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5,6-Dihydrostigmasterol

BY ABRAHAM MAZUR

Among the sterols isolated from sponges are spongosterol,[1] clionasterol,[2] and microclionasterol[3] all reported to contain 27 carbon atoms. These sterols have not been well characterized and doubt exists as to their purity.

In a preliminary communication[4] the author reported the study of a sterol mixture obtained from a fresh-water sponge.[5] Since no further purification could be attained by recrystallization of the acetates, the free sterols were separated by means of repeated chromatographic adsorption on activated alumina. In this manner two distinct fractions were finally obtained, and one of them was characterized as a new sterol.

The more strongly adsorbed fractions gave a positive Rosenheim reaction, evidence for the presence of a conjugated double bond. The acetate of this fraction distilled *in vacuo*, displayed absorption maxima which correspond to those obtained with ergosteryl acetate (see figure) and those reported[6] for 7-dehydrocholesterol. The molecular[7] extinction coefficient at 282.5 mμ was 7200 as compared to 12,900 for ergosterol, indicating approximately 56% purity. Too little of this material was available for further study.

The less strongly adsorbed fractions were converted to the acetates and subjected repeatedly to chromatographic analysis until no change in melting point or rotation occurred. The sterol and its various derivatives analyzed for $C_{29}H_{50}O$. It proved to have one double bond, which added one mole of hydrogen to yield stigmastanol.

The position of the double bond was not at 5,6 because the sterol displayed unmistakable differences from the 22,23-dihydrostigmasterol[8] prepared by Fernholz and Ruigh.[9] It yielded neither a crystalline dibromide nor an α,β-unsaturated ketone. Catalytic hydrogenation in cyclohexane was extremely slow and incomplete except in the presence of a trace of acetic acid, an effect not observed with cholesterol.

The sterol formed an ozonide which on decomposition yielded an aldehyde, apparently ethyl isopropyl acetaldehyde isolated as the 2,4-dinitrophenylhydrazone. Stigmasterol,[10] on similar treatment, yielded a derivative which melted 12° higher, but caused only a 2° depression on admixture. Since no optical rotation could be obtained with the derivative of the sponge sterol, whereas the hydrazone obtained from stigmasterol displayed optical activity, it may be concluded that racemization had occurred. A similar discrepancy has been reported by Fernholz[11] for brassicasterol. The double bond, therefore, appears to be at position 22,23 and the sterol should be named 5,6-dihydrostigmasterol.

After our preliminary study had been reported, Valentine and Bergmann[12] published a re-investigation of clionasterol and suggested the formula $C_{29}H_{50}O$. From the physical constants reported it would appear that clionasterol is identical with 5,6-dihydrostigmasterol.

The presence in an invertebrate of a sterol with the stigmasterol nuclear structure is of interest since the sterols of plants and of the lower animal forms are structurally related to stigmasterol and not to cholesterol, which seems to be the principal sterol of the vertebrates.

Experimental

Preparation of the Sterol Mixture.—One kilogram of the siliceous sponge, *Spongilla lacustris*, was collected and stored under acetone. The acetone extract was evaporated to dryness and the residue extracted with petroleum ether. The residual sponge was extracted with petroleum ether and both extracts pooled. The total lipin extracted by the petroleum ether weighed 88.6 g. This fraction was dissolved in petroleum ether and extracted with 0.3% potassium carbonate in 70% aqueous methyl alcohol to remove any free acids. A crystalline sterol separated at this point. The neutral lipins were saponified and the soaps removed. The unsaponifiable fraction was dried,

