Summary

Two kinds of hydroxycholesteryl benzoate, A and B, were prepared from 7-bromocholesteryl benzoate and their respective dehydration by heating in vacuum provided provitamin D_3. On the other hand, dehydration by boiling in glacial acetic acid gave bicholestatriene, an yellow hydrocarbon, $C_{54}H_{82}$, in a good yield.

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51. Ko Arima and Ryoichi Hayatsu: Studies on Cholestapolyenes. II. Structure of Bicholestatriene A.

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Some reports have been made regarding the formation of a small amount of yellow steroidal hydrocarbon, bicholestatriene, $C_{54}H_{82}$, as a by-product[1]. Its structure, however, still remanins obscure, the assumed structures given in these reports being quite unfounded. Tsuda and others[2] determined the ultraviolet absorption spectrum of this substance and also carried out its titration with perbenzoic acid, proving the presence of six double bonds. In the first paper of this series[3], it was shown that yellow steroidal hydrocarbon was obtained in an average yield of 6% by boiling the two kinds of hydroxycholesteryl benzoate A and B with glacial acetic acid and that the substance was identical with the yellow steroidal hydrocarbon obtained by Tsuda and others.

In the present series of experiments, attempts were made to hydrogenate part or whole of the six double bonds by catalytic reduction. At the present moment, three kinds of colored bicholestatrienes have been isolated by different methods of preparation and these have tentatively been designated A, B, and C. The bicholestatriene reported here and in the previous paper is the one designated as A.

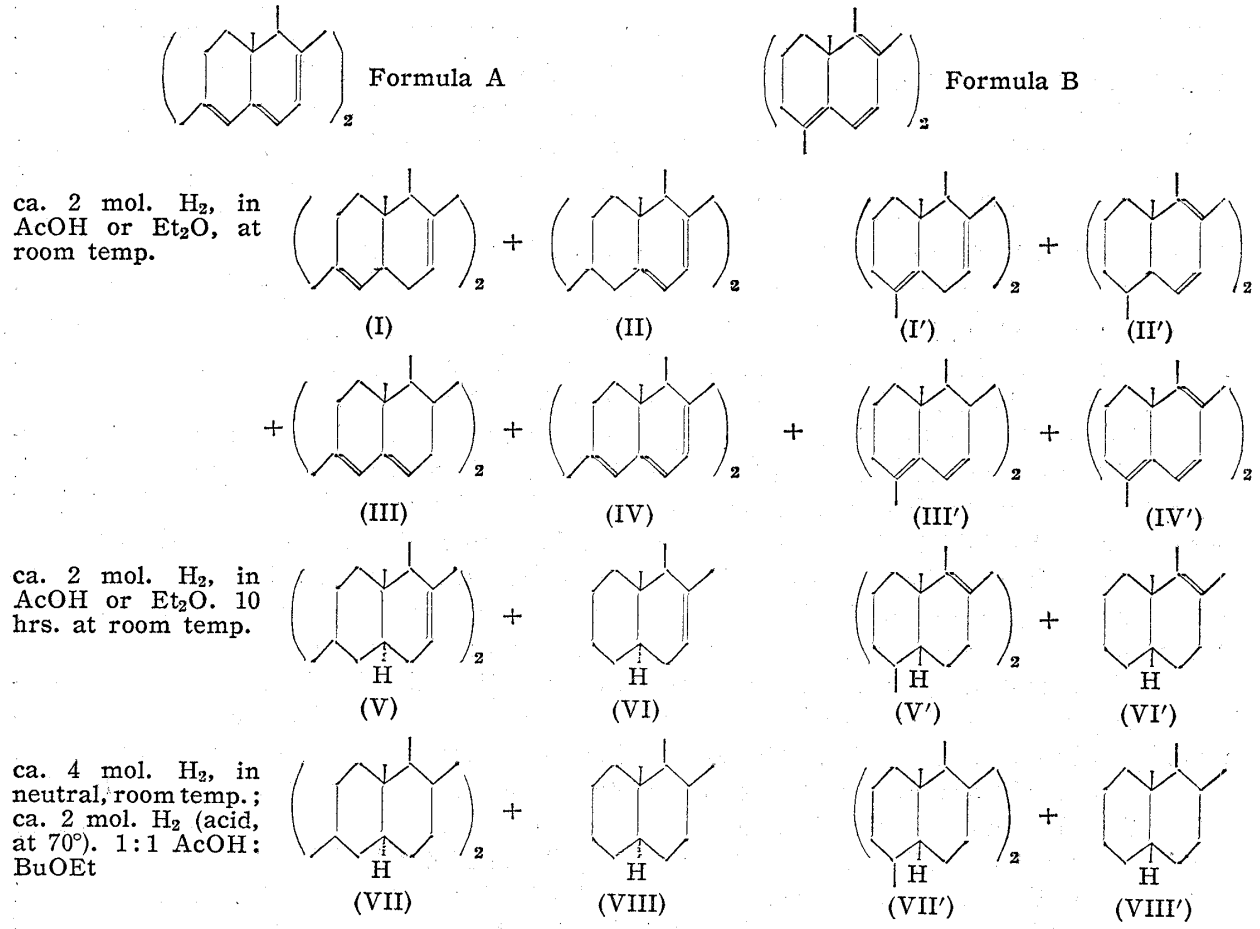
Squire[4] carried out the reduction of cholestenone with sodium amalgam to cholestenone pinacol and obtained 3,3′-bis(3,5-cholestadiene) by its dehydration. The ultraviolet absorption maxima of 3,3′-bis(3,5-cholestadiene) were found to be $\lambda_{max}^{Et_2O}$ 293, 305, and 321 mμ, and log ε=4.73, 4.80, and 4.66. In general, when one double bond conjugates with the double bonds of conjugated diene system, the third double bond is called an extended conjugative double bond, and its maximum absorption is known to shift to a longer wave length range by 30 mμ[5]. Cholestadiene possesses the maxima of $\lambda_{max}^{Et_2O}$ 229, 235, and 244 mμ, and since 3,3′-bis(3,5-cholestadiene) possesses two more extended conjugative double bonds than bicholestadiene, its absorption maxima should shift 60 mμ to the longer wave length range. The calculated values for 3,3′-bis(3,5-cholestadiene) would, therefore, be 289, 295, and 305 mμ. Two of these values coincide with those of 3,3′-bis(3,5-cholestadiene) with less than 2 mμ difference. This fact shows that the foregoing general law is applicable in a certain amount of precision when the increase of the extended conjugative double bond is between 2 and 4. Comparision of the calculated values of $\lambda_{max}^{Et_2O}$

