Sterols and other Unsaponifiable Substances in the Fats of Shell Fishes, Crustacea and Echinodermata. X. Corbisterol

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In the 2nd report of this series,(1) a new sterol named corbisterol was separated from the fat of *Corbicula leana*, and the formula $C_{29}H_{44}O$ or $C_{28}H_{42}O$ was assigned to this sterol on the basis of analysis, iodine value and saponification value of its acetate. The iodine value was determined by the pyridine sulfate dibromide method. In a later study described in the 8th report,(2) however, the determination of unsaturation of this sterol by perbenzoic acid showed a value for its acetate, which was half the value obtained by the pyridine sulfate dibromide method and corresponded to the value calculated for two ethylenic linkages. The determination of hydrogen value may afford a sure means to know the numbers of ethylenic linkages of corbisterol, but both corbisterol and its acetate absorbed hydrogen very slowly, even at an elevated temperature, when hydrogenation proceeded to a certain degree, and the quantitative hydrogenation could not be attained. Bergmann and Ottke(3) pointed out that 7-dehydrosterols are prone to give iodine values considerably higher than the theoretical values, and suggested that corbisterol is a 7-dehydrosterol, probably identical with 22, 23-dihydroergosterol.

The present paper is concerned with a further study of corbisterol by bromination, reduction and ozonolysis of corbisteryl acetate and absorption spectrum of corbisterol. In order to ascertain whether or not the reaction involved in the iodine value determination accompanies substitution, and to what extent if it does, the bromination product was prepared by allowing the pyridine sulfate dibromide solution to react with corbisteryl acetate under the same condition as the iodine value determination. The bromine content of bromination product was found to be approximately 6 atom equivalents per one mole of bromination product, while the total consumption of bromine corresponding to the iodine value was 8 atom equivalents per one mole of corbisteryl acetate. These results may be interpreted by assuming that of the total bromine consump-

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(1) Taro Matsumoto and Yoshiyuki Toyama, *J. Chem. Soc. Japan*, **64**, 326 (1943).

(2) Minoru Kita and Yoshiyuki Toyama, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **70**, 451 (1949).

(3) W. Bergmann and R. C. Ottke, *J. Org. Chem.*, **14**, 1085 (1949).

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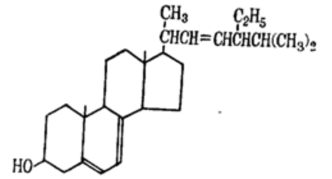
tion 4 atom equivalents are attributed to addition reaction while the remaining 4 atom equivalents are attributed to substitution reaction which causes 2 atom equivalents of bromine to enter into the bromination product and forms 2 moles of hydrogen bromide. Formation of hydrogen bromide in bromination was ascertained in another experiment in which a solution of bromine in chloroform was allowed to react with corbisteryl acetate and hydrogen bromide formed during the reaction was estimated by titration with alkali in a specially devised vessel. The result of titration indicated the formation of as much as 2.9 moles of hydrogen bromide per one mole of corbisteryl acetate while the total consumption of bromine was found to be 8 atom equivalents. Although the titration with alkali can not be thought to give the correct value of the amount of hydrogen bromide formed by substitution, the above results show convincingly that substitution occurs to a considerable extent in bromination. However, it can not be assumed that addition of bromine to all ethylenic linkages has occurred quantitatively in this case, though substitution occurs to a considerable extent. Bromination product prepared from corbisteryl acetate by bromination under thorough cooling showed bromine content lying between 4 and 6 atom equivalents per one mole.

If perbenzoic acid titration represents the real unsaturation, corbisterol should be diunsaturated sterol. It is, however, known that perbenzoic acid titration gives a lower value than the theoretical value for some compounds having conjugate double bonds, as shown by Pummerer, Rebmann and Reindel(4) in the case of several carotenoids and isoprene, the unsaturation of the latter based on perbenzoic acid titration being found to correspond to only one double bond. The presence of conjugate double bonds in corbisterol was suspected from the fact that pyridine sulfate dibromide solution developed a peculiar red coloration with corbisteryl acetate as in the case with tung oil which consists substantially of glyceride of elaeostearic acid having conjugate double bonds. Meanwhile Bergmann advised one of the present authors, Toyama, in a private communication to take an ultraviolet absorption spectrum of corbisterol. Fig. 1 shows ultraviolet absorption curve of corbisterol. The typical absorption spectrum of 5, 7-dienoic sterols is seen in the curve. Corbisteryl acetate shows also a positive Tortelli-Jaffe color reaction.

Corbisteryl acetate was subjected to ozonolysis, and a volatile aldehyde was separated from the product of ozonolysis by its 2, 4-dinitrophenyl hydrazone which formed yellow colored needles and had nearly the same melting point 115–116° as 2, 4-dinitrophenyl hydrazone of ethyl isopropyl acetaldehyde obtained by Bergmann and Low(5) from the product of ozonolysis of poriferasterol. These results seem to indicate fairly certainly that corbisterol has the formula $C_{29}H_{46}O$ and that its structure is 5, 7, 22-triunsaturated C_{29}-sterol as shown below.



It differs from ergosterol in the group attached to 24–C, the former having ethyl group instead of methyl group at 24–C. It is also to be noted that comparing corbisterol and 7-dehydrostigmasterol prepared from stigmasterol by Linsert,(6) both sterols have nearly the same melting point and specific rotation, but their acetates differ markedly in their melting points.

	Corbisterol	7–Dehydrostigmasterol
M. p.	151–152°	154°
$[\alpha]_D$	–105.5°	–113.1°
M. p. of acetate	152–153°	172°

The results of the biological assay of irradiated corbisterol will be published elsewhere.

