Summary

A number of 3-methyl-3,4-dihydroisoquinoline and 3-methyl-1,2,3,4-tetrahydroisoquinoline derivatives are described. The substituents are 6,7-dimethoxyl, 6,7-methylenedioxyl and 6,7-dihydroxyl. The compounds are closely related to norhydrastinine and dihydronorhydrastinine. A series of general reactions for their preparation is given.

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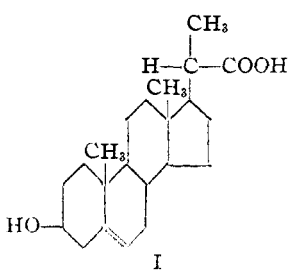
[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

Brassicasterol. II. Degradation by Ozone

BY ERHARD FERNHOLZ AND HOMER E. STAVELY

In a previous communication[1] it was shown that completely hydrogenated brassicasterol, brassicastanol, is not identical with stigmastanol, the common hydrogenated derivative of the phytosterols stigmasterol, β-sitosterol, and α-spinasterol. This was somewhat unexpected since our analytical data favored an empirical formula with 29 carbon atoms, rather than one with 28 carbon atoms as originally proposed by Windaus and Welsch.[2] Analyses reported[1] for brassicasteryl *m*-dinitrobenzoate fitted almost equally well for a C_{28} or a C_{29} sterol but analyses for brassicastyl *m*-dinitrobenzoate definitely favored a C_{29} sterol. In spite of this, on the basis of ozonization experiments we are now forced to conclude that brassicasterol contains only 28 carbon atoms.



I

After addition of one mole of bromine to the nuclear double bond of brassicasteryl acetate, treatment with ozone and subsequent debromination, β-3-hydroxy-bisnorcholenic acid (I) was isolated, the same acid obtained by similar treatment of stigmasteryl acetate.[3] This degradation product accounts for the largest part of the molecule and leaves no doubt as to the position of the two double bonds and the hydroxyl group. Another sample of brassicasterol was ozonized under conditions favorable for the isolation of a volatile aldehyde, the other molecular fragment. A crystalline semicarbazone of the aldehyde was obtained which analyzed for six carbon atoms from the aldehyde. It is therefore conclusively proved that the empirical formula of brassicasterol is $C_{28}H_{46}O$.

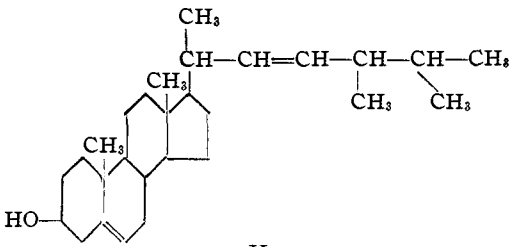
A comparison of the properties of the aldehyde semicarbazones obtained by ozonizing ergosterol,[4] stigmasterol[5] and brassicasterol seems to indicate that the aldehyde from brassicasterol is slightly racemized 1-methylisopropylacetaldehyde, as shown in Table I. A mixed sample of the semicarbazone from brassicasterol with the semicarbazone of 1-methylisopropylacetaldehyde from ergosterol (m. p. 128) melted at 120–122°, showing no depression.

TABLE I

	Ethylisopropylacetaldehyde semicarbazone (from stigmasterol)	Methylisopropylacetaldehyde semicarbazone (from ergosterol)	Semicarbazone from brassicasterol
M. p., °C.	128	128	119
$[\alpha]_D$	+9°	−52°	−40°
C, %	56.14	53.46	53.65
H, %	9.95	9.62	9.70
N, %	24.58	26.74	26.76

This is noteworthy because all precautions were taken to prevent racemization and in a trial run with ergosterol no racemization occurred. The assumption that brassicasterol contains a certain amount of an isomer with the opposite configuration on C_{24} seems to offer an attractive explanation for this observation, although it is also possible that brassicasterol contains a closely related isomeric sterol with a structurally differing side chain. Our degradation experiments leave, however, little doubt that formula II must be assigned to brassicasterol, which could be called 7,8-dihydroergosterol.

(1) Fernholz and Stavely, THIS JOURNAL, **61**, 142 (1939).
(2) *Ber.*, **42**, 612 (1909).
(3) Fernholz, *Ann.*, **507** 128 (193
(4) Guiteras, Nokamiya and Inhoffen, *Ann.*, **494**, 119 (1932).
(5) Guiteras, *Z. physiol. Chem.*, **214**, 89 (1933).



II

The difficulty of obtaining a pure sample of brassicasterol is also illustrated by a marked difference in the rotation of its hydrogenation product and pure ergostanol and their derivatives, except the *m*-dinitrobenzoate as shown in Table II.

TABLE II

	M. p., °C.	$[\alpha]^{25}D$
Brassicastanol	142	+24
Brassicastyl acetate	143	+15
Brassicastyl *m*-dinitrobenzoate	202	+14
Ergostanol	144	+15
Ergostyl acetate	145	+ 6
Ergostyl *m*-dinitrobenzoate	203	+13

In view of these discrepancies it was not possible to assume their identity in spite of the absence of mixed melting point depressions throughout the series without the more convincing evidence obtained by ozone degradation of brassicasterol.

