[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

THE CONFIGURATION OF CHOLESTEROL DIHALIDES

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The addition of halogen to the double bond of cholesterol can theoretically give rise to four stereoisomers, two of which possess the *cis* or coprostane skeleton and two the *trans* or cholestane arrangement. However, only one isomer has as yet been isolated from the direct bromination or chlorination of the free sterol.

On the basis of earlier observations that saturated 3-keto steroids with rings A and B fused in the *cis* position are brominated at C_4 and those with an A/B: *trans*-ring-union are brominated at C_2 (1), Butenandt and Schramm (2) and Inhoffen (3) showed that dibromocholestan-3-one, and hence dibromocholesterol, possesses a *cis* or coprostane structure. The validity of this work rests on the assumption that the same directive influences are present when hydrogen at C_5 is replaced by halogen. This assumption appears to be well-founded, since bromination of a 3-keto-cholestane-5,6(β)-diol, the configuration of whose hydroxyl group at C_5 is known (4), yields 2-bromo-3-ketocholestane-5,6(β)-diol (5).

In this connection it is interesting to note that Décombe and Rabinowitch (6) have advanced a *trans* or cholestane structure for dichlorocholesterol because it yields 3(β)-hydroxycholestane on catalytic hydrogenation. It must be noted, however, that these authors provide no evidence to support this assumption, and as will be shown later in this paper, this assignment of a *trans* configuration is incorrect.

That there is the possibility of the formation of isomeric dihalides of $\Delta^{5,6}$-sterols was first shown by Berg and Wallis (7), who obtained two isomeric dichlorides by the action of iodobenzene dichloride on certain $\Delta^{5,6}$-sterol esters. This phenomenon was observed with cholesteryl benzoate and *p*-toluenesulfonate, β-sitosteryl benzoate, and stigmasteryl benzoate.

In this paper we wish to describe the results of our investigations on the determination of the configurations of the two chlorine atoms at C_5 and C_6 of one of these dichloro esters, namely the lower-melting isomer of 3-benzoxy-5,6-dichlorocholestane (m.p. 120°). This compound on hydrolysis with alkali yields a 5,6-dichlorocholesterol identical with the cholesterol dichloride obtained in poor yield by direct chlorination of cholesterol. It is also identical with the cholesterol dichloride obtained in good yield by the action of iodobenzene dichloride on the free sterol. This dichlorocholesterol was oxidized to the corresponding 3-keto compound and treated with one mole of bromine in acetic acid solution. We submit the following evidence as indicative that the bromine atom in the resulting bromo compound is situated at C_4. Dehalogenation with zinc and acetic acid yields cholestene-4-one-3. Treatment with potassium acetate in boiling ethanol yields a compound which Inhoffen (3), who obtained it from the corresponding

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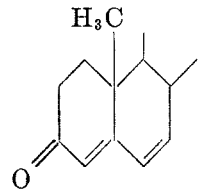
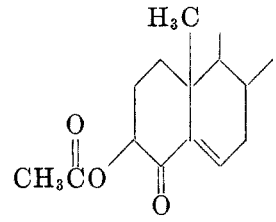
tribromo compound, has formulated as (I) but which has recently been shown by spectroscopic means to be (II).



I



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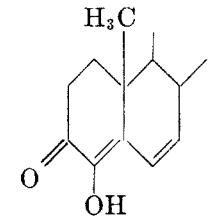


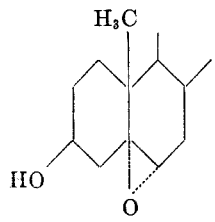
III

With hot hydrochloric acid (II) was converted to 4-hydroxy-4,6-cholestadiene-3-one (III). The products (II) and (III) which had previously been obtained from 4,5,6-tribromocholestan-3-one are identical with those which we prepared from bromodichlorocholestan-3-one.

It should be noted, however, that the stability of the bromodichlorocholestan-3-one is far greater than that of the corresponding tribromo compound, for whereas 4,5,6-tribromocholestan-3-one on heating with sodium iodide in ethanol yields the enol ethyl ether of 4-cholestene-3,6-dione, 4-bromo-5,6-dichlorocholestan-3-one under the same conditions is recovered largely unchanged.

Accordingly we must conclude that rings A and B in dichlorocholesterol are fused in the *cis* position in the same way as in dibromocholesterol and in cholesterol hydrochloride (8) and not in the *trans* position as assumed by Décombe and Rabinowitch (6).

