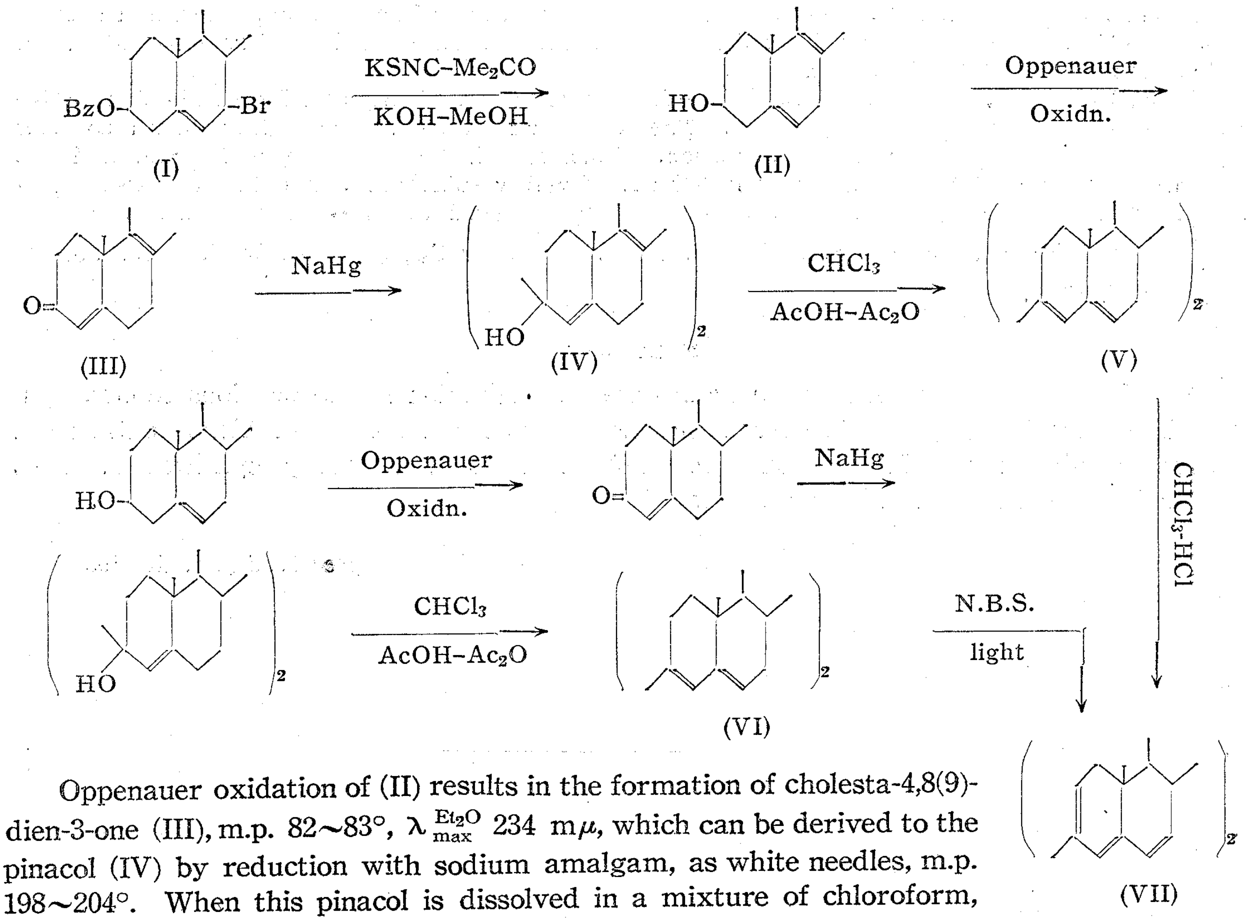
53. Ko Arima: Studies on Cholestapolyenes. IV. Synthesis of Bicholestatriene C.