The great structural similarity of stigmasterol and brassicasterol has led us to apply the partial debromination reaction recently described by us[6] for stigmasteryl acetate tetrabromide also to the brassicasterol derivative. A 22,23-dibromobrassicasteryl acetate of m. p. 236–238° was obtained.

Experimental

Ergostyl *m*-Dinitrobenzoate.—Five and one-half grams of ergostanol, 5 g. of *m*-dinitrobenzoyl chloride and 70 cc. of pyridine were heated for one-half hour on the steam-bath, then diluted with water, filtered, and recrystallized from ethanol–chloroform, m. p. 202–203°; $[\alpha]^{24}D$ +14° (12.7 mg. in 2.0 cc. chloroform, $\alpha^{24}D$ +0.09°, 1 dm. tube).

Anal.[7] Calcd. for $C_{35}H_{52}O_6N_2$: C, 70.43; H, 8.78. Found: C, 70.18; H, 8.66.

β-3-Hydroxy-bisnorcholenic Acid.—Brassicasteryl acetate (3.82 g.) was dissolved in chloroform and 1.34 g. of bromine (1 mole) in chloroform was added dropwise. After twice the theoretical amount of ozone had been passed through the solution it was evaporated *in vacuo* below 30°, and the residue debrominated with zinc and acetic acid. Water was added and the mixture extracted with ether, the ether washed with water and an excess of 2 *N* sodium hydroxide. A precipitate, which appeared between the ether and an aqueous layer, was washed with 2 *N* sodium hydroxide and ether, then acidified with dilute acid and extracted with ether. The ether was evaporated and the residue refluxed with alcoholic potassium hydroxide for two hours, water was then added and the mixture extracted with a large amount of ether. The ether residue was recrystallized from acetic acid, m. p. (dec.) 293°

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.23; H, 9.91. Found: C, 76.15; H, 10.10. Mixed m. p. with an authentic sample of β-3-hydroxy-bisnorcholenic acid showed no depression.

β-3-Hydroxy-bisnorcholenic Acid Methyl Ester.—The methyl ester was prepared from 160 mg. of β-3-hydroxy-bisnorcholenic acid by means of diazomethane; recryst. from aqueous methanol, m. p. 139–140°.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.58; H, 10.10. Found: C, 75.45; H, 9.96.

A mixed m. p. with an authentic sample of the methyl ester of β-3-hydroxy-bisnorcholenic acid showed no depression.

β-3-Acetoxy-bisnorcholenic Acid Methyl Ester.—Seventy milligrams of β-3-hydroxy-bisnorcholenic acid methyl ester stood overnight in a solution of 0.3 cc. of acetic anhydride in 3 cc. of pyridine. The solvent was evaporated *in vacuo* and the residue crystallized from methanol, m. p. 138°.

Anal. Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.54. Found: C, 74.77, 74.82; H, 9.47, 9.49.

A mixed m. p. with an authentic sample of β-3-acetoxy-bisnorcholenic acid methyl ester showed no depression.

Ozonization of Brassicasteryl Acetate.—Two grams of brassicasteryl acetate was suspended in 20 cc. of glacial acetic acid and an excess of ozone passed through the suspension. It was poured immediately into 100 cc. of water and distilled until no more oil drops distilled over. The distillate was neutralized with sodium bicarbonate, 200 mg. of semicarbazide hydrochloride was added, and more sodium bicarbonate to slight alkalinity. The semicarbazone crystallized on shaking. It was filtered, washed with water, and recrystallized from petroleum ether. The melting point could not be raised above 119° by recrystallization or sublimation; $[\alpha]^{23}D$ −39.4 ± 2.0 (8.65 mg. in 2.0 cc. of ethanol, $\alpha^{23}D$ −0.17, 1 dm. tube).

Anal. Calcd. for $C_7H_{15}ON_3$: C, 53.46; H, 9.62; N, 26.74. Calcd. for $C_8H_{17}ON_3$: C, 56.14; H, 9.95; N, 24.58. Found: C, 53.65; H, 9.70; N, 26.76.

A mixed m. p. with the semicarbazone of 1-methylisopropylacetaldehyde from ergosterol (m. p. 128) melted at 120–122°.

Brassicasteryl Acetate-22,23-dibromide.—Brassicasteryl acetate tetrabromide (180 mg.) was debrominated with sodium iodide by the method used for stigmasteryl acetate tetrabromide.[6] The product was crystallized from ethanol–benzene, m. p. 236–238°.

Anal. Calcd. for $C_{30}H_{48}O_2Br_4$: **Br**, 42.1. Calcd. for $C_{30}H_{48}O_2Br_2$: Br, 26.7. Found: **Br**, 27.9.

Summary

On ozonizing brassicasteryl acetate, a C_{22} acid,

(6) THIS JOURNAL, **61**, 2956 (1939).

(7) Analyses herein reported are by Mr. J. F. Alicino, Fordham University.