Our next experiments were directed to the solution of the problem of determining the configuration of the halogen atom in the 6-position of the cholesterol dihalide molecule. To our knowledge no attempt has as yet been made to determine the stereochemical configuration of the halogen atom in this position in either dibromo- or dichloro-cholesterol. Any methods involving substitution of this halogen atom at C_6 are liable to doubt because of uncertainty in establishing whether *cis* or *trans* elimination has occurred. However, we have succeeded in determining the arrangement of this halogen atom in the following manner. When "α"-cholesterol oxide, which has been shown to be 5,6(α)-oxidocholestane-3-ol (IV) (4), is treated in the form of its acetate with aqueous dioxane at 150°, or with acetic acid, it forms the 3-acetoxy, or the 3,6-diacetoxy derivative respectively, of the so-called "trans triol" (9, 4), the configuration of which has been established as cholestane-3(β),5,6(β)-triol (V) (4, 5). Similar treatment of the 3-acetoxy or the 3-benzoxy derivatives of (IV) with hydrochloric acid yields a product which has been shown to possess a tertiary hydroxyl group and which on hydrolysis with quinoline or sodium carbonate again yields (IV) (9, 10, 11). This product is therefore the 3-acetoxy or the 3-benzoxy derivative of 6(β)-chlorocholestane-3,5(β)-diol (VI), inversion having as before occurred at C_6 (4). Treatment of the corresponding derivatives of "β"-cholesterol oxide (VII) with hydrochloric acid gives derivatives of 5-chlorocholestane-3(β),6(β)-diol (VIII) with inversion at C_5 (4, 12).



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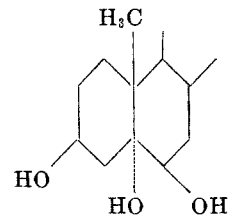

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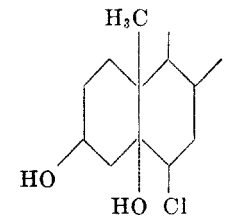

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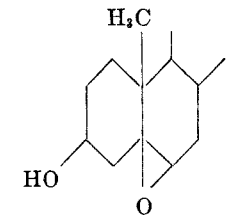
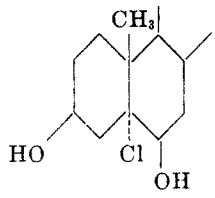
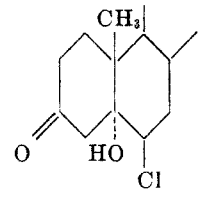


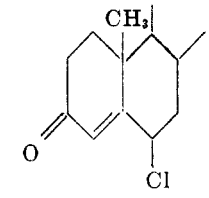
VII



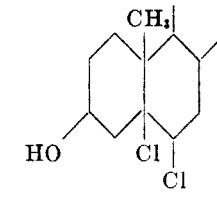
VIII



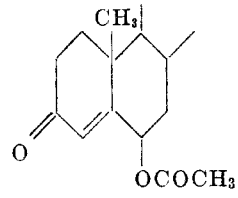
IX



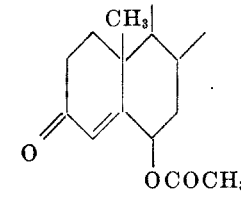
X



XI



XII



XIII

We have obtained (VI) by reacting the *free* sterol "α"–oxide with hydrochloric acid. This compound (VI) was oxidized with chromic acid to the corresponding 3-keto steroid (IX). Dehydration with thionyl chloride and pyridine gave 6(β)-chloro-4-cholestene-3-one (X). Dichlorocholesterol on oxidation to the ketone followed by dehydrochlorination with potassium acetate in ethanol yielded a product identical in optical rotation, ultra-violet absorption spectrum, melting point, and mixed melting point with this compound. Thus both chlorine atoms in the particular dichlorocholesterol under discussion are on the same side of the molecule and configurationally the compound has the structure (XI).

When both 6(β)-chloro-4-cholestene-3-one (X) and the corresponding 6-bromo compound, obtained by oxidation and dehydrobromination of dibromocholesterol (13), were allowed to react with acetate ions the same product was obtained. It possessed an ultra-violet absorption band similar to the starting materials and analyzed correctly for the corresponding acetate. However, it differs both in melting point and rotation from the known 6(β)-acetoxy-4-cholestene-3-one (XII). We believe that it is 6(α)-acetoxy-4-cholestene-3-one (XIII) which as yet has not been reported. The appearance of the same 6-acetoxy compound from both the 6-bromo and the 6-chloro compounds indicates that the stereochemical arrangement of the halogen atoms in both these steroids is the same. Accordingly, dibromocholesterol is correctly designated as 5,6(β)-dibromocoprostan-3-ol.