(*Takamine Research Laboratory, Sankyo Co., Ltd.*)

Systematic synthesis of 3,3′-bis(3,5,7-cholestatriene) was reported in the previous paper[1]. A series of experiments were then carried out for the same purpose using, as the First Route method, non-conjugated dienol, cholesta-5,8(9)-dien-3-ol, as the starting material, and the Second Route of brominating 3,3′-bis(3,5-cholestadiene) with N-bromosuccinimide followed by dehydrobromination. In both cases, however, the objective 3,3′-bis(3,5,7-cholestatriene) could not be obtained and only a bicholestatriene of orange red color was the product from both methods. This compound was tentatively designated as bicholestatriene C.

Dehydrobromination of 7β-bromocholesteryl benzoate (I) sometimes fails to form 7-dehydrocholesteryl benzoate, only yielding the benzoate, m.p. 148~150°, of non-conjugated dienol (II). For example, reaction of (I) with thiocyanate in acetone, at a room temperature, results in the sole formation of (II) whose saponification with methanolic potash gives needle crystals of m.p. 147~148°, not exhibiting any ultraviolet absorption maximum. Since Tsuda and others[2] have shown that the positions of the double bonds in this dienol are at $\Delta^{5(6)}$ and $\Delta^{8(9)}$, the synthesis of 3,3′-bicholestatriene was attempted by the Squire's method[3], using (II) as the starting material.

Fig. 1. Synthesis of Bicholestatrine C



Oppenauer oxidation of (II) results in the formation of cholesta-4,8(9)-dien-3-one (III), m.p. 82~83°, $\lambda_{\max}^{\mathrm{Et_2O}}$ 234 mμ, which can be derived to the pinacol (IV) by reduction with sodium amalgam, as white needles, m.p. 198~204°. When this pinacol is dissolved in a mixture of chloroform,

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[1] Part III: This Bulletin, 1, 220 (1953).
[2] Unpublished.
[3] Squire: J. Am. Chem. Soc., 73, 2586 (1951).

glacial acetic acid, and acetic anhydride and refluxed, dehydration occurs and a bicholestatriene is formed as white needles, m.p. 245~246°. Since it shows an ultraviolet absorption maxima at 306, 315, and 322 mμ, the same as those of Squire's 3,3′-bis(3,5-cholestadiene), the compound was assumed to be 3,3′-bis(3,5,8(9)-cholestatriene)(V).

This fact forms one of the bases for proving that the double bond at C_8 in the non-conjugated dienol used as the starting material is not in $\Delta^{7(8)}$. If the latter configuration is correct and, consequently, the other double bond is in $\Delta^{4(5)}$, the product from the foregoing reaction should show ultraviolet absorption maxima at 352 and 369 mμ, identical with those of 3,3′-bis(3,5,7-cholestatriene), but such absorptions were not observed at all.

Saturation of dry hydrogen chloride in chloroform solution of (IV) and allowing this solution to stand at room temperature for 24 hours, resulted in coloration of the solution to red. Removal of the solvent under a reduced pressure and recrystallization of the residue from a mixture of benzene and acetone yielded orange red needles, m.p. 234~240°(decomp.). The analytical values of this compound agreed with those of bicholestatriene and its ultraviolet absorption maxima were: $\lambda_{\max}^{Et_2O}$ 306, 322, 368, 370, and 390 mμ; $\lambda_{\max}^{\text{cyclohexane}}$ 307, 320, 379, and 394 mμ. It is seen that the ultraviolet absorption has shifted to a longer wave length range compared to 3,3′-bis(3,5,7-cholestatriene). Since this compound is comparatively easily soluble in ether and is reddish, it is thought to be a bicholestatriene and a tentative designation of bicholestatriene C is given to it.

If there occurred only the transition of $\Delta^{8(9)}$ to $\Delta^{7(8)}$ by dry hydrogen chloride, 3,3′-bis(3,5,7-cholestatriene) should have been formed. The bonding position is certain to be at 3 and 3′, so that no other conjugated system but $\Delta^{2,4,6}$ can be assumed. However, this is inconsistent with known facts that in cholestadienes, the configuration of $\Delta^{2,4}$ is so unstable that it transits to $\Delta^{3,5}$ configuration by hydrogen chloride[4].

