[CONTRIBUTION FROM THE PHARMACEUTICAL INSTITUTE, MEDICAL FACULTY, UNIVERSITY OF KYUSHU]

Cholesterol and Related Compounds. II. Bromination of 7-Ketocholestanol and 7-Ketocholestenol

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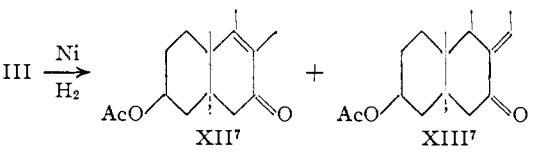
Reaction of 7-ketocholestanyl acetate (I) with N-bromosuccinimide under infrared irradiation gives the 6,8-dibromide, which on dehydrohalogenation yields as the primary product an acetoxydienone, considered to be 7-keto-$\Delta^{5,8(9)}$-cholestadienyl acetate (III). Bromination of 7-ketocholestenyl acetate under the same conditions gives the 8-bromo derivative, IX. The two products have been related by conversion to common reaction products.

When 7-ketocholestanyl acetate (I) is treated with two moles on N-bromosuccinimide with concomitant infrared irradiation, an unstable dibromide II, m.p. 80°, is formed. II readily loses hydrogen bromide when refluxed with pyridine. Three products have been separated by chromatography from this reaction. One of these, m.p. 152–153°, $\lambda_{max}^{C_2H_5OH}$ 239.5 mμ[2] (E 26,650), is identical with a substance described by us in a previous paper,[3] and to which we assigned the structure of 7-keto-$\Delta^{5,8(9)}$-cholestadienyl acetate (III, see below). A second product, V, m.p. 119–120°, λ_{max} 281 mμ (E 25,300), has the empirical formula $C_{27}H_{40}O$, and consequently we considered that it probably is formed from III by loss of the elements of acetic acid with extension of the unsaturated system to give 7-keto-$\Delta^{3,5,8(9)}$-cholestetriene. And indeed we converted III into V by saponification to the free alcohol, conversion to the tosylate and detosylation with dimethylaniline. Presumably V is not an initial product but is formed from III during chromatography on alumina.[4] Indeed the crude dehydrohalogenation product, m.p. 125–135°, shows ultraviolet absorption maxima at 239 mμ (0.862 optical density) due to III, and at 278–280 mμ (optical density, 0.190), due to V. The difference in intensity shows that III is the predominant product.

In addition to these two products, a small amount of another substance, IV, m.p. 109–110°, was obtained, but it has been characterized only by ultraviolet absorption data, λ_{max} 241 mμ (E 5020), 278 mμ (E 14,500).

Since our first paper[3] in which we assigned the structure of 7-keto-$\Delta^{5,8(9)}$-cholestadienyl acetate to III, Inhoffen[5] has assigned this structure to a substance which clearly differs from III both in melting point, m.p. 169–171°, and in the absorption maximum, $\lambda_{max}^{CH_3OH}$ 248 mμ (E 12,800). Substances believed to have structures corresponding to III and to V but in the ergosterol series have recently been described by Glaxo Laboratories[6] and also differ from our products in ultraviolet absorption characteristics. Thus the substance assigned structure III (but with the ergosterol side chain) by the English group has an absorption maximum at 246 mμ (E 12,800), and that corresponding to V (ergosterol side chain) has two absorption maxima, 217 mμ (E 19,000) and 272 mμ (E 14,600). Unfortunately it is not yet possible to predict absorption maxima of cross-conjugated systems. Since our original assignment of structure to III rested mainly on catalytic hydrogenation (Pd catalysis, acetic acid) to 7-ketocholestanyl acetate, we have extended our reduction studies. Catalytic hydrogenation with Raney nickel as catalyst results in absorption of one mole of hydrogen; the product was separated by chromatography on acid-washed alumina into two products, one of which corresponded in physical properties to 7-keto-$\Delta^{8(9)}$-cholestenyl acetate, and the other to 7-keto-$\Delta^{8(14)}$-cholestenyl acetate.[7] The latter substance is assumed to arise by migration of the double bond



XII[7] XIII[7]

during hydrogenation from the 8,9- to the 8,14-position. In addition to these two products, starting material was recovered. Although this result supports structure III which we have assigned to our dienolone, we do not consider this structure as proved in view of the discrepancies noted above and hope that further work will provide conclusive evidence.