(1) Henze, *Z. physiol. Chem.*, **41**, 109 (1904).
(2) Dorée, *Biochem. J.*, **4**, 72 (1909).
(3) Bergmann and Johnson, *Z. physiol. Chem.*, **222**, 220 (1933).
(4) Mazur, THIS JOURNAL, **63**, 883 (1941).
(5) *Spongilla lacustris*, kindly collected by Dr. G. L. Clarke at Trout Lake, Vilas County, Wisconsin, and stored under acetone.
(6) Windaus, Lettré and Schenck, *Ann.*, **520**, 98 (1935).
(7) The molecular weight was assumed to be the same as that of ergosteryl acetate.
(8) The author is indebted to Dr. W. L. Ruigh of the Squibb Institute for Medical Research for several specimens of this compound and its derivatives.
(9) Fernholz and Ruigh, THIS JOURNAL, **62**, 3346 (1940).
(10) The author is indebted to Dr. R. Schoenheimer of these laboratories for a specimen of stigmasterol.
(11) Fernholz and Stavely, THIS JOURNAL, **62**, 428 (1940).
(12) Valentine and Bergmann, *J. Org. Chem.*, **6**, 452 (1941).

dissolved in dry pyridine and treated with an excess of succinic anhydride on the steam-bath for one hour and overnight at room temperature. The sterol acid succinates were isolated in the usual manner. The resulting semi-crystalline product was recrystallized several times from ethyl alcohol. The total product amounted to 7.5 g., or 0.75% of the original sponge.

The crystalline material displayed a strong Liebermann test and a positive Rosenheim reaction. It was entirely precipitable by digitonin and could not be further purified after several recrystallizations in the free forms or as the acetates.

Sterol with Conjugated Double Bonds.—A solution of 1.0 g. of the crystalline sterol mixture in a pentane–benzene mixture (5:2) was percolated through a column of activated alumina. This was washed successively with 50:1, 25:1, 10:1, 5:1, 2:1 and 1:1 mixtures of pentane and benzene. The sterols were completely retained on the column. This was cut into four equal parts and each was treated in a soxhlet with ether, the extracts were recrystallized from alcohol, and the melting point, rotation and absorption spectrum determined. The fraction most strongly adsorbed showed the strongest absorption in the ultraviolet. The chromatographic procedure was repeated systematically. The fraction most strongly adsorbed, which weighed 55.3 mg., was converted to its acetate and distilled *in vacuo*. The resulting 25 mg. of white crystalline product had m. p. 148°; $[\alpha]D$ −93.9°[13] (ergosteryl β-acetate[14] has m. p. 179–180°; $[\alpha]D$ −90.0°). Absorption maxima (see figure) were found at 272.5, 283.5 and 294.5 mμ; the maximum at 283.5 mμ had an extinction coefficient (ε) of 7200. Ergosteryl β-acetate displayed maxima at 273, 283.5 and 293 mμ, with ε = 12,900 at 283.5 mμ.

5,6-Dihydrostigmasteryl Acetate.—The remaining sterol mixture was converted to the acetates by refluxing with acetic anhydride. The acetates were dissolved in a 1:1 pentane–benzene mixture and passed through a column of activated alumina followed by mixtures of pentane–benzene in the ratio of 1:2, 1:3, 1:4, 1:6, 1:8, 1:10 and finally pure benzene. The various washings were collected separately in 10 fractions. Each was evaporated to dryness, the residue recrystallized from alcohol and melting point and rotation determined. Those fractions whose rotations were close to each other were united and again adsorbed on alumina. In this way a fraction was obtained weighing 3.3 g. which could not be separated further by this technique; m. p. 137°; $[\alpha]D$ −47.6°.

Anal. Calcd. for $C_{31}H_{51}O_2$: C, 81.5; H, 11.5. Found: C, 81.4; H, 11.6.

5,6-Dihydrostigmasterol.—The acetate was saponified by refluxing for two hours with a 5% potassium hydroxide solution in 95% alcohol. The free sterol was crystallized several times from alcohol; m. p. 136.5–137°; $[\alpha]D$ −41.8°.

5,6-Dihydrostigmasteryl benzoate had m. p. 137.5°; $[\alpha]D$ −17.1°.

Anal. Calcd. for $C_{36}H_{54}O_2$: C, 83.2; H, 10.5. Found: C, 83.0; H, 10.3.

5,6-Dihydrostigmasteryl 3,5-dinitrobenzoate had m. p. 200°; $[\alpha]D$ −18.3°.