Since the object of the present experiments was the preparation of 3,3′-bis(3,5,7-cholestatriene), attempt was made for the Third Route of synthesis. Two moles of N-bromosuccinimide was added to the carbon tetrachloride solution of 3,3′-bis(3,5-cholestadiene) and the mixture was irradiated with two 375-watt infrared lamps by which the reaction started and the solution turned red color, with succinimide floating on the surface. After about 15 minutes, when the reaction was completed, the solvent was removed by distillation and the residue was recrystallized from a mixture of benzene and acetone to crystals which gave identical melting points, analytical values, and absorption maxima as those of the bicholestatriene crystals obtained by the first method. The dibromo compound, assumed to be formed as an intermediate, could not be isolated due probably to its unstability.

The bromination may possibly occur at C_7 and C_2. It is assumed that the bromine atom which entered C_7 had undergone a dehydrobromination with the hydrogen at C_6 forming $\Delta^{6(7)}$, and resulted in the transition of the double bond of $\Delta^{3,5}$ to $\Delta^{2,4}$. The bromine atom, if it enters C_2, would undergo dehydrobromination with the hydrogen at C_1 and subsequently form 3,3′-bis(1,3,5-cholestatriene) but the formation of such a bistriene cannot be assumed from the first route reactions. It seems more likely, therefore, that the bromination occurred only at C_7 and not at C_2. It may also be that $\Delta^{1,3,5}$ had undergone transition to $\Delta^{2,4,6}$, but there are so many vague and obscure points that these are all mere assumptions.

The fact that the ultraviolet absorption has shifted to a longer wave length range as compared to those of 3,3′-bis(3,5,7-cholestatriene) may be explained by assuming cross-conjugation of two cholestatriene systems and a diene system but this point will also have to be confirmed by further studies.

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[4] Stavely, Bergmann: J. Org. Chem., 1, 567, 575(1937); Bergmann, Hismann: *Ibid.*, 4, 401(1940).

Vice-Director of the same, for their kind guidance and advices. Grateful acknowledgement is made to Messrs. Hirai, Kawamoto, Furukawa, and Maruyama for analysis and spectrography.

Experimental

1) **Preparation of cholesta-5,8(9)-dienol (I)**—To a suspension of 10 g. of 7β-bromocholesteryl benzoate⁵⁾ in 200 cc. of dehydrated acetone, stirred at a room temperature, an excess of ammonium thiocyanate was added and the mixture was stirred for about 30 minutes. The silky crystalline mass thereby formed was collected by suctional filtration, washed with water, and recrystallized from a mixture of chloroform and methanol to scaly crystals of m.p. 146∼148° (becoming clear at 180∼186°). After recrystallization from ether, the crystals melted at 148∼150° (becoming clear at 180∼186°). Saponification with 5% methanolic potassium hydroxide by boiling and recrystallization of the crystals that separated out on cooling yielded needle crystals of m.p. 145∼147°. Yield, approx. 5 g. *Anal.* Calcd. for $C_{34}H_{48}O_2$ (Benzoate): C, 83.54; H, 9.91. Found: C, 83.82; H, 10.23. *Anal.* Calcd. for $C_{27}H_{44}O$ (Dienol); C, 83.85; H, 11.45. Found: C, 84.10; H, 11.22.

2) **Preparation of cholesta-4,8(9)-dien-3-one (II)**—To a solution of 5 g. of (I) dissolved in 150 cc. of dehydrated benzene, 10 g. of aluminum isopropoxide and 60 g. of dehydrated acetone were added, and the mixture was boiled with reflux for 10 hours. When cool, the reaction mixture was transferred to a separatory funnel, washed several times with diluted sulfuric acid, then consecutively with water, diluted sodium carbonate solution, and water, and dried over calcium chloride over night. The solvent was removed by distillation at a reduced pressure, 100 cc. of acetonitrile added to the residue, and the wall of the vessel was scratched with a glass rod by which the oily residue crystallized. There crystals were collected by filtration and recrystallized from methanol to colorless needles, m.p. 76∼78°. Repeated recrystallization from methanol raised the melting point to 80∼81°. Ultraviolet absorption, $\lambda_{\max}^{Et_2O}$ 248 mμ, log ε=3.995, and an extremely faint one at $\lambda_{\max}^{Et_2O}$ 279 mμ, log ε=0.72, showing occlusion of a minute amount of cholesta-4,6-dien-3-one. Yield, 3 g. *Anal.* Calcd. for $C_{27}H_{42}O$: C, 84.82; H, 11.00. Found; C, 85.0; H, 11.31.

