Preparation of 3-[N-(+)Pantothenyl)-2-aminoethylthio]-5-hydroxy-1,4-naphthoquinone (Juglone–Pantetheine Adduct) (VII).—Seventy cc. of a butanol solution of pantetheine prepared by catalytic reduction of pantethine and containing 0.028 meq. RSH/cc. (2.0 meq. pantetheine) was treated at room temperature with 348 mg. (2 mmoles) of 5-hydroxy-1,4-naphthoquinone. The red quinone dissolved to give a very dark brown solution. The solution was immediately evaporated at 40° (*in vacuo* with slow air stream) to a dark brown solid residue. This residue was dissolved in 5 cc. of methanol and the deep red solution was filtered to remove some black granular insoluble material. The filtrate was cooled and reddish-brown crystals were obtained; 270 mg. (30% yield), m.p. 143–148°. The derivative was recrystallized from methanol, orange-red needles, m.p. 153–155° (dec.). A mixed m.p. with juglone (m.p. 160–161°) was 130–134°; dried at 60° *in vacuo* for analysis.

Anal. Calcd. for $C_{21}H_{26}O_7N_2S$: C, 55.99; H, 5.82; N, 6.22; S, 7.12. Found: C, 55.90; H, 6.01; N, 6.13; S, 7.35.

In some of the preparations which were made, particularly when incompletely reduced pantethine was the starting material, the separation of the black amorphous by-product was quite troublesome. Dilution of the methanol solution with ether caused the material to separate, but since the desired adduct is very sparingly soluble in ether, some loss may be incurred by this treatment. The derivative is fairly soluble in water and absolute ethanol.

The ultraviolet spectrum in ethanol showed three bands: λ 232 mμ, ε 15,900; λ 307 mμ, ε 6,700; λ 413 mμ, ε 7,100. The derivative was so highly colored that an accurate rotation was impossible; a value of $[\alpha]^{25}D$ +13 ± 3° (*c* 0.466 in abs. EtOH) was obtained.

The microbiological activity of this derivative was somewhat erratic, values of 9,100 to 11,300 LBF units/mg. were obtained. The value was not changed significantly when the derivative was added aseptically to the medium and assayed without autoclaving. This potency of 10,000 units/mg. corresponds to 85% of the pantetheine content.

The yield of this derivative was not improved by using excess juglone; the adduct was more difficult to isolate in this case. Attempts to form similar adducts with 2-methylnaphthoquinone or with benzalacetophenone were unsuccessful.

DETROIT 32, MICHIGAN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Configuration of Steroid Bromoketones. I. Methyl 4β-Bromo-3-ketocholanate

BY LOUIS F. FIESER AND RENATO ETTORRE

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The configuration of the bromoketone (II) mentioned in the title was established by sodium borohydride reduction to two bromohydrins identified as *cis* (V) and *trans* (VI) by their respective conversion with base to a ketone (I) and an oxide (X), and by hydrogenation of the *cis*-bromohydrin to the 3β-hydroxy compound (IV). Both bromohydrins and their acetates are convertible in excellent yield to methyl Δ³-cholenate (IX). The free acid corresponds in three constants with an acid tentatively formulated by Wieland as Δ²-cholenic acid.

Although the last step in the synthesis of several steroid hormones consists in dehydrobromination of a 4-bromo-3-ketone of the normal or bile acid series, chemical evidence of the configuration of the predominant products of bromination has been lacking.[1] In this investigation of the problem we employed as a model compound the 4-bromo derivative (II) of methyl 3-ketocholanate,[2] available in high yield by oxidation of methyl lithocholate[2] with sodium chromate in acetic acid. The bromo ketone, isolated in 58% yield by crystallization, appeared to be homogeneous when chromatographed and its behavior on dehydrohalogenation conformed to the usual pattern. Thus refluxing in pyridine afforded methyl 3-keto-Δ⁴-cholenate[3] (III) in low yield, whereas in the non-stereospecific reaction of Mattox and Kendall[4] it gave the 2,4-dinitrophenylhydrazone of III in high yield.