1) Tsuda, *et al.*: J. Pharm. Soc. Japan, 71, 282 (1951) (C.A., 45, 8151 (1951)); Hafez: Nature, 165, 401 (1950); Tsuda, *et al.*: J. Pharm. Soc. Japan, 71, 275 (1951) (C.A., 45, 8150 (1951)); Ottke, Bergmann: Nature, 166, 997 (1950).
2) Tsuda, *et al.*: J. Pharm. Soc. Japan, 72, 182 (1952) (C.A., 47, 2190 (1953)).
3) Part I: This Bulletin, 1, 212 (1953).
4) E. N. Squire: J. Am. Chem. Soc., 73, 2586 (1951).
5) L. Fieser, M. Fieser: "Natural Products Related to Phenanthrene," 3rd Ed., p. 185 (1949).

352 and 369 mμ of bicholestatriene A with those of 3,3′-bis(3,5-cholestadiene) shows that the former values coincide to the value obtained by the addition of 60 mμ to the latter within 4 mμ error. This fact indicates that the two extended conjugative double bonds have been introduced into the conjugated system and, consequently, the six double bonds in bicholestatriene may be assumed to be wholly conjugated. When the position of the bonding is tentatively assumed as at 3,3′ or 4,4′, the formulae A and B (Fig. 1) can be proposed.

Squire carried out high pressure hydrogenation of 3,3′-bis(3,5-cholestadiene) and derived it to 3,3′-bicholestanyl proving it with that obtained by a different method. If the structure of bicholestatriene A is that shown by formula A, exhaustive hydrogenation should form 3,3′-bicholestanyl (VII) which should coincide with that prepared by Squire. The exhaustive hydrogenation of a compound of formula B under such conditions that no transition of Δ^4 would take place should yield 4,4′-bicoprostanyl (VII′), or if the transition of Δ^4 to Δ^5 does take place, 4,4′-bicholestanyl should be formed. In either case, substances different from 3,3′-bicholestanyl obtained by Squire would be formed.

Fig. 1. Assumed Structural Formula for Bicholestatriene A and Various Derivatives to be formed by its Partial and Exhaustive Hydrogenation.