3) **Cholesta-4,8(9)-dien-3-one pinacol (III)**—The method of Squire²⁾ was followed. To a solution of 2 g. of (II), m.p. 78∼80°, dissolved in 150 cc. of 1:1 mixture of isopropanol and purified glacial acetic acid, while being stirred vigorously, 300 g. of freshly prepared 2% sodium amalgam was added in small portions, during 1.5 hours. The temperature of the reaction mixture gradually increased until the mixture began to reflux gently towards the end. This reaction mixture was poured into 600 cc. of water and the crystals that gathered on an upper layer were collected by filtration. After washing with water, the crystals were dissolved in 100 cc. of benzene, dried over sodium sulfate, filtered, and the filtrate was evaporated to 25 cc. Addition of the same amount of hot acetone and standing separated crystals of m.p. 198∼204°. Yield, 70%. *Anal.* Calcd. for $C_{54}H_{88}O_2$: C, 84.37; H, 11.45. Found: C, 84.71; H, 12.23.

Under some conditions, dehydration occurred during the reduction and about one-half the amount of 3,3′-bis(3,5,8(9)-cholestatriene) was formed.

4) **Preparation of 3,3′-bis(3,5,8(9)-cholestatriene) (IV)**—One g. of (III), m.p. 197∼200°, was refluxed for 1 hour in a mixture of 40 cc. chloroform, 2 cc. glacial acetic acid, and 2 cc. acetic anhydride. After 3 hours, sparingly soluble (IV) began to separate out as long needle crystals and the boiling was continued for further 1 hour. The mixture was filtered when cool and white needle crystals, m.p. 245∼250°(decomp.), were obtained in an 80% yield. Ultraviolet absorption maxima: $\lambda_{\max}^{\text{cyclohexane}}$ 294, 306, and 321 mμ; log ε=4.46, 4.54, and 4.33. *Anal.* Calcd. for $C_{54}H_{82}$: C, 88.76; H, 11.23. Found; C, 88.54; H, 11.42.

5) **Preparation of bicholestatriene C (3,3′-bis(2,4,6-cholestatriene))**—i) Dry hydrogen chloride gas was saturated in the cold solution of 300 mg. of 3,3′-bis(3,5,8(9)-cholestatriene) (IV) dissolved in 100 cc. of chloroform and the mixture was allowed to stand at a room temperature for 24 hours by which the colorless solution gradually turned red color. Chloroform was removed by low-pressure distillation and the residue was recrystallized from a mixture of benzene and acetone to orange red needles, m.p. 240∼245° (decomp.). Ultraviolet absorption: $\lambda_{\max}^{Et_2O}$ 306, 322, 368, 370, and 390 mμ, log ε=4.31, 3.37, 4.59, 4.57, 4.59; $\lambda_{\max}^{\text{cyclohexane}}$ 307, 320, 379, and 394 mμ, log ε=3.99, 4.14, 4.40, and 4.44. *Anal.* Calcd. for $C_{54}H_{82}$: C, 88.76; H, 11.23. Found: C, 88.39; H, 11.02.

ii) To a solution of 200 mg. of 3,3′-bis(3,5-cholestadiene)⁵⁾ dissolved in 100 cc. of carbon tetrachloride, 100 mg. (2 moles) of N-bromosuccinimide was added and the mixture was irradiated with two 325-watt infrared lamps. After about 30 minutes, succinimide began to separate out and floated on the surface while the solution became red colored. The imide was removed by filtra-

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5) H. Schalteger: Helv. Chim. Acta, 29, 285 (1946).

tion and the filtrate was treated as in the foregoing (i) by which bicholestatriene C was obtained as orange crystals of m.p. 240~243° (decomp.). Ultraviolet absorption: $\lambda_{\max}^{Et_2O}$ 308, 326, 379, and 390 mμ, log ε=4.00, 4.06, 4.28, and 4.30. *Anal.* Calcd. for $C_{54}H_{82}$: C, 88.76; H, 11.23. Found: C, 88.20; H, 11.21.