The plan for determination of the orientation of the bromine atom was to reduce the carbonyl group of the bromo ketone, see if the resulting product behaved as a *cis*- or a *trans*-bromohydrin, and determine the orientation of the hydroxyl group by removal of the bromine substituent. Sodium borohydride seemed a promising reagent for effecting the first step because of Chaikin and Brown's[5] brief mention of the successful reduction of ω-bromoacetophenone and because the reagent reduces 3-ketones of the bile acid series in methanol (absolute) without attack of the ester group in the side chain.[6] Reduction of the 4-bromo derivative of methyl 3-ketocholanate with sodium borohydride in methanol at 25° gave a mixture of 3-epimeric bromohydrins from which one isomer was isolated by direct chromatography; by chromatography after acetylation of the total mixture both epimeric acetates were isolated. Each acetate afforded the corresponding bromohydrin on saponification with methanolic alkali at room temperature and also on methanolysis with boron fluoride as catalyst. The observation that deacetylation by the latter method proceeded notably slower with the more dextrorotatory of the two acetates indicated that this isomer probably has the *cis* orientation of the substituents at 3 and 4. Conclusive evidence that the more hindered, more dextrorotatory bromohydrin is indeed *cis* and the epimer *trans* was found in the behavior of the bromohydrins on dehydrohalogenation: refluxing alcoholic alkali converted the former into the 3-ketone I and the latter into an oxide. The validity of this method of diagnosis was established by Bartlett[7] in the cyclohexane series. Finally, the orientation of the hydroxyl group in the *cis*-bromohydrin was established by debromination. Reduction proceeded smoothly in alcoholic potassium hydroxide solution at room temperature and gave 3β-hydroxycholanic acid,[8,9] identified by comparison (as acid and as ester) with a sample

(1) Evidence from physical properties will be discussed in paper II.

(2) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **72**, 5530 (1950).

(3) R. Schoenheimer and F. Berliner, *J. Biol. Chem.*, **115**, 19 (1936).

(4) V. R. Mattox and E. C. Kendall, THIS JOURNAL, **70**, 882 (1948); **72**, 2290 (1950).

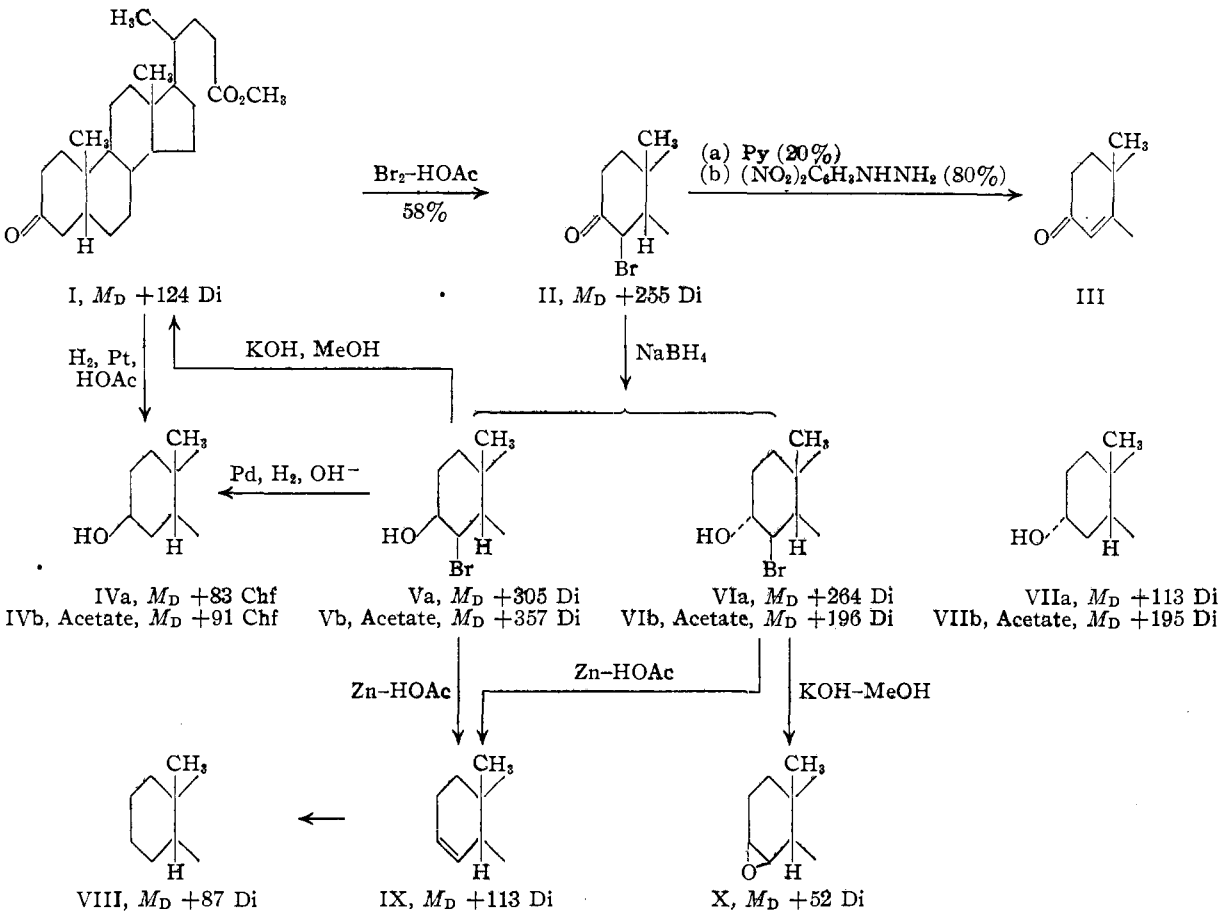
(5) S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

(6) H. Heymann and L. F. Fieser, *ibid.*, **73**, 5252 (1951).

