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.

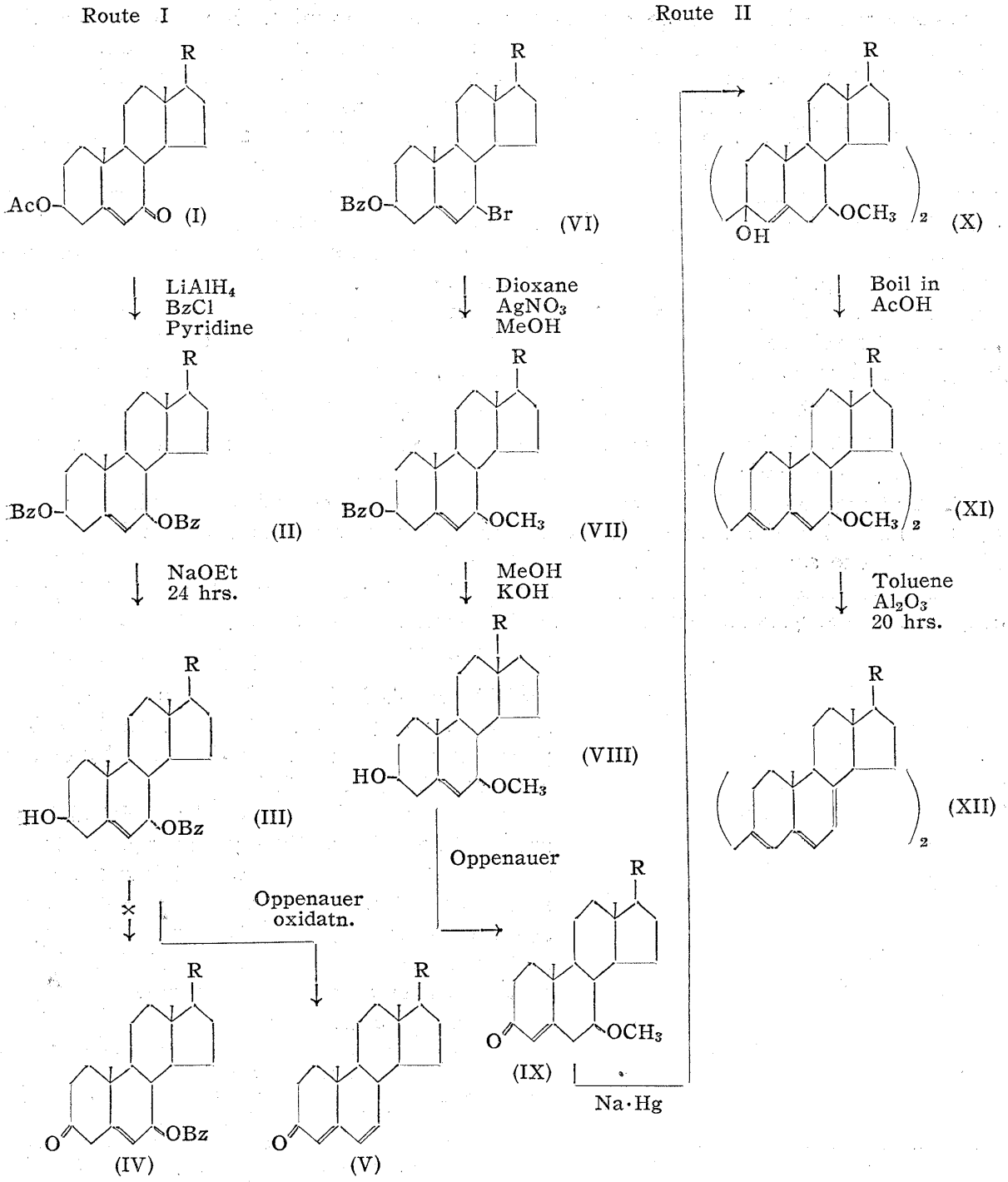
For the method of bonding the sterol nuclei at 3 and 3′, there are the Wurz and Grignard reactions of 3-halogeno compound, and the reduction of 3-keto compound with sodium amalgam to 3-ketopinacol with subsequent dehydration[2], but for the preparation of a compound of bitriene series, the latter method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

Fig. 1. Synthetic Confirmation of Bicholestatriene A.



of (III) failed to give the expected 7α-benzoxycholest-4-en-3-one (IV) and only cholest-4, 6-dien-3-one (V) was formed, the latter having been confirmed by deriving the oily reaction product to a semicarbazone whose analytical value for nitrogen having agreed with that of the semicarbazone of (V). Even under a much milder conditions, such as gently boiling (III) in ether, with acetone and aluminum isopropoxide for 24 hours, resulted in the liberation of the polar bond at C_7.