Summary:

For the purpose of structural determination by the synthesis of bicholestatriene A (3,3'-bis(3,5,7-cholestatriene)), 3,3'-bis(3,5,8(9)-cholestatriene) was prepared from cholesta-4,8(9)-dien-3-one and transition of $\Delta^{8(9)}$ was attempted by dry hydrogen chloride. Unexpectedly, however, colored bicholestatriene C was the sole product. The same substance was also obtained when 3,3'-bis(3,5-cholestadiene) was brominated with N-bromosuccinimide. Bicholestatriene C thereby formed was assumed, from its ultraviolet absorption, as 3,3'-bis(2,4,6-cholestatriene) but much is still vague and obscure as to its structure.

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54. Ko Arima and Ryoichi Hayatsu: Studies on Cholestapolyenes. V. Synthesis of 3,3'-Bis(3,5,7-cholestatriene) and Bicholestatriene B.

(*Takamine Research Laboratory, Sankyo Co., Ltd.*)

The preparation of 3,3'-bis(3,5,7-cholestatriene) (II) was described in the first paper of this series⁽¹⁾ but a new method of such synthesis giving a better yield was found by the present experiments. At the same time, a new yellow bicholestatriene B was obtained as a by-product both of which are described in the present paper.

As shown in Fig. 1, both Hafez⁽²⁾ and Ottke and Bergmann⁽³⁾ reported that (II) was formed as a by-product during the bromination of Δ^{5}-isocholestadiene (1) and cholesteryl acetate (III) with N-bromosuccinimide. Tsuda and others⁽⁴⁾ also obtained (II) by the bromination of cholesteryl bromide (VI) and by an N-bromosuccinimide bromination of cholesteryl tosylate (IV). The present authors prepared (II) in a 6% yield by boiling hydroxycholesteryl benzoate (V) with glacial acetic acid⁽¹⁾ but its structure and reaction mechanism of its formation were totally unknown.

By subsequent experiments by Arima⁽⁵⁾, it became almost certain that this yellow steroidal hydrocarbon is 3,3'-bis(3,5,7-cholestatriene) but the reaction mechanism of its formation is still not clear unless the formation of a free radical of cholestatriene as an intermediate can be supposed. It may be assumed that the application of a dehydration agent, such as acids, to compounds that could form such a free radical, would result in the formation of (II). However, attempt at the preparation of a bischolestadiene by the heating of isocholestadiene with glacial acetic acid failed totally.

A new substance (VIII), m.p. 78~80°, yet unknown in literature, was obtained when 7β-bromocholesteryl tosylate (VII) was heated with fused potassium acetate in dehydrated methanol. Analytical values and ultraviolet absorption, $\lambda_{\max}^{Et_2O}$ 254 and 272 mμ, indicated that the new substance (VIII) might be a hydrocarbon of a probable structure of $\Delta^{1,8(9)}$ or $\Delta^{6,8(14)}$-isocholestatriene. During this reaction, a small amount of needle crystals, m.p.

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1) Part I: This Bulletin, 1, 212 (1953).
2) Hafez: Nature, 165, 401 (1950).
3) Ottke, Bergman: Nature, 166, 997 (1950).
4) Tsuda, *et al.*: J. Pharm. Soc. Japan, 71, 282(1951) (C. A., 45, 8151 (1951); *ibid.*, 71, 275(1951) (C. A., 45, 8150(1951)); *ibid.*, 72, 182(1952)(C. A. 47, 2190(1953)).
5) Part III: This Bulletin, 1, 220 (1953).