(7) P. D. Bartlett, *ibid.*, **57**, 224 (1935).

(8) F. Reindel and K. Niederländer, *Ber.*, 1243 (1935).

(9) K. Yamasaki and K. Kyogoku, *Z. physiol. Chem.*, **235**, 43 (1935).



(All constants given are for methyl ester derivatives.)

made by hydrogenation of 3-ketocholanic acid (or the ester) in acetic acid solution. The possibility might be entertained that the reaction proceeds through the 3-ketone, although the bromohydrin is stable to alkali in the cold for periods much longer than that required for hydrogenation. However this is excluded by the observation of Reindel and Niederländer that 3-ketocholanic acid on hydrogenation in presence of base yields chiefly the 3α-hydroxy acid. The *cis*-bromohydrin is thus methyl 3β-hydroxy-4β-bromocholanate (Va) and the product of initial bromination is the 4β-bromo-3-ketone II. Attempts to secure independent evidence of the configuration of the *trans*-bromohydrin VIa were unsuccessful, since hydrogenation under various conditions gave either oils or material that appeared to be the impure 3α-hydroxy ester VIIa.[2] That no epimerization of the bromine substituent had occurred in the formation of VIa was shown by oxidation and recovery of the bromoketone II.

In contrast to methyl 3β-hydroxycholanate (IVa), the 3β,4β-*cis*-bromohydrin Va is not precipitated by digitonin. The case is analogous to that reported by Spring and Swain[10]: Δ^4-cholesten-3β,4β-diol forms a sparingly soluble digitonide, but its 4-benzoate does not. The hindered character of the *cis*-bromohydrin Va, noted above, may be due in part to the *cis* orientation and in part to the greater hindrance inherent in a polar-oriented 3β-hydroxyl group in contrast to that of the equatorial 3α-hydroxyl group of the epimer VIa (Barton[11]). It is to be noted also that in the bromination of the 3-ketone I, which probably proceeds through the enol, the 4-bromine substituent assumes the less hindered equatorial orientation (β), in accord with the principle postulated by Barton.[11]

Since debromination of methyl 3α-hydroxy-4β-bromocholanate (VIa) did not proceed smoothly by catalytic hydrogenation, an attempt was made to achieve the same result by chemical reduction. To our surprise, this *trans*-bromohydrin, as well as its acetate, was found to react with zinc dust and acetic acid with great readiness and to afford, in high yield, an unsaturated ester identified as a methyl cholenate by hydrogenation to methyl cholanate. We later learned of the work of Mori,[12] who found that 3-esters of 5α-chlorocholestane-3β,6β-diol are convertible into cholesteryl esters by brief treatment with zinc dust in acetic acid. In view of these experiences with 3,4- and 5,6-*trans*-halohydrins, it is curious that Ott and Reichstein[13] tried the same reaction on methyl 3α-acetoxy-11β-hydroxy-12α-bromocholanate and recovered unchanged starting material. Mori[14] found that 6β-chlorocholestane-3β,5α-diol 3-acetate is converted by zinc and acetic acid not into cholesteryl acetate

(10) F. S. Spring and G. Swain, *J. Chem. Soc.*, 83 (1941).

(11) D. H. R. Barton, *Experientia*, **6**, 316 (1950).

(12) S. Mori, *J. Chem. Soc. Japan*, **70**, 303 (1949); see *C. A.*, **45**, 4733 (1951).

(13) G. H. Ott and T. Reichstein, *Helv. Chim. Acta*, **26**, 1809 (1943).

(14) S. Mori, *J. Chem. Soc. Japan*, **70**, 257 (1949); see *C. A.*, **45**, 4733 (1951).

but into cholestane-3β,5α,6β-triol 3,6-diacetate. The limits of the reaction, as applied to *trans*-halohydrins, are thus not well defined. That the elimination reaction is not restricted to *trans*-halohydrins is established by the observation that the *cis* epimer, methyl 3β-hydroxy-4β-bromocholanate (Va), and its acetate also reacted readily with zinc and acetic acid to give an olefinic ester identical with that from VIa. Yields of over 90% are obtainable from either bromohydrin, and the unsaturated ester is thus readily prepared from the unseparated reduction mixture.