Experiments are now in progress designed to determine the configuration of the two halogen atoms in the higher-melting isomer of 3-benzoxy-5,6-dichlorochol-

estane (m.p. 251°), originally prepared by Berg and Wallis (7) by the action of iodobenzene dichloride on cholesteryl benzoate, and in the free sterol dichloride corresponding to this benzoate subsequently prepared by Décombe and Rabinowitch (14), by the action of the same reagent on cholesteryl formate followed by gentle hydrolysis. The results of these investigations will be reported at a later date.

Perhaps in this connection it should be noted that it has been reported (15) that two isomeric bromides are obtained on direct bromination of cholesteryl benzoate. Evidence, however, submitted to substantiate this claim is weak and the problem needs further investigation.

EXPERIMENTAL[2]

Preparation of 4-bromo-5,6-dichlorocholestan-3-one. Dichlorocholesterol, m.p. 138°, prepared by the method of Berg and Wallis (7), was oxidized with chromic acid according to the directions of Décombe and Rabinowitch (6) to yield 5,6-dichlorocholestan-3-one, m.p. 97° (dec.), softening at 94°; $(\alpha)_D^{22}$ −27° (*c*, 4.9).

To a solution of 1.90 g. of 5,6-dichlorocholestan-3-one in 150 ml. of acetic acid was added a few drops of a 50% mixture of hydrobromic and acetic acids followed by 1 mole of bromine dissolved in acetic acid (5.9 ml., 0.707 *M*.). The solution was decolorized almost immediately. After the solution stood at room temperature for a half-hour 300 ml. of water was added and the resulting precipitate filtered off, washed with water, and dried. Recrystallization from 60 ml. of a 1:1 mixture of ethyl acetate and ethanol gave 1.25 g. of feathery needles, m.p. 163–165° (dec.). Repeated recrystallization from aqueous acetone gave material which melted constantly at 166–167° (dec.); $(\alpha)_D^{22}$ −20.5° (*c*, 2.9).

Anal. Calc'd for $C_{27}H_{43}BrCl_2O$: C, 60.7; H, 8.11.
Found: C, 61.0; H, 8.24.

Reactions of 4-bromo-5,6-dichlorocholestan-3-one. (a) A mixture of 0.20 g. of 4-bromo-5,6-dichlorocholestan-3-one, 0.5 g. of zinc dust, and 10 ml. of acetic acid was refluxed for 2½ hours. It was then poured into water, extracted with ether, the ethereal solution washed with aqueous bicarbonate, dried, and evaporated. The residue was dissolved in ethyl acetate, the solution decolorized with Darco and methanol added until an incipient turbidity occurred. After ice-box storage, crystals were obtained which on further recrystallization from the same solvent had m.p. 80°, undepressed in admixture with an authentic specimen of cholestene-4-one-3, m.p. 80°.

(b) Hot solutions of 1.00 g. of 4-bromo-5,6-dichlorocholestan-3-one in 4 ml. of benzene and of 0.70 g. of potassium acetate in 25 ml. of ethanol were combined and the mixture gently boiled in an open flask for 20 minutes. There occurred a rapid separation of inorganic material from the solution. Water was added until a faint turbidity formed, the solution was cooled, and the resulting crystalline precipitate filtered off. Recrystallization from 25 ml. of ethanol gave 0.57 g. of 3-acetoxy-2,5-cholestadiene-4-one (II) as prisms, m.p. 158–159°; $(\alpha)_D^{24}$ +11.9° (*c*, 3.1). A mixed melting point with a specimen, m.p. 158–159°, prepared from 4,5,6-tribromocholestan-3-one, was 158–159°. As a further means of identification II was hydrolyzed with ethanolic hydrochloric acid by Inhoffen's procedure (3) to 4-hydroxy-4,6-cholestadiene-3-one (III), m.p. 159–160°, undepressed in admixture with an authentic specimen, m.p. 158–159°; $(\alpha)_D^{24}$ +39.7° (*c*, 2.1).

