal. Calcd. for $C_{10}H_{21}ON_3$: N, 21.09. Found: N, 21.01.

A mixed m. p. with a sample of *l*-methyl isoheptyl ketone semicarbazone m. p. 153–154° showed no depression. The *l*-methyl isoheptyl ketone semicarbazone, $[\alpha]^{24}D$ −14.4°, was prepared from α-ergostenol acetate by following the above procedure. Nakayima[8] gives the constants m. p. 150°, $[\alpha]^{17}D$ −15.8° from ergostanol, and m. p. 156°, $[\alpha]^{18}D$ −16.45° from α-ergostenol.

Campestanol.—Two tenths of a gram of campestanyl acetate was refluxed with 20 cc. of 5% alcoholic potassium hydroxide for an hour. After dilution with water, the precipitated sterol was filtered, dried and crystallized from alcohol, m. p. 143–145°. From acetone campestanol forms narrow leaflets m. p. 146–147°, $[\alpha]^{24}D$ +31° (16.7 mg. in 2 cc. chloroform, αD +0.26°, l = 1 dm.).

Anal. Calcd. for $C_{28}H_{50}O \cdot \frac{1}{2}H_2O$: C, 81.69; H, 12.50. Found: C, 81.52, 81.56; H, 12.42, 12.49.

Campestanyl *m*-Dinitrobenzoate.—The bulk of the campestanol was treated with 1 g. of *m*-dinitrobenzoyl chloride and 10 cc. of dry pyridine and worked up in the usual manner. The campestanyl *m*-dinitrobenzoate formed leaflets, m. p. 198°, $[\alpha]^{24}D$ +22° (17.8 mg. in 2 cc. chloroform gave $\alpha^{24}D$ +0.20; l = 1 dm.).

Anal. Calcd. for $C_{35}H_{52}O_6N_2$: C, 70.43; H, 8.78. Found: C, 70.35, 70.58; H, 9.03, 8.91.

***i*-Campesterol *p*-Toluenesulfonate.**—Half a gram each of campesterol and *p*-toluenesulfonyl chloride dissolved in 5 cc. of pyridine was kept overnight at room temperature then diluted with water. The solution was extracted with ether and the ether extract washed with dilute hydrochloric acid, water, sodium carbonate, and water. The tosyl ester was crystallized from acetone, m. p. 150–152°; yield 0.44 g.

***i*-Campesterol Methyl Ether.**—Four hundred mg. of the tosyl ester was converted to the *i*-methyl ether by refluxing in a methanol solution of potassium acetate and the crude product purified by chromatographing over alumina as in the case of the *i*-stigmasteryl methyl ether.[4]

The *i*-methyl ether crystallized from acetone–methanol in leaflets, m. p. 61–63°, $[\alpha]^{24}D$ +62° (15.5 mg. in 2 cc. of chloroform gave $\alpha^{24}D$ +0.48°, l = 1 dm.).

Anal. Calcd. for $C_{28}H_{50}O$: C, 83.98; H, 12.16. Found: C, 83.53; H, 12.09.

Summary

From the chromic acid oxidation products of campesteryl acetate were isolated acetone and *d*-methyl isoheptyl ketone and from campestanyl acetate β-3-hydroxy-*nor-allo*-cholanic acid. Campesteryl *p*-toluenesulfonate refluxed with methanolic potassium acetate forms a characteristic campestryl *i*-methyl ether. These facts confirm the empirical formula $C_{28}H_{48}O$ and show that campesterol is an isomer of 22,23-dihydrobrassicasterol which differs only in the optical configuration on C-24.

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