Partial hydrogenation should not result in any difference of the product from A or B formula by ultraviolet spectral analysis. About two moles of hydrogen absorption should result in the interruption of the conjugated polyene system and the appearance of the absorption of conjugated diene system. Disappearance of the conjugated system could be expected by approximately 4 moles of hydrogenation, and the three kinds of double bonds in connection with C_8-position of the sterol nucleus are assumed to remain without re-

duction. From these considerations, five kinds of reaction conditions were chosen, as iterated below.

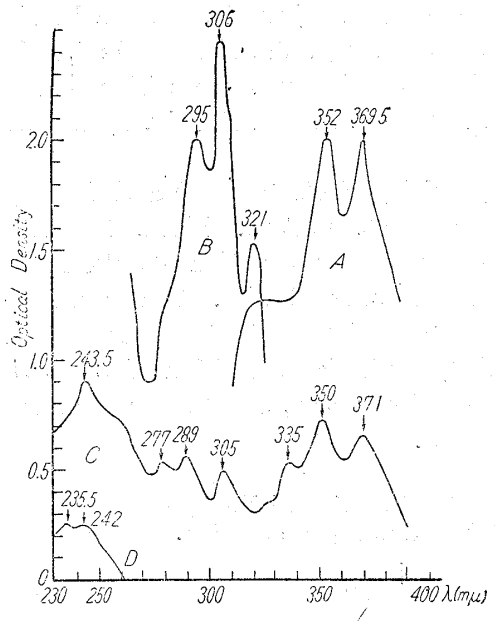


Fig. 2. Partial Catalytic Reduction of Bicholestatriene A.
A—Bicholestatriene A (0.005% ether solution)
B—3,3′-Bis(3,5-cholestadiene) (cyclohexane)
C—ca. 2 mol. Hydrogen absorbed
D—ca. 4 mol. Hydrogen absorbed.

The first was the acetic acid catalytic hydrogenation in organic solvent, at room temperature, allowing absorption of approximately two moles of hydrogen. Curve C, in Fig. 2, shows the ultraviolet absorption spectrum of the reduction product in 0.005% ether solution and Curve A is the absorption spectrum of bicholestatriene A determined under the same conditions. The comparison of these curves will show that the reduction did not proceed selectively but the expected absorption of the diene system, (I) and (I′), $\lambda_{max}^{Et_2O}$ 243.5 mμ has appeared in comparatively strong intensity, showing that the hydrogenation of Δ^5 in Formula A and Δ^3 in Formula B had been effected. Other maxima, 277 and 289 mμ, give rise to the presence of (II) and (II′) in the conjugated system, in which $\Delta^3-\Delta^{3'}$ in Formula A and $\Delta^4-\Delta^{4'}$ in Formula B, had been reduced. This absorption maximum at 306 mμ may also be related to (III) and (III′), in which the double bond in connection with Δ^8 had been reduced, but this is not very clear. Some of the starting material that failed to be hydrogenated may have been occluded.

In the second condition, the hydrogenation was allowed to proceed until absorption of approximately four moles of hydrogen, under the same conditions as in the first. Curve D in Fig. 2 is that of this product in which the absorption of conjugated diene system has practically disappeared.

In the third condition, exhaustive hydrogenation was carried out at room temperature for 10 hours, in organic solvent with acetic acid. Absorption of hydrogen continued, although in extremely slow rate, even after absorption of 4 moles. By the utilization of the difference of solubility in acetone, some crystals of m.p. 220~225°, assumed to be 3,3′-bicholesteryl (V) or 3,3′-bicoprosteryl (V′), and a hydrocarbon, m.p. 76~78°, assumed to be (VI) or (VI′), were obtained as the products. No final determination as to their structures has yet been made. Neither of the products show ultraviolet absorption.

The fact that a hydrocarbon is formed by catalytic reduction indicates that exhaustive hydrogenation under more drastic conditions will probably afford cholestane (VII′) as well as bicholestanyl (VII). Moreover, if ca. 4 moles of hydrogen is allowed to be absorbed by conditions such as would not allow transition of the double bond at Δ^4 (Fig. 1, V′)[6], then by exhaustive hydrogenation carried out in acidity at 70°, coprostane (VIII′) should be formed as well as bicoprostanyl (VIII′) (Fifth Method of Catalytic Reduction).

In the fourth condition, the substance was dissolved in a 1:1 mixture of butyl ethyl ether and glacial acetic acid, and hydrogenated exhaustively for 5 hours at 70°. Bicholestatriene A and the substance of m.p. 220~225° obtained by the third method both yielded the same bicholestanyl (VII). Cholestane (VIII) was isolated from the acetone-soluble portion.