(13) All rotations were determined in chloroform.

(14) Bills and Honeywell, *J. Biol. Chem.*, **80**, 15 (1928).

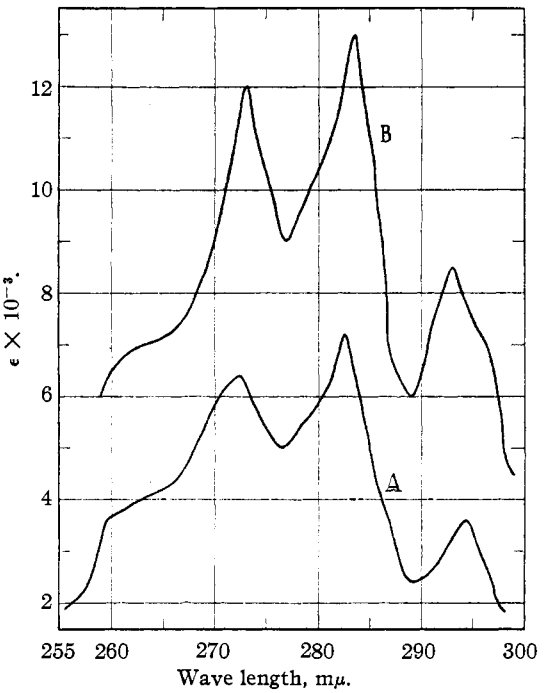

Fig. 1.—Absorption spectra: A, sterol acetate; B, ergosterol acetate.

Anal. Calcd. for $C_{36}H_{52}N_2O_6$: C, 71.0; H, 8.6. Found: C, 71.2; H, 8.7.

5,6-Dihydrostigmastanyl Acetate.—Ninety-seven and eight-tenths milligrams of the sterol acetate was dissolved in 10 ml. of glacial acetic acid and hydrogenated in the presence of 5 mg. of Adams catalyst. The uptake of hydrogen corresponded to 1 double bond. In a similar hydrogenation in cyclohexane, little hydrogen was absorbed during two hours. On addition of 1 drop of glacial acetic acid, the theoretical uptake of hydrogen was observed. The stanol acetate was recrystallized from alcohol; m. p. 129°; $[\alpha]D$ +11.5°.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.9. Found: C, 81.1; H, 11.7.

Stigmastanyl acetate was prepared from stigmasteryl acetate by hydrogenation in acetic acid in the same manner; m. p. 128°; $[\alpha]D$ +15.7°; mixed m. p. 128°.

5,6-Dihydrostigmastanol prepared by hydrolysis of the above acetate had m. p. 134–135°; $[\alpha]D$ +23.3°. Stigmastanol, prepared from its acetate, had m. p. 135°; $[\alpha]D$ +28°; mixed m. p. 135°.

5,6-Dihydrostigmastanyl 3,5-Dinitrobenzoate.—The stanol was converted to its dinitrobenzoate, which, after recrystallization from benzene alcohol, had m. p. 210°; $[\alpha]D$ +139°.

Anal. Calcd. for $C_{36}H_{54}O_6N_2$: C, 70.8; H, 8.9. Found: C, 70.7; H, 8.9.

Stigmastanyl dinitrobenzoate, prepared in a similar manner, had m. p. 210°; $[\alpha]D$ +14°; mixed m. p. 210°.

5,6-Dihydrostigmastanone.—The stanol in glacial acetic acid was treated with chromic acid overnight at room temperature. The product as twice recrystallized from aqueous alcohol, had m. p. 155°; $[\alpha]D$ +38.9°. Stigmas-

tanone, similarly prepared, had m. p. 155°; $[\alpha]_D$ +40.6°; mixed m. p. 155°.

5,6-Dihydrostigmastanone oxime, recrystallized from aqueous alcohol, melted at 210°.

Anal. Calcd. for $C_{29}H_{51}ON$: C, 81.0; H, 12.0. Found: C, 80.8; H, 12.1.