(c) A solution of 200 mg. of 4-bromo-5,6-dichlorocholestan-3-one in 1 ml. of benzene was refluxed with 160 mg. of sodium iodide and 10 ml. of ethanol for 2½ hours. Water was added and the solution extracted with ether. The ethereal solution was washed with aqueous

[2] All melting points are uncorrected. All rotations were taken in chloroform with a 1-dm. semimicro tube and light-absorption data were determined in ethanolic solutions with a Beckman spectrophotometer.

sodium bisulfite, water, and evaporated. The residue, recrystallized once from ethanol, gave 110 mg. of material melting at 158–159°, and a mixed melting point determination with starting material showed no depression.

Preparation of 6(β)-chlorocholestane-3,5(β)-diol (VI). "α"-Cholesterol oxide (3.2 g.) m.p. 139–140°, prepared by the method Ruzicka and Bosshard (16) was dissolved in 150 ml. of dry chloroform and a steady stream of dry hydrogen chloride was passed through the solution for a half-hour. A crystalline precipitate formed after about 5 minutes. The solution was kept overnight at room temperature and then this precipitate was filtered off. Recrystallization from 150 ml. of ethyl acetate-methanol (1:1) gave 2.5 g. of felted needles, m.p. 163–164° (dec.). Further recrystallization from hexane gave material melting at 164–165° (dec.); $(\alpha)_D^{23}$ −10° (*c*, 2.7).

Anal. Calc'd for $C_{27}H_{47}ClO_2$: C, 73.8; H, 10.8.
Found: C, 73.5; H, 10.9.

Acetylation of this compound with acetic anhydride gave 6(β)-chloro-5-hydroxy-3(β)-acetoxycholestane, m.p. 190–191°; $(\alpha)_D^{22}$ −29.5° (*c*, 1.9). A mixed melting point determination with an authentic specimen, m.p. 190–191°, prepared from "α"-cholesterol oxide acetate by the method of Baxter and Spring (12) showed no depression. These authors report $(\alpha)_D^{18}$ −26.7° and m.p. 186–187°.

Preparation of 6(β)-chloro-5-hydroxy-cholestan-3-one (IX). A solution of 1.9 g. of chromic acid in 50 ml. of acetic acid was added during 1.5 hours with continuous stirring to a solution of 6.2 g. of 6(β)-chlorocholestane-3,5(β)-diol in 850 ml. of acetic acid. The mixture, in which a crystalline precipitate had formed, was allowed to stand at room temperature overnight. Excess chromic acid was then destroyed by the addition of a little ethanol and 2 liters of water was added with stirring. The precipitate was washed with water, and dried. Recrystallization from 330 ml. of ethyl acetate gave 4.2 g. of needles, m.p. 190° (dec.), which on further recrystallization from the same solvent melted at 193° (dec.); $(\alpha)_D^{22}$ +3° (*c*, 0.9). These needles are only slightly soluble in ether, chloroform, and ethanol but are moderately soluble in hot ethyl acetate.

Anal. Calc'd for $C_{27}H_{45}ClO_2$: C, 74.2; H, 10.38.
Found: C, 74.6, H, 10.48.

Preparation of 6(β)-chloro-4-cholestene-3-one (X). A solution of 0.30 g. of 6(β)-chloro-5-hydroxycholestan-3-one in 6 ml. of dry pyridine was treated dropwise at 0° with 0.15 ml. of thionyl chloride. The mixture was kept at this temperature for 20 minutes and then poured into ice-water. The solution was made acid to Congo Red with hydrochloric acid and extracted with ether. The ethereal solution was washed twice with water, filtered from some gelatinous material, and evaporated. The residue crystallized spontaneously on the addition of a little methanol. Two crystallizations from ethyl acetate-methanol gave 80 mg. of short needles, m.p. 127–128°; $(\alpha)_D^{25}$ +13° (*c*, 2.7); λ_{max} (ultra-violet) 2410 Å (ε 14,000).