It was assumed that when C_7 is in an equatorial bonding, the 7β-benzoxyl group would remain but, lacking any data for partial saponification, the method of Route II using 7β-methoxycholesterol was carried out.

Treatment of 7β-bromocholesteryl benzoate (VI) with aqueous solution of silver nitrate in dioxane resulted in immediate precipitation of silver bromide. Removal of the salt by filtration and evaporation of the filtrate under a reduced pressure yielded an oil. Warming of this oil with methanol yielded 7β-methoxycholesteryl benzoate (VII), m.p. 135°, in approximately 30% yield. Saponification by boiling with methanolic potash gave 7β-methoxycholesterol (IX), m.p. 158~159°, which was boiled in dehydrated benzene with dehydrated acetone and aluminum isopropoxide (existing data call for aluminum *tert*-butoxide) for Oppenauer oxidation and 7β-methoxycholest-4-en-3-one (IX), m.p. 143°, and cholest-4,6-dien-3-one (IV), were obtained in approximately 50% and 30% yield, respectively[5].

(IX) was then reduced by the Squire's method, with sodium amalgam, and, some needle crystals of m.p. 209~211° (decomp.) were obtained whose analytical values agreed well with those of 7β-methoxycholest-4-en-3-one pinacol (X). Boiling (X) as a suspension in glacial acetic acid for two hours resulted in the separation of yellow crystals, which, after being washed with a mixture of acetone and ether, melted at 240~250° with decomposition. The ultraviolet absorption of this substance were $\lambda_{max}^{Et_2O}$ 292, 306, 321, 364, and 377 mμ, which were identical with those of 3,3′-bis(3,5-cholestadiene) and proving the formation of 3,3′-bis(7,7′-methoxy-3,4-cholestadiene) (XI) (cf. Fig. 2).

The absorption maxima at 364 and 377 mμ show the occlusion of a substance formed by the liberation of 7,7′β-methoxyl group and the foregoing reaction was found to give bicholestatriene as a by-product.

(XI) was heated with alumina in toluene at 100° for 20 hours, filtered, and the filtrate evaporated. Recrystallization of the residue from benzene gave yellow needle crystals, m.p. 242~244° (decomp.), $\lambda_{max}^{Et_2O}$ 351 and 368 mμ; log ε=4.462 and 4.462. From the course of the present synthesis, the positions of the double bond in the yellow needles of m.p. 242~244° hereby obtained, seem certain to be at 3, 5, 7.

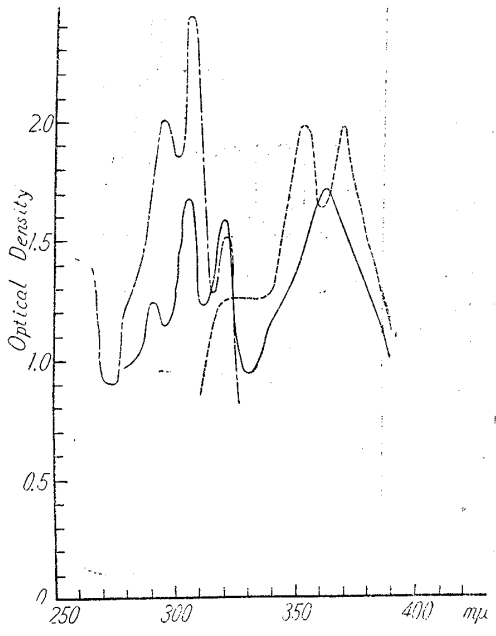


Fig. 2. Crude 3,3′-bis(7,7′β-methoxy-3,5-cholestatriene)
—— Crude 3,3′-bis(7,7′β-methoxy-3,5-cholestadiene) (0.005% Et_2O)
—·— 3,3′-Bis(3,5-cholestadiene) (0.005% cyclohexane)
..... 3,3-Bis(3,5,7-cholestatriene) (Bicholestatriene A) (0.005% ether)

On the other hand, the ultraviolet absorption and melting point of these yellow crystals are identical with those of the bicholestatriene from which it seems practically certain that the substance designated tentatively as bicholestatriene A by the author possesses the structure of 3,3′-bis(3,5,7-cholestatriene) as previously assumed. This substance has an antirachtic action[6].