Experimental

Crude corbisteryl acetate prepared from the sterol fraction of the fat of *Corbicula leana* was purified by repeated recrystallization. The purified product had m. p. 152–153°, $[\alpha]_D$–72.8°, saponification value 122.1 (calculated for $C_{31}H_{48}O_2$:123.9), and iodine value (pyridine sulfate dibromide method) 221.6 (calculated for $C_{31}H_{48}O_2$:168.2).

Found: C, 81.94 %; H, 11.13 %.

Calculated for $C_{31}H_{48}O_2$: C, 82.24%; H, 10.69%.

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(4) R. Pummerer, L. Rebmann and W. Reindel, *Ber.*, **62**, 1411 (1929).

(5) W. Bergmann and E. M. Low, *J. Org. Chem.*, **12**, 67 (1947).

(6) O. Linsert, *Hoppe-Seyler's Z. physiol. Chem.*, **241**, 125 (1936).

Corbisterol obtained from its acetate showed m. p. 151–152°* and $[\alpha]_D$ –105.5° after recrystallization from ethanol.

Found: C. 84.55%; H, 11.58%.

Calculated for $C_{29}H_{46}O$: C, 84.81%; H, 11.29%.

Bromination of corbisteryl acetate.—Bromine in the form of pyridine sulfate dibromide was allowed to react with corbisteryl acetate under the same condition as the iodine value determination. The bromination product was separated using ether, the ether solution washed with water and dried over anhydrous sodium sulfate, and the ether distilled off. Bromide thus obtained was a dark colored solid (Found : Br, 50.00%. Calculated for $C_{31}H_{48}O_2Br_6$: Br, 51.44%). Bromine in chloroform was allowed to react with corbisteryl acetate in a similar way as the iodine value determination. Titration with thiosulfate gave an iodine value 222.5. After titration with thiosulfate, the solution was titrated with sodium hydroxide and it was found that the total amount of acids formed in the course of reaction was 2.9 moles per one mole of corbisteryl acetate.

Corbisteryl acetate (0.123 g.) was dissolved in 5 cc. of ether, and bromine in acetic acid (1:2.5) was added dropwise to the solution under cooling below –10° until the solution became colored by excess of bromine. After keeping the solution under cooling for 2 hours, the insoluble bromide formed was filtered, washed with cold ether and dried under vacuum, yielding 0.0685 g. of white crystalline powder. It melted at 186–188° with darkening (Found : Br, 45.50 %. Calculated for $C_{31}H_{48}O_2Br_6$: Br, 51.44%. Calculated for $C_{31}H_{48}O_2Br_4$: Br, 41.39 %). The filtrate from this bromide was washed with thiosulfate solution for the removal of excess bromine. Ether was distilled off under vacuum until the solution became concentrated. On cooling the concentrated solution, 0.046 g. of white crystalline powder was separated. Fractional crystallization of crystalline powder from ether gave 2 fractions, a fraction with m. p. 189–189.5° (decomp.) and Br 46.78 % and the other fraction with m. p. 183–184.5° (decomp.) and Br 42.32 %.

Absorption spectrum of corbisterol. — Fig. 1 shows ultraviolet absorption spectrum of corbisterol in ethanol (0.0018 g. in 15.5857 g.) The maximum absorptions occurred at 272, 282 and 294 mμ and the molecular extinction coefficients were 9,974, 10,008 and 6,016 respectively.

Reduction of corbisteryl acetate.—Crude corbisteryl acetate (0.085 g.) was dissolved in 20 cc. of warm ethanol, and 3.3 g. of metallic sodium was added in small portions while the solution was refluxed. To the solution then cold water was added and it was extracted with ether. On distilling off ether, there was obtained 0.077 g. of crystalline substance, which had m. p. 144–145° and iodine value 140.6 (calculated for $C_{29}H_{48}O$: 123.0) after recrystallization from ethanol. The molecular extinction coefficients of the reduction product were 503 at 272 mμ, 515 at 282 mμ and 298 at 294 mμ, so that the bulk of original conjugation in corbisteryl acetate must have disappeared by the reduction.

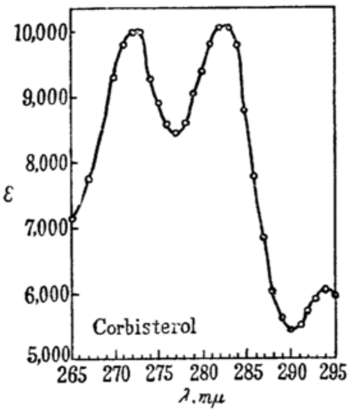

Fig. 1.—Absorption spectrum of corbisterol.

Ozonolysis of corbisteryl acetate.— Corbisteryl acetate (1.4 g.) was suspended in 14 cc. of acetic acid and ozonized oxygen with 3 % of ozone was passed through the solution at a flow rate of 300–400 cc. per minute. After 50 minutes the solution became clear. Ozonized oxygen was passed for further 15 minutes. Zinc powder and a few drops of silver nitrate solution were added to the solution while stirring. After 30 minutes zinc was centrifuged. The solution was poured into water and the emulsion was heated. The volatile matter was distilled and caught in 500 cc. of 2 N-hydrochloric acid solution containing 1 g. of 2, 4-dinitrophenyl hydrazine. The precipitate formed was filtered after standing 20 hours. Yield 0.2 g. On recrystallization from ethanol, its melting point was unaltered at 115–116°. It was optically inactive or very slightly dextrorotatory, if at all.

Summary

From the results of a further study of corbisterol by bromination, reduction and ozonolysis of corbisteryl acetate and absorption spectrum of corbisterol, the structure of corbisterol is believed to be 5, 7, 22-triunsaturated C_{29}-sterol.

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* The melting points of corbisterol and its acetate recorded in the 8 th report seem to be a little too high. There might have been some error in the melting point determination.