In the fifth condition, approximately 4 moles of hydrogen was allowed to be absorbed by neutral reduction and this was then treated under the same conditions as in the fourth

6) L. Fieser, M. Fieser: "Natural Products Related to Phenanthrene," 3rd Ed., 121 (1949).

method. Only bicholestanyl (VII) and cholestane (VIII) were obtained. (VII) showed no depression of the melting point on admixture with 3,3′-bicholestanyl, prepared by the Grignard reaction of 3β-chlorocholestane. Both gave a melting point of 270~300° with decomposition, and no optical rotation. Cholestane (VIII) was also admixed with a sample synthesized by a different method and showed no depression of the melting point. The fact that coprostane was not formed by these reductions may be taken as a proof of $\Delta^{3,5,7}$-structure.

From the fact that 3,3′-bicholestanyl is formed by the exhaustive hydrogenation, the position of the bonding was assumed to be at 3 and 3′, and by the ultraviolet spectral analysis of partially hydrogenated product and non-formation of coprostane, $\Delta^{3,5,7}$-structure was assumed, from which bicholestatriene A probably corresponds to 3,3′-bis(3,5,7-cholestatriene).

The authors take this opportunity in expressing their deep gratitude to Prof. K. Tsuda of the University of Kyushu, Mr. Sudo, Director of the Shinagawa Plant of this firm, and Mr. Matsui, the Vice-Director of the same, for their kind and unfailing guidance throughout the course of this work. The authors' thanks are also due Messrs. Hirai, Kawamoto, Furukawa, and Maruyama for spectral measurement and elemental analyses.

Experimental

1) Acid catalytic reduction of bicholestatriene A (Absorption of ca. 2 moles hydrogen) (Fig. 2 C)—The catalyst was prepared from 250 mg. of platinum oxide in a mixture of 800 cc. of dehydrated ether and 100 cc. of purified glacial acetic acid in a 1000-cc. reduction vessel. To this was added 500 mg. of the yellow crystals of bicholestatriene A and hydrogenation carried out. Approximately 2 moles of hydrogen (ca. 30 cc.) was absorbed rapidly and the yellow color disappeared from the solution. The reduction was stopped at this juncture, the reaction mixture was filtered, and the filtrate was concentrated. Glacial acetic acid was removed under a diminished pressure until the whole was evaporated. The pale yellow residue was recrystallized from a mixture of chloroform and acetone and 400 mg. of pale yellow needles, m.p. 200~220°, apparently a mixture of substances, were obtained (cf. the main text and Fig. 2C).

2) Acid catalytic reduction of bicholestatriene A (Absorption of ca. 4 moles hydrogen)—The hydrogenation was carried out as in the above method (1), allowing approximately 4 moles of hydrogen to be absorbed, and treated as in the foregoing to 300 mg. of needles, m.p. 210~215° (cf. Fig. 2D).

3) Exhaustive acid hydrogenation of bicholestatriene A—The catalytic reduction was carried out under the same conditions as in (1) until approximately 4 moles of hydrogen was absorbed and the vessel was then shaken for about 10 more hours, during which hydrogen was absorbed slowly but surely. The reaction mixture was filtered, the filtrate evaporated to dryness, and the residue was extracted with acetone. Cooling of the acetone extract separated about 50 mg. of needles, m.p. 78~80°. *Anal.* Calcd. for $C_{27}H_{46}$: C, 87.70; H, 12.18. Found: C, 87.42; H, 12.07.

The acetone-insoluble portion was recrystallized from a mixture of chloroform and acetone to 200 mg. of white needles, m.p. 220~225° *Anal.* Calcd. for $C_{54}H_{90}$; C, 87.70; H, 12.18. Found: C, 87.27; H, 12.22.

4) Neutral hydrogenation followed by exhaustive acid reduction—The catalyst was prepared in the usual manner from 250 mg. of platinum oxide in 150 cc. of butyl ethyl ether contained in a 300-cc. reduction vessel. To this was added 500 mg. of bicholestatriene A which absorbed approximately 4 moles of hydrogen during about 1 hour and the reaction mixture showed a faint greenish yellow fluorescence. To this mixture was added 150 cc. of glacial acetic acid, irradiated with a 350-watt infrared lamp to maintain the inner temperature at 70°, and the hydrogenation was carried on for another 5 hours. The absorption of 2 moles of hydrogen during this period was not well observed. The reaction mixture was filtered, the filtrate evaporated under a reduced pressure, and the residue was extracted with hot acetone. Cooling of the acetone extract yielded 20 mg. of needles, m.p. 184°. This substance was found to have two sterol nuclei from the result of molecular weight determination. *Anal.* Calcd. for $C_{54}H_{94}$: C, 87.26; H, 12.74. Found: C, 87.36; H, 12.49; Mol. Wt., 729.9.