Our acid, which, from the method of preparation, can surely be assigned the structure of Δ^3-cholenic acid, is probably identical with a cholenic acid that Wieland and co-workers[15] isolated as the chief product of pyrolytic dehydration of lithocholic acid. The Wieland acid, purified by regeneration from either a less soluble dibromide, m.p. 233°, or a more soluble stereoisomer, m.p. 171°, had the constants: m.p. 156°, α_D +17° Al. Our acid melts at 155–156°, α_D +18° Al; less soluble dibromide, m.p. 232–233°. Wieland tentatively formulated his acid as Δ^2-cholenic acid because on oxidation with selenium dioxide it afforded two hydroxycholenic acids, but from what is now known of the course of selenium dioxide oxidations and of allylic rearrangements this evidence seems inconclusive. We plan to study Wieland's hydroxycholenic acids further.

Experimental

Methyl 3-Ketocholanate.[2]—A solution of 16.6 g. of sodium chromate tetrahydrate in 30 cc. of acetic acid was added slowly with swirling to a solution of 13.8 g. of methyl lithocholate[2] (m.p. 125–127°, α_D +29° Di) in 110 cc. of acetic acid and the temperature was kept at 35 ± 2° by cooling under the tap. Trials of reaction periods of 2–19 hr. indicated that the oxidation is complete in 6 hr. The solution was poured slowly with stirring into 500–600 cc. of ice-water. A granular white precipitate separated and was easily washed and dried (m.p. 108–110°). Crystallization from methanol gave a poor product (m.p. 114–116°) that was tedious to filter, but slow recrystallization from 30–60° petroleum ether afforded prismatic needles, m.p. 119–120°, α_D +32° Di. The total yield of product of m.p. 115–116° or better was 12.6 g. (91%).

Anal. Calcd. for $C_{25}H_{40}O_3$ (388.57): C, 77.27; H, 10.38. Found: C, 77.25; H, 10.39.

Methyl 4β-Bromo-3-ketocholanate (II).—A solution of 10 g. of methyl 3-ketocholanate (m.p. 114–116°) in 150 cc. of acetic acid was stirred at 25° during dropwise addition of 4.8 g. of bromine (1.1 moles) in 15 cc. of acetic acid. Bromine absorption was slow at the start and then proceeded more rapidly (HBr catalysis). Addition of water produced a gummy precipitate that was extracted with ether. The washed and dried solution was concentrated to 20–25 cc., chilled in Dry Ice–acetone, and scratched until a paste resulted. The solid was filtered rapidly and washed with a little ether chilled in Dry Ice–acetone. Colorless, crystalline material (7 g.) was thus separated from a brown mother liquor. Crystallization from acetone–methanol afforded 6.1 g. (58%) of plates, m.p. 98–99°. On chromatography, the material appeared to be homogeneous. Recrystallization of chromatographed material gave plates, m.p. 99–100°, α^{23}_D +54.6 ± 2° Di.

Anal. Calcd. for $C_{25}H_{39}O_3Br$ (467.59): C, 64.22; H, 8.27. Found: C, 64.41; H, 8.40.

Methyl 2,4-Dibromo-3-ketocholanate.[16]—Bromination of 10 g. of methyl dehydrolithocholate was conducted as above but with twice the amount of bromine solution. The resulting yellow solution was poured with stirring onto ice-water, and after several hours the precipitate coagulated to a granular solid that could be collected by filtration. The air-dried product was dissolved in 300 cc. of acetone and 200 cc. of methanol (hot) and the filtered solution concentrated to about 250 cc.; an oil separated and then solidified (6 g.). Slow evaporation of the mother liquor at 25° afforded a second crop of 2.5 g. of solid. Crystallization from methanol gave a total of 7.1 g. (50%) of dibromide, m.p. 143–145°. Repeated recrystallization from methanol gave needles, m.p. 145–146°, α^{23}_D +18.4 ± 2° Di. The substance was eluted from acid-washed alumina by 1:1 petroleum ether–benzene and no variations in fractions were observed.

Anal. Calcd. for $C_{25}H_{38}O_3Br_2$ (546.39): C, 54.95; H, 7.06. Found: C, 54.81; H, 7.20.

The combined material (5.2 g.) recovered from the mother liquors was refluxed with 3 g. of zinc dust in 70 cc. of acetic acid for several hours and the product extracted with ether. Crystallization of the residue from petroleum ether gave 3.3 g. of Beilstein-negative methyl dehydrolithocholate, m.p. 113–115°, identified by mixed m.p. determination.

Methyl 3α-Acetoxy-4β-bromocholanate (VIb) and Methyl 3β-Acetoxy-4β-bromocholanate (Vb).—A solution of 4 g. of methyl 4β-bromodehydrolithocholate (m.p. 98–99°) in 160 cc. of absolute ethanol was cooled to 25°, 120 mg. (1.5 equiv.) of sodium borohydride in 20 cc. of ethanol was added and the solution let stand at 25° for 18–20 hr., poured into 400 cc. of water, and the product extracted with ether. The crude product was then acetylated in pyridine (50 cc.)–acetic anhydride (25 cc.) overnight at 25° and the acetate mixture (4.2 g.) chromatographed on acid-washed alumina.[17]

Fractions 2–5 (1:1 PE–B), m.p. between 103 and 114° (2.5 g.), on repeated crystallization from methanol (chilling in salt–ice) afforded 1330 mg. of the **3α-acetate VIb** as needles, m.p. 119–120°, α_D +38.3°, +38.2 ± 1° Di; a second crop of 200 mg. melted at 116–117°.