Preparation of 6(β)-chloro-4-cholestene-3-one (X) *from 5,6-dichlorocholestan-3-one.* Three and seven-tenths grams (8.2×10^{-3} mole) of 5,6-dichlorocholestan-3-one, m.p. 97° (dec.), was refluxed in a solution of 2.4 g. (25×10^{-3} mole) of freshly fused potassium acetate and 150 ml. of ethanol for 2 hours. Potassium chloride began to separate from the solution after about 10 minutes. Water (300 ml.) was carefully added, the solution cooled, and the resulting crystalline precipitate washed with water. The filtrate was analyzed for chloride ion by the Volhard method. It contained 8.2×10^{-3} mole of chloride ion. The precipitate was recrystallized from 80 ml. of aqueous acetone to give 1.95 g. of short needles, m.p. 124–126°. One crystallization from ethyl acetate-methanol gave material which melted constantly at 125–127°; $(\alpha)_D^{26}$ +14.0 (*c*, 2.6); λ_{max} (ultra-violet) 2410 Å (ε 14,000). A mixed melting point determination with the 6(β)-chloro-4-cholestene-3-one, m.p. 127–128°, prepared from 6(β)-chloro-5-hydroxycholestan-3-one showed no depression.

Anal. Calc'd for $C_{27}H_{43}ClO$: C, 77.4; H, 10.34.
Found: C, 77.6; H, 10.16.

Ruzicka (17) mentions the preparation of this compound but gives no details of its properties.

Preparation of 6(α)-acetoxy-4-cholestene-3-one (XIII). To a solution of 6.0 g. of freshly fused potassium acetate in 25 ml. of dry acetic acid was added 1.12 g. (2.67×10^{-3} mole) of 6(β)-chlorocholestene-4-one-3, m.p. 124–126° and the mixture was refluxed gently for 80 minutes with the exclusion of moisture. Water was added and the solution extracted with ether. The aqueous solution contained 2.60×10^{-3} mole of chloride ion as determined by the Volhard method. The ethereal solution was washed thoroughly with dilute sodium bicarbonate solution, water, and dried. The ether was removed and the remaining oil dissolved in a little acetone. This solution was decolorized with Darco and methanol was added until a slight turbidity formed. The crude crystalline product (90 mg.), m.p. 134–136°, which slowly formed was recrystallized twice from acetone-methanol to give long colorless needles which melted constantly at 139–139.5°; $(\alpha)_D^{25}$ +62°; λ_{max} (ultra-violet) 2420 Å (ε 14,500).

Anal. Calc'd for $C_{29}H_{46}O_3$: C, 78.7; H, 10.47.
Found: C, 78.4; H, 10.37.

When 6-bromocholestene-4-one-3, m.p. 131°, prepared by the method of Dane, Wang, and Schulte (13), was treated with potassium acetate and acetic acid as above, the same product, m.p. 139–139.5°, was obtained and in similar yield. A mixed melting point determination with the material, m.p. 139–139.5°, obtained from the 6-chlorocholestene-4-one-3 showed no depression. Attempts to improve the yield of this product were fruitless. Ellis and Petrow (5) record for 6(β)-acetoxycholestene-4-one-3, m.p. 101.5° and $(\alpha)_D^{19}$ +36°.

Preparation of 6(α)-hydroxy-4-cholestene-3-one. A solution of 50 mg. of 6(α)-acetoxy-4-cholestene-3-one in 4 ml. of 0.2 *N* ethanolic sodium hydroxide was kept at room temperature overnight and acidified. Isolation of the product with ether followed by three crystallizations from ether-methanol gave needles m.p. 116–118°. A mixed melting point determination with starting material showed a depression to 90–100°.

Anal. Calc'd for $C_{27}H_{44}O_2$: C, 81.1; H, 11.08.
Found: C, 81.4; H, 11.31.

In ethanolic solution this material gives a purple coloration with ferric chloride. The mother liquors from this product, m.p. 115–118°, were worked up to yield crude material m.p. 106–109°. This was acetylated with acetic anhydride and pyridine at room temperature to yield material identical with the 6(α)-acetoxy-4-cholestene-3-one described above. 6(β)-Hydroxy-4-cholestene-3-one has m.p. 192°.

Acknowledgment. We wish to take this opportunity to express our thanks to the Rockefeller Foundation, New York City, for a grant in aid, and to Merck and Company, Inc., Rahway, New Jersey, for the analyses published in this paper.

SUMMARY

Cholesterol dichloride prepared either by the action of iodobenzene dichloride on cholesterol or by direct chlorination of cholesterol, has been shown to possess the *cis* or coprostane structure and not the *trans* or cholestane arrangement as previously reported. The halogen atom at C_6 in the above cholesterol dichloride and also in cholesterol dibromide has been proved to possess the (β)-configuration.

PRINCETON, NEW JERSEY

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