β-3-hydroxy-bisnorcholenic acid, and a C_6 aldehyde are obtained. The empirical formula of brassicasterol is therefore $C_{28}H_{46}O$.

The C_6 aldehyde appears to be a partially racemized 1-methylisopropyl-acetaldehyde, which is a degradation product of ergosterol.

The structural formula of 7,8-dihydroergosterol is proposed for brassicasterol.

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The Vitamin K Activity of Naphthoquinones

BY ERHARD FERNHOLZ, S. ANSBACHER AND H. B. MACPHILLAMY

Soon after McKee, *et al.*,[1] reported that vitamins K_1 and K_2 have a quinoid structure, publications from various laboratories appeared on the vitamin K activity of naphthoquinones. Because of its outstanding potency 2-methyl-1,4-naphthoquinone and its derivatives have been investigated most thoroughly. A discussion of the pertinent literature concerning this compound has been presented in a foregoing publication.[2] A suggestion also has been made to use 2-methyl-1,4-naphthoquinone as a basic standard for the assay of vitamin K;[3] under the conditions used in our laboratory one milligram contains 2000 units.

The following summarizes our assay results with other naphthoquinones: inactive, 1,2-naphthoquinone; weakly active in a dose of one milligram, 2,6-dimethyl, 2-allyl-, 2,3-diallyl-, 2-*n*-hexadecyl-,2-*n*-octadecyl-1,4-naphthoquinone; one unit per milligram, 1,4-naphthoquinone, 2-ethyl-, 2-propyl-, and 2-methyl-3-*n*-octadecyl-1,4-naphthoquinone; 20 units per milligram, 2,3-dimethyl-1,4-naphthoquinone; 70 units per milligram, 2-methyl-3-phytyl-1,4-naphthoquinone(vitamin K_1).

Thayer, *et al.*,[4] reported that 1,2-naphthoquinone is inactive whereas 1,4-naphthoquinone is active in a dose of 1 mg.; our results confirm this report. Almquist and Klose,[5] however, stated that they had obtained entirely negative results with the latter compound. The interesting contrast between the comparatively active 2,3-dimethyl-1,4-naphthoquinone and the rather weakly active 2,6-dimethyl-1,4-naphthoquinone[6] has been reported also by Tishler and Sampson.[7] There is some uncertainty concerning the activity of 2-ethyl-1,4-naphthoquinone. Thayer, *et al.*,[4] state that it is fully active at a level of 125 γ, and according to Tishler and Sampson it shows activity above 200 γ. In our experience the compound is not nearly as active as reported by Thayer, *et al.*, since 1 mg. was found to be necessary to give a unit response. The propyl derivative has been assayed and found inactive at a level of 0.4 mg.;[7] we found it active in a dose of 1 mg. Fieser, *et al.*,[8] originally reported that 2-allyl-1,4-naphthoquinone is very active but Thayer, *et al.*,[4] stated in the same issue of THIS JOURNAL that the compound was inactive in a dose of 2 mg. In a later paper Fieser, *et al.*,[9] agreed with Thayer. We also found that 2-allyl-1,4-naphthoquinone and 2,3-diallyl-1,4-naphthoquinone have little, if any, activity at a level of 1 mg. We have prepared and assayed the *n*-hexadecyl, *n*-octadecyl, and 2-methyl-3-octadecyl-1,4-naphthoquinones. These compounds have shown rather low potencies.[10] The last of the three, containing the methyl group in the 2-position, is definitely more active than the two others.

The long-chain naphthoquinones were prepared by chromic acid oxidation of the corresponding hydrocarbons. With the exception of the *n*-octadecyl compound, substitution of the naphthalene nucleus in the β-position was ensured by using the method of Barbot[11] for the production of β-substituted tetralin ketones. The ketones were reduced by the Clemmensen

(1) McKee, Binkley, MacCorquodale, Thayer and Doisy, THIS JOURNAL, **61**, 1295 (1939).

(2) Ansbacher, Fernholz and Dolliver, THIS JOURNAL, **62**, 155 (1940).

(3) Thayer, Binkley, MacCorquodale, Doisy, Emmet, Brown and Bird, *ibid.*, **61**, 2563 (1939).

(4) Thayer, Cheney, Binkley, MacCorquodale and Doisy, THIS JOURNAL, **61**, 1932 (1939).

(5) *Ibid.*, **61**, 2557 (1939).

(6) We are indebted to Professor Fieser for sending us samples of these two substances as well as of allyl-naphthoquinone and diallyl-naphthoquinone.

(7) *Ibid.*, **61**, 2563 (1939).

(8) Fieser, Bowen, Campbell, Fry and Gates, *ibid.*, **61**, 1926 (1939).

(9) Fieser, Campbell and Fry, THIS JOURNAL, **61**, 2206 (1939).

(10) After this paper had been submitted a paper by Fieser [THIS JOURNAL, **61**, 3467 (1939)] appeared wherein it is postulated that vitamin K activity begins to appear as the alkyl groups are increased in size. Obviously our results do not support this idea.

(11) Barbot, *Bull. soc. chim.*, [4] **47**, 1314 (1930).