Anal. Calcd. for $C_{27}H_{43}O_4Br$ (511.53): C, 63.39; H, 8.47. Found: C, 63.54; H, 8.41.

Fractions 6–8 (100 B), m.p. from 89 to 99° (1.8 g.) were combined and crystallized several times from methanol to give 890 mg. of pure **3β-acetate Vb** as hexagonal plates, m.p. 105–106°, α_D +69.8 ± 2° Di.

Anal. Calcd. for $C_{27}H_{43}O_4Br$ (511.53): C, 63.39; H, 8.47. Found: C, 63.49; H, 8.41.

Methyl 3α-Hydroxy-4β-bromocholanate (VIa).—A solution 300 mg. of the acetate VIb (119–120°) in 20 cc. of methanol was treated with 0.3 cc. of boron fluoride etherate and let stand at 25° for 72 hr. Ether extraction after addition of water gave an oily material that was taken up in a small amount of petroleum ether. On cooling and scratching the substance separated as prismatic needles; yield 210 mg., m.p. 104.5–105° (well dried), α_D +56.2 ± 1° Di.

Anal. Calcd. for $C_{25}H_{41}O_3Br$ (469.50): C, 63.94; H, 8.80. Found: C, 64.09; H, 8.49.

In another experiment a solution of 580 mg. of acetate VIb was refluxed with 20 cc. of methanol and 0.4 cc. of boron fluoride etherate for one-half hour and the reaction product collected by ether extraction and chromatographed. Fractions 2–5, m.p. 129–135° (100 B) on crystallization from methanol, afforded 200 mg. of blades m.p. 149–150°, α_D +35°, +35.8 ± 1° Di.

Anal. Calcd. for $C_{27}H_{43}O_4Br$ (511.53): C, 63.39; H, 8.47. Found: C, 63.40, 63.34; H, 8.76, 8.75.

The substance corresponds in rotation and analysis to unreacted starting material but the melting point is considerably higher. It probably is an isomorphic form of VIb, although we were unable to transform either substance into the other by seeding melts or saturated solutions.

The acetate VIb was also hydrolyzed by dissolving 150 mg. (118–119°) in 12 cc. of 1% methanolic potassium hydroxide and letting the solution stand overnight. After dilution and acidification the product was extracted with ether and esterified with diazomethane, after which several crystallizations from petroleum ether gave 93 mg. of VIa, m.p. 104–105°, undepressed on admixture with the product of methanolysis.

(15) H. Wieland, K. Kraus, H. Keller and H. Ottawa, *Z. physiol. Chem.*, **241**, 47 (1936).

(16) Structure assignment by analogy, see C. Djerassi and G. Rosenkranz, *Experientia*, **7**, 93 (1951); H. H. Inhoffen, G. Kölling, G. Koch and I. Nebel, *Ber.*, **84**, 361 (1951).

(17) Solvents for chromatography are indicated thus PE = 30–60° petroleum ether, B = benzene, An = acetone.

Oxidation of VIa with chromic acid and crystallization from methanol gave plates, m.p. 96–99°, of the ketone II (mixed m.p.).

Methyl 3β-Hydroxy-4β-bromocholanate (Va). (a) **From the Reduction Mixture.**—The mixture of epimers resulting from reduction of 4 g. of methyl 4β-bromodehydrolithocholate as described above on being chromatographed afforded first oils (1:2 PE-B), then two fractions (5 and 6; 100 B) that gave 825 mg. of solid, m.p. 65–77° on crystallization from petroleum ether, then a sequence of oils (fractions 8–11; 100 B, 100 E). The solid material from fractions 5 and 6 on crystallization from methanol (chilling in salt–ice) gave 370 mg. of soft needles of Va, m.p. 81–82°, α_D +64.9 ± 2° Di. The alcohol is not precipitated by digitonin.

Anal. Calcd. for $C_{25}H_{41}O_3Br$ (469.50): C, 63.94; H, 8.80. Found: C, 63.44; H, 8.95.

The oily chromatogram fractions 8–11 were combined and rechromatographed, but afforded no crystalline products. However, acetylation of the total oily product gave material that solidified when rubbed with methanol, and two crystallizations from this solvent afforded 1.45 g. of methyl 3α-acetoxy-4β-bromocholanate as white needles, m.p. 119–120°, identified by mixed m.p. determination.