The structure of this substance is still unknown.

Concentration of the acetone mother liquor provided 100 mg. of needles, m.p. 80°. *Anal.* Calcd. for $C_{27}H_{48}$: C, 87.09; H, 12.90. Found: C, 87.20; H, 12.90. No depression of the melting point on admixture with cholestane.

The portion insoluble in hot acetone was recrystallized from a mixture of chloroform and

acetone to 200 mg. of crystals melting with decomposition at 270~300°. *Anal.* Calcd. for $C_{54}H_{94}$: C, 87.26; H, 12.74. Found: C, 87.21; H, 12.27. $[\alpha]_D^{20}$: ±0° (15 mg. of the substance dissolved in 5 cc. $CHCl_3$). (This substance coincides with bicholestanyl obtained by Squire). The same results are obtained when bicholestatriene A is exhaustively hydrogenated in acid solution at 70° from the beginning.

The crystals of m.p. 220~225°, obtained by the exhaustive hydrogenation of bicholestatriene A in acid solution at a room temperature for 10 hours, in acid solution at 70°, also gave the bicholestanyl, m.p. 270~300° (decomp.), of Squire's. Cholestane could not be isolated from 100 mg. of the material.

Summary

Partial catalytic reduction of the yellow hydrocarbon, bicholestatriene A, $C_{54}H_{82}$, was carried out. From the results of the ultraviolet absorption spectral analyses of the reduction product and from the fact that the exhaustive hydrogenation derived it to the structurally known 3,3′-bicholestanyl, the structure of the hydrocarbon was assumed to be 3,3′-bis(3,5,7-cholestatriene), with the bonding at 3 and 3′ and the double bonds at 3, 5, and 7.

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52. Ko Arima: Studies on Cholestapolyenes. III. Synthesis of 3,3′-Bis-(3, 5, 7-cholestatriene) (Bicholestatriene A).

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In the previous report[1], the bonding position of bicholestatriene A was assumed to be at 3 and 3′, and the distribution of the double bond as $\Delta^{3,5,7}$, from the ultraviolet spectral analysis of the partial catalytic reduction product of the bicholestatriene and by the derivation of the compound to a known substance by an exhaustive hydrogenation. In the present series of experiments, based on these assumptions, 3,3′-bis(3, 5, 7-cholestatriene) was systematically synthesized and this was found to be identical with bicholestatriene A, details of which are herein reported.

For the method of bonding the sterol nuclei at 3 and 3′, there are the Wurz and Grignard reactions of 3-halogeno compound, and the reduction of 3-keto compound with sodium amalgam to 3-ketopinacol with subsequent dehydration[2], but for the preparation of a compound of bitriene series, the latter method of passing through 3-ketopinacol compound is more useful.

The synthesis of 3,3′-bis(3, 5, 7-cholestatriene)(XII) was attempted by the Oppenauer oxidation of 7α-benzoxycholesterol (III) and 7β-methoxycholesterol (VIII) to 7α-benzoxycholest-4-en-3-one (IV) and 7β-methoxycholest-4-en-3-one (IX), respectively, and then follow the methods of Routes I and II in Fig. 1.

Route I: According to the method of Fieser[3], 7-ketocholesteryl acetate was prepared whose reduction with lithium aluminum hydride gave a mixture of 3, 7-diol isomers which was derived, without isolation, to 3, 7-dibenzoate with benzoyl chloride and pyridine. Recrystallization of the dibenzoate from methanol resulted in the separation of 3β, 7α-dibenzoate (II) first as crystals of m.p. 169~171°. Then, by the method of Wintersteiner[4], only the C_3 portion alone was partially saponified with sodium ethoxide from which 7α-benzoxycholesterol (III), m.p. 110~115°, was obtained in a good yield. Oppenauer oxidation

1) Part II: This Bulletin, 1, 216 (1953).
2) Squire: J. Am. Chem. Soc., 73, 2586 (1951).
3) L. Fieser: *Ibid.*, 71, 2226 (1949).
4) Wintersteiner: *Ibid.*, 64, 1177 (1942).