If structure III is correct, the dibromo derivative II can be considered as the 6,8-derivative. It is known that monobromination of 7-ketocholestanyl acetate leads to the 6-bromo derivative.[8]

We have also investigated the reaction of 7-ketocholesteryl acetate (VII) with N-bromosuccinimide, again under infrared irradiation. The product is a monobromo derivative IX, which retains the characteristic ultraviolet absorption maximum of VII, m.p. 152°, λ_{max} 233 mμ. On dehydrohalogenation with pyridine (followed by chromatography on alumina) it is converted into a mixture of III and V, and hence it is regarded as the 8-bromo derivative IX. Chromatography of this product results in loss of the elements of acetic acid; the product VIII is regarded as the 8-bromo derivative of $\Delta^{3,5}$-cholestadiene-7-one, since the absorption maxima in the ultraviolet (λ_{max} 279 mμ, E 17,100) is almost identical with that of the unsubstituted dienone (λ_{max} 277 mμ, E 24,400).

(1) Takamine Research Laboratory, Sankyo Co., Ltd., Tokyo, Japan.

(2) An earlier value of 238 mμ[3] is now corrected to 239.5 mμ.

(3) Part I, K. Tsuda, K. Arima and R. Hayatsu, THIS JOURNAL, **76**, 2933 (1954).

(4) A similar reaction has been reported by J. Elks, *et al.*, *J. Chem. Soc.*, 463 (1954).

(5) H. H. Inhoffen and W. Mengel, *Ber.*, **87**, 146 (1954).

(6) J. Elks, *et al.*, *J. Chem. Soc.*, 451, 463 (1954).

(7) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953).

(8) T. Barr, L. M. Heilbron, E. R. H. Jones and F. S. Spring, *J. Chem. Soc.*, 334 (1938).

On dehydrohalogenation with pyridine it is converted into the trienone V. This bromodienone is isomeric with a bromo derivative prepared by Karrer[9] by treatment of $\Delta^{3,5}$-cholestadiene-7-one (XI) with N-bromosuccinimide (without irradiation) and which on dehydrohalogenation gives a trienone isomeric with V. The simplest explanation of these differences is that Karrer's product is the 2-bromo derivative of XI, whereas our product is the 8-bromo derivative. We have treated the dienone XI with N-bromosuccinimide with infrared irradiation and obtained an oil, which on dehydrohalogenation (pyridine) yielded the trienone V together with an isomer, m.p. 150–151°, $\lambda_{max}^{C_2H_5OH}$ 294 mμ (E 10,700), of unknown structure.

Experimental[10]

6,8-Dibromo-7-ketocholestanyl Acetate (II).—To a solution of 5 g. of 7-ketocholestanyl acetate (I) in 50 cc. of carbon tetrachloride, 5 g. (2 moles) of N-bromosuccinimide (NBS) and 0.2 g. of dibenzoyl peroxide were added; the mixture was irradiated with a 375-watt infrared lamp at a distance of 40 cm. and refluxed on a water-bath with exclusion of moisture for 35–40 minutes. After filtration from succinimide, the solvent was removed under reduced pressure below 40°, and the reddish-brown, oily residue that solidified was recrystallized from acetone; pale yellow microcrystals, m.p. 80° dec., yield 2.1 g., recovery of succinimide, 2.55 g.

Anal. Calcd. for $C_{29}H_{46}Br_2O_3$: C, 57.80; H, 7.64. Found: C, 57.64; H, 7.89.