(b) By **Hydrolysis of the Acetate Vb.**—When 390 mg. of methyl 3β-acetoxy-4β-bromocholanate (Vb, 102–103°) was refluxed for 30 min. with 0.2 cc. of boron fluoride etherate in 10 cc. of methanol, ether extraction and crystallization from methanol afforded starting material (m.p. 103–104°) in close to quantitative yield. A solution of 100 mg. of Vb in 10 cc. of methanol plus 0.1 cc. of catalyst was then allowed to stand at 25° for several days before processing. Crystallization of the extracted product from petroleum ether gave 60 mg. of the β-hydroxy compound Va, m.p. 81–82°, identified by mixed m.p. comparison.

Alkaline hydrolysis of 150 mg. of Vb, conducted exactly as described for the epimer, gave an oily product that was chromatographed. Fractions 5–7 (1:1 PE–B) on crystallization from methanol and then petroleum ether gave 75 mg. of 3β-hydroxy compound, identified by mixed m.p. determination. Fractions 9 and 10 (100 B; 100 E) on crystallization from methanol afforded a small amount of material, m.p. 115–116°, undepressed on admixture with methyl dehydrolithocholate.

Methyl 3β-Hydroxycholanate (IVa) from Va.—A 100-mg. portion of Va (m.p. 77–79°) was dissolved in 10 cc. of ethanol containing 750 mg. of potassium hydroxide, 50 mg. of 5% palladium–charcoal was added, and the mixture was shaken with hydrogen at 25°. Absorption of hydrogen was complete in one hour (6.4 cc.), after which the solution was acidified, diluted, extracted with ether, and the extracted material esterified with diazomethane. The residue from ether extraction crystallized from methanol, and on recrystallization the product melted at 114–115°. Addition of 1 cc. of 1% digitonin solution in 90% ethanol to a solution of 5 mg. of the product in 1 cc. of 90% ethanol gave, after a few seconds delay, a heavy precipitate of digitonide.

In another experiment[18] the product of hydrogenation was crystallized twice from acetone and melted at 175–176°, α_D +21.2° Chf; a mixed m.p. with authentic 3β-hydroxycholanic acid (see below) showed no depression. The mother liquor material was recovered and esterified with diazomethane; the ester, crystallized twice from aqueous methanol, melted at 110–113° and gave no depression on admixture with an authentic sample.

3β-Hydroxycholanic Acid.[18]—Since this acid and its derivatives have not been fully characterized, samples were prepared as follows. A mixture of 1057 mg. of methyl 3-ketocholanate, 109 mg. of platinum oxide, 1 cc. of 36% hydrochloric acid and 50 cc. of acetic acid absorbed 92 cc. of hydrogen in 2 hr. The crude ester was obtained by dilution of the filtered solution, saponified, and the **hydroxy acid** crystallized twice from acetone: m.p. 177–177.5°, α_D +20.4° Chf (Reindel[8]: 176–177°, α_D +25.9° Al); the acid separates from aqueous acetone as a hydrate melting about 130–145°. The **methyl ester** (diazomethane), crystallized twice from methanol, melted at 114–116°, α_D +21.3° Chf. (Yamasaki,[9] m.p. 115–116°); it was also obtained by hydrogenation of the ketone methyl ester in acetic acid (m.p. 113–114°). A mixture with the above sample showed no depression in m.p. The **methyl ester acetate** crystallized from chloroform–methanol in small plates, m.p. 165–167°, α_D +21.1° Chf.

Anal. Calcd. for $C_{27}H_{44}O_4$ (432.62): C, 74.95; H, 10.25. Found: C, 74.89; H, 10.25.

Hydrogenation of VIb.—Hydrogenation of the alcohol VIa by the procedure applied to Va gave material that could not be characterized. Hydrogenation of the acetate Vb (200 mg.) in 50 cc. of ethanol in the presence of 1 cc. of diethylamine and 1 g. of 2% palladium-on-calcium carbonate,[19] conducted overnight, and crystallization from methanol gave a small crop of Beilstein-negative crystals, m.p. 94–96°, α_D +42° Di. A mixture with methyl lithocholate 3-acetate,[2] m.p. 133–134°, α_D +45° Di, sintered at 95° and melted at 117–119°.

Methyl 3-Ketocholanate (I) from the *cis*-Bromohydrin Va.—A solution of 300 mg. of Va (m.p. 80–81°) and 150 mg. of potassium hydroxide pellets in 20 cc. of methanol was refluxed for 3 hr., made acid with dilute hydrochloric acid, and the product extracted with ether. The residue after treatment with diazomethane, gave crystals from methanol and recrystallization from methanol and then from petroleum ether yielded 150 mg. of needles, m.p. 113–114°, undepressed by admixture with authentic I, m.p. 118–119°.