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Experimental

1) **Formation of cholest-4,6-dien-3-one**—To a solution of 1 g. of (III) dissolved in 50 cc. of dehydrated ether, 2 g. of aluminum isopropoxide and 12 g. of acetone were added and gently boiled under reflux for 24 hours. When cooled, the ether layer was washed first

5) H. B. Henhest, E. R. H. Jones: J. Chem. Soc., 1798 (1949).
6) Paper read before the General Meeting of Vitamin Society (May, 1953) in Nagoya by Yutaka Wakao of the National Nutrition Institute.

with dil. sulfuric acid and then with sodium carbonate solution, dried over calcium chloride, and filtered. The filtrate was evaporated to dryness under a reduced pressure, and the residual brown oil, which failed to crystallize, was dissolved in 20 cc. of pyridine, excess of semicarbazide hydrochloride added, and heated at 100° for 2 hours. The insoluble matter that separated out on the addition of water was collected by filtration, washed with water, and recrystallized from a mixture of chloroform and acetone to sandy crystals of m.p. 240~243° (decomp.), which were sparingly soluble in organic solvents. Yield, 600 mg. The analytical values of this substance were identical with those of the semicarbazone of 4,6-cholestadienone, possessing no benzoyl group in C_7. *Anal.* Calcd. for $C_{35}H_{52}O_3N_3$ (7β-Benzoxycholest-4-en-3-one semicarbazone): C, 74.70; H, 9.25; N, 7.48. Calcd. for $C_{28}H_{45}ON_3$: (Cholest-4,6-dien-3-one semicarbazone): C, 76.60; H, 10.25; N, 9.56. Found: (I) C, 75.47; H, 10.22; N, 9.76. (II) 9.76.

Since the analytical value for nitrogen is in good agreement, this compound must be 4,6-cholestadienone and it became certain that 7β-benzoxycholest-4-en-3-one had not been formed.

2) The course of syntheses from (VI) to (VIII), shown in Fig. 1, was carried out as given in the literature of Henbest and Jones[5].

3) 7β-Methoxycholest-4-en-3-one pinacol (X)—A solution obtained by dissolving 4 g. of (IX) in an equimolar mixture of isopropanol and glacial acetic acid was vigorously stirred and 600 g. of freshly prepared 2% sodium amalgam was added in small portions during 1.5 hours, the rate of addition being adjusted so that the reaction mixture refluxed towards the end. The reaction is exothermic and there is no necessity of warming. The whole reaction mixture was poured into 1 L. of water and the crystals that floated were collected by filtration. After washing with water, the crystals were dissolved in 100 cc. of benzene, dried over calcium chloride, filtered, and evaporated to 25 cc. Addition of 25 cc. of hot acetone and standing resulted in the separation of crystals of m.p. 209~211°. Yield, 3.1 g. *Anal.* Calcd. for $C_{56}H_{94}O_4$: C, 80.73; H, 11.46. Found: C, 81.23; H. 11.56.

4) **3,3′-Bis(7,7′β-methoxy-3,5-cholestadiene)(XI)**—Refluxing of 3 g. of the pinacol (X) with 100 cc. of glacial acetic acid for 2 hours resulted in its change to yellow crystals. When cooled, the crystals were collected by filtration, washed with a mixture of acetone and ether, and showed m.p. 240~250°. These were used for the next demethoxylation without further purification (cf. main text for ultraviolet absorption data).

5) **3,3′-Bis(3,5,7-cholestatriene)(XII)**—A mixture of 1 g. of (XI) and 5 g. of alumina in 100 cc. of toluene was refluxed for 20 hours. The cooler and the flask were tightly bound so that they would not separate on sudden ebullition. When cooled, the mixture was filtered, and the solvent removed by which approximately 600 mg. of yellow substance was obtained. Recrystallization from benzene yielded 200 mg. of crystals that melted at 242~244° with decomposition. $\lambda_{max}^{Et_2O}$ 351 and 368 mμ, log ε=4.462 and 4.462. *Anal.* Calcd. for $C_{54}H_{82}$: C, 88.76; H, 11.23. Found: C, 88.25; H, 10.94.

Summary

Based on the assumption drawn from catalytic reduction in the previous report, systematic synthesis of 3,3′-bis(3,5,7-cholestatriene) was carried out and bicholestatriene A was found almost certain to be the same substance as that hereby synthesized. This substance possesses an antirachtic activity.

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