Stigmastanone oxime had m. p. 210°; mixed m. p. 210°.

Ozonization of 5,6-Dihydrostigmasteryl Acetate.—One gram of the sterol acetate was suspended in 10 ml. of glacial acetic acid which had been freed of aldehydes by distillation over chromic oxide. Ozone in excess was passed through the suspension for one hour. The clear solution was added to 100 ml. of water and the mixture distilled to one-quarter the volume into a suspension of 800 mg. of 2,4-dinitrophenylhydrazine in 100 ml. of 95% alcohol containing 1 ml. of concentrated hydrochloric acid. The mixture of the hydrazone and unchanged reagent was washed with water and dried. It was suspended in benzene and percolated through a column of activated alumina. Benzene was used to elute the yellow hydrazone, while the dinitrophenylhydrazine reagent remained strongly adsorbed at the top of the column. The benzene solution was evaporated to dryness; the residue, recrystallized several times from alcohol, yielded 100 mg. of a yellow crystalline compound; m. p. 109°; $[\alpha]_D$ 0°.

Anal. Calcd. for $C_{13}H_{18}N_4O_4$: C, 53.02; H, 6.19; N, 19.03. Found: C, 52.93; H, 6.13; N, 18.95.

Stigmasterol was treated in the same manner and the dinitrophenylhydrazone isolated; m. p. 121°; $[\alpha]_D$ −5.8° in benzene; mixed m. p. 119°.

In another ozonization of 250 mg. of the sterol acetate, some of the volatile aldehyde, collected as such, displayed a positive fuchsin test.

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Summary

The sterols of a fresh-water sponge, *Spongilla lacustris*, were separated by means of repeated chromatographic adsorption on activated alumina. One fraction, strongly adsorbed, yielded an impure sterol with conjugated double bonds, displaying an absorption spectrum similar to that obtained with ergosterol. The fraction less strongly adsorbed by the alumina yielded a pure monounsaturated sterol identified as 5,6-dihydrostigmasterol.

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Isolation of Ecgonidine Methyl Ester from Coca Seeds[1]

BY JOHN R. MATCHETT AND JOSEPH LEVINE

Although a copious literature exists regarding the alkaloids of the coca leaf, nothing appears to have been reported regarding the chemistry of the seeds. In this Laboratory we have had occasion to examine the seeds of two varieties, *Erythroxylon coca*, Lam., obtained from Peru and *Erythroxylon novogranatense*, (Morris) Hieron, obtained from Java, and have isolated from each an alkaloid which proved to be the methyl ester of ecgonidine. Neither ecgonine nor its esters were present in amounts sufficient for identification.

Ecgonidine and certain of its derivatives are well known, though the methyl ester does not appear to have been described previously. The ethyl ester has been isolated in small yield from the by-alkaloids of the coca leaf by Liebermann,[2] who expressed the opinion that it did not occur there naturally, but was probably formed from ecgonine during the long processing required to separate the ecgonine alkaloids.

The conditions under which ecgonidine methyl ester was isolated from the seeds were sufficiently mild to ensure against decomposition of ecgonine or its esters; hence it appears certain that it existed as such in the seeds. Whether it is actually formed by the plant or is a product of chemical change after the ripening of the seed can only be disclosed by examination of fresh seeds. Those examined by us were of unknown age. It is worthy of note, however, that they were from widely separated sources, and that in both ecgonidine methyl ester was found to be the only alkaloid present in substantial amounts. If it were formed from an ecgonine ester, the change must have been sensibly complete before the seeds reached this Laboratory.

Ecgonidine (I or II), or anhydroecgonine, has been prepared by treatment of ecgonine (III) with dehydrating agents, such as phosphorus pentachloride[3] or phosphorus oxychloride.[4] The struc-

(1) Not copyrighted.

(2) Liebermann, *Ber.*, **40**, 3602 (1907).

(3) Merck, *ibid.*, **19**, 3002 (1886).

(4) Einhorn, *ibid.*, **20**, 1221 (1887).