Methyl 3α,4α-Oxidocholanate (X) from the *trans*-Bromohydrin Acetate VIb.—A solution of 500 mg. of the 3α-acetate VIb (m.p. 119–120°) and 900 mg. of potassium hydroxide in 35 cc. of methanol was let stand at 25° overnight and then refluxed for 3 hr. Careful neutralization of the cooled solution with dilute hydrochloric acid gave a precipitate that was taken up in ether. The washed and dried extract was evaporated and the residue esterified with diazomethane. Evaporation of the solvent left a gummy residue that was chromatographed. The bulk of the material was eluted by 1:1 petroleum ether–benzene, and these fractions were Beilstein-negative and melted in the range 50–68° (a minor late fraction eluted by benzene, m.p. 84–97°, was also Beilstein-negative). Four crystallizations from methanol gave a total of 200 mg. of oxide, m.p. 72–73°, α_D +13.3 ± 2° Di.

Anal. Calcd. for $C_{25}H_{40}O_3$ (388.57): C, 77.27; H, 10.38. Found: C, 77.59; H, 10.71.

The substance was found stable to hydrogenation at room temperature, and the intensity of absorption at 5.8 μ was distinctly less than that noted for methyl 3-ketocholanate.

Methyl Δ^3-Cholenate (IX). (a) **Zinc–Acetic Acid Procedure.**—The unsaturated ester IX was obtained by the following procedure from the two bromohydrins, Va and VIa, as well as from their acetates, Vb and VIb. A mixture of 100 mg. of bromohydrin or acetate and 300 mg. of zinc dust was refluxed gently with 20 cc. of acetic acid for 10 min. and the solution was filtered, the zinc washed, and the combined solutions diluted with water. The acetic acid was neutralized by addition of sodium bicarbonate and the mixture extracted with ether. A chilled solution of the crude product in methanol on scratching afforded needles, m.p. 80–82°, in 80–85% yield. Two further crystallizations raised the m.p. 83–84°, α_D +30.5 ± 2° Di. In another experiment (L.F.F.) the filtered acetic acid solution was diluted to turbidity and seeded; 477 mg. of VIb gave 320 mg. (92%) of IX, m.p. 80–81°.

Anal. Calcd. for $C_{25}H_{40}O_2$ (372.57): C, 80.59; H, 10.82. Found: C, 80.15; H, 11.13.

The ester gave a positive test for unsaturation with tetranitromethane, it decolorized bromine solution instantly, and gave a strong Liebermann–Burchard test. Hydrogenation in acetic acid in the presence of Adams catalyst was complete in 15–20 min.; the filtered solution was diluted to turbidity and scratched, and on cooling a heavy precipitate of needles separated. Two recrystallizations afforded needles, m.p. 86–87°, α_D +23.2 ± 2° Di; no depression in m.p. with authentic **methyl cholanate** (VIII).

Anal. Calcd. for $C_{25}H_{42}O_2$ (374.59): C, 80.15; H, 11.30. Found: C, 79.79; H, 11.21.

(b) **Zinc–Ethanol Procedure.**—A solution of 100 mg. of either *cis*- or *trans*-bromohydrin acetate (Vb or VIb) in 15 cc. of ethanol was refluxed for 3 hr. with about 400 mg. of activated zinc.[20] The filtered alcoholic solution on cooling in ice gave needles of IX, m.p. 78–80°, in 70–80% yield.

(18) Experiment by Dr. Wei-Yuan Huang.

(19) M. Busch and H. Stove, *Ber.*, **49**, 1063 (1916).

(20) L. F. Fieser and W. S. Johnson, THIS JOURNAL, **62**, 576 (1940).

(c) **From the Bromohydrin Mixture.**—The crude mixture resulting from sodium borohydride reduction of 2 g. of methyl 4β-bromo-3-ketocholanate (II) was reduced directly as in (a) and the product (940 mg., 69%) m.p. 68–72°) purified by elution from alumina with petroleum ether–benzene.

Δ³-Cholenic Acid (L.F.F.)—The total Δ³-cholenate resulting from reduction of 320 mg. of the *cis*-bromohydrin acetate Vb was heated with alcoholic alkali and the solution diluted with water, when a sparingly soluble salt separated. This was filtered from a slightly yellowish mother liquor, dissolved in a large volume of water, and the solution acidified. The yield of pure white crude acid, m.p. 147–150°, was 220 mg. (quant.). The acid is readily soluble in hot methanol but crystallizes well at 25° in fibrous needles, m.p. 152–154°; recrystallized, m.p. 155–156°, α_D +18° Al.

Anal. Calcd. for $C_{24}H_{38}O_2$ (358.54): C, 80.39; H, 10.68. Found: C, 80.40; H, 10.58.

Bromination was conducted according to Wieland[15] and the product crystallized from ether–petroleum ether. The first crop formed prismatic needles, m.p. 228–229°; recrystallized, m.p. 232–233°. (Comparison with the Wieland samples was not possible since the latter were destroyed by fire in a bombing attack during the war.)

Methyl 3-Keto-Δ⁴-cholenate[3] (III).—A solution of 500 mg. of methyl 4β-bromo-3-ketocholanate (II) in 50 cc. of pyridine (distilled over barium oxide) was refluxed for 12 hr., cooled, acidified with dilute hydrochloric acid, and the product extracted with ether. The solution was washed, dried, clarified with Norit and evaporated. On scratching an ice-cold methanol solution, crystals, m.p. 111–113°, were obtained. Several further crystallizations gave needles m.p. 123–125° (lit.[3] 124–125°), λ^{EtOH} 238 mμ (12,600).

Dehydrobromination in pyridine in a sealed tube at 136°[21] was tried but the starting material was recovered unchanged.

Methyl 3-Keto-Δ⁴-cholenate 2,4-Dinitrophenylhydrazone (a).—A solution of 200 mg. of methyl 4β-bromo-3-ketocholanate (97–99°) in 5 cc. of acetic acid was treated with 90 mg. of 2,4-dinitrophenylhydrazine and heated on a hot-plate under nitrogen for 4–5 min. The orange precipitate that separated was crystallized from chloroform–methanol to give orange-red microcrystals, m.p. 231–232°, yield 242 mg. (80%). A sample prepared from the ketone III melted at 232–233°.

Anal. Calcd. for $C_{31}H_{42}O_6N_4$ (566.68): C, 65.70; H, 7.47. Found: C, 65.70; H, 7.53.

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Configuration of Steroid Bromoketones. II. 4β-Bromotestane-17β-ol-3-one Acetate and 2β-Bromocholestane-3-one

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Application of the method of diagnosis reported in paper I affords chemical evidence that the bromoketones listed in the titles have the configurations indicated. Discrepancies are noted between our conclusions and those based on analysis of molecular rotation data (Djerassi) and upon shifts in carbonyl absorption in the infrared (R. N. Jones) and the possibility is suggested that both methods of deducing configurations from physical constants may be invalidated by variations in the conformation of ring A depending upon the nature of the substituents in ring A and at C_{17}.

The method for determination of the configuration of α-bromoketones described in paper I[2] was applied to a further 3-ketone of the coprostane series, namely, Butenandt's[3] etiocholane-17β-ol-3-one acetate (I), for which we shall use the Ciba Conference[4] name testane-17β-ol-3-one acetate. The 4-bromo derivative (II), obtained in 60–70% yield, showed the usual resistance to dehydrohalogenation by pyridine. Reduction of II with sodium borohydride afforded only one bromohydrin (III) in 65% yield. This main product was identified as a *trans*-bromohydrin by its transformation on prolonged refluxing with alcoholic potassium hydroxide into the oxido alcohol IV, distinguished from a ketonic product by the absence of infrared carbonyl absorption. The hydroxyl group of the *trans*-bromohydrin (III) was shown to be α-oriented by hydrogenation in methanolic potassium hydroxide solution in the presence of palladium–charcoal to testane-3α,17β-diol (V); an identical product was obtained in 54% yield by the action of sodium borohydride on the starting ketone I. The 4-bromine substituent of the 4-bromo-3-ketone II is thus identified as β-oriented, as found in the analogous case in the bile acid series.[2] Also, the bromohydrin III was converted smoothly to an olefinic product (VI) on reaction with zinc and acetic acid.

The known 2-bromocholestane-3-one[5] (VIII) was obtained in high yield, and chromatography of the mother liquor afforded only more of the same product and a little of the 2,2-dibromoketone. Reduction of VIII with sodium borohydride gave a single bromohydrin, identified as *cis* because it gave cholestanone on dehydrohalogenation, and as having a 3β-hydroxyl group by debromination to cholestanol (which resulted in 76% yield by reduction of cholestanone with sodium borohydride). The evidence thus indicates that the halogen atom of 2-bromocholestane-3-one is β-oriented. The infrared spectrum of the bromohydrin acetate showed an undivided, strong band at 8.08 μ, which Jones[6] has shown to distinguish 3β-acetoxy allosteroids from the 3α-epimers. The bromohydrin IX on reduction with zinc and acetic acid gave a hydrocarbon corresponding in constants and in the properties of the dibromide with Mauthner's[7] Δ²-cholestene (X).

Djerassi[8] analyzed the molecular rotation data for seven 3-ketoallosteroids differently substituted at C_{17}, found that the M_D effect of introduction of the 3-keto group is constant (av. +70, range 58 to

(1) Fellow of the Camille and Henry Dreyfus Foundation.

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