Dehydrobromination of the Dibromide II.—A solution of 3 g. of II in 30 cc. of pyridine was refluxed for 6 hr. The reaction mixture was poured after cooling into ice-chilled dilute HCl and extracted with ether. The ether layer was washed with 10% HCl and water, dried and the ether distilled under reduced pressure to give 1.2 g. of brownish oil. Crystallization from acetone–alcohol gave crude crystals, m.p. 125–135°. The ultraviolet absorption measurement of this material showed λ_{max} 239 mμ (0.862 optical density) and 278–280 mμ (0.190 optical density) (0.004% EtOH). After chromatographic purification through alumina, the amount of the substance showing λ_{max}^{EtOH} 239 mμ was smaller than that in the foregoing ratio. Therefore the 3-acetoxy group had been eliminated during chromatographic purification owing to use of alumina not treated with acid. In this chromatography, 1.1 g. of the crude crystals was dissolved in 300 cc. of benzene–petroleum ether (1:1) and placed on 50 g. of alumina (Brockmann Grade II); the eluate was divided into 40-cc. portions. Fractions 1–4 afforded a colorless oil which solidified and was crystallized three times from ethanol; colorless needles (V), m.p. 119–120°, yield 210 mg., ultraviolet absorption: λ_{max}^{EtOH} 281 mμ (E 25,300), $\alpha^{18}D$ −260.8° (c 1.07 $CHCl_3$), γ_{max} 1670, 1642, 1625 and 1600 cm.$^{-1}$ in Nujol.

Anal. Calcd. for $C_{27}H_{40}O$: C, 85.20; H, 10.59. Found: C, 85.01; H, 10.22.

Fractions 6–8 (same solvent 2:1) afforded a colorless oil which after crystallization from ethanol afforded colorless needles, m.p. 108–109°, λ_{max}^{EtOH} 241 and 278 mμ (E 5020 and 14,500), yield 40 mg.

Fractions 11–15 (same solvent 4:1) afforded a colorless oil which when crystallized from ethanol gave colorless prisms (III), m.p. 152–153°, λ_{max}^{EtOH} 239.5 mμ (E = 26,506), $\alpha^{22}D$ −67.3° (c 0.951 $CHCl_3$), γ_{max} 1775, 1685 and 1620 cm.$^{-1}$ in Nujol, yield 180 mg.

Anal. Calcd. for $C_{29}H_{44}O_2$: C, 79.1; H, 10.0. Found: C, 79.32; H, 9.86.

This substance gave no depression of the melting point on admixture with a sample of 7-keto-$\Delta^{5,8(9)}$-cholestadiene-3-ol acetate described previously.[3]

Conversion of III to V.—A solution of 3 g. of III in 40 cc. of ethanol was treated with 0.7 g. of potassium hydroxide, allowed to stand for 15 hours, and then poured into water and repeatedly extracted with ether. The ether layer was washed with water, dried and the solvent was removed by distillation. The residue (2.1 g.) soon solidified and was dissolved in 10 cc. of pyridine, and after drying, 1.5 g. of tosyl chloride added and the mixture was allowed to stand for 40 hours. The reaction mixture was poured into a mixture of ice-water and dilute hydrochloric acid and extracted with ether. The ether solution was washed with 10% sodium carbonate and water, dried, and the solvent was removed under reduced pressure. The residue (1.9 g.) was crystallized from acetone, colorless needles, m.p. 108–109° (135° clear), yield 1.4 g.

Anal. Calcd. for $C_{34}H_{48}O_4S$: C, 73.91; H, 8.69. Found: C, 73.61; H, 8.60.

One g. of 7-keto-$\Delta^{5,8(9)}$-cholestadiene-3-tosylate thus obtained was refluxed for 14 hours with 40 cc. of dimethylaniline, poured into ice-cooled dilute hydrochloric acid, and extracted with ether. The ether layer was washed with water, dried and the solvent was removed under reduced pressure. The brown oily residue (400 mg.) was dissolved in 40 cc. of petroleum ether–benzene (1:1) and passed through a column of 30 g. of alumina (Brockmann Grade II). The column was eluted with 200 cc. of petroleum ether–benzene (1:1) and the eluate was fractionated into 40 cc. each.

Fractions 3–5 afforded a colorless oil which soon solidified and was crystallized from ethanol; m.p. 119–120°, λ_{max}^{EtOH} 281 mμ (E 25,800), identical with V, yield 120 mg.

7-Keto-6-bromocholestanol Acetate (VI).—To a solution of 3 g. of I in 30 cc. of carbon tetrachloride, 3 g. (2 moles) of NBS and 150 mg. of dibenzoyl peroxide were added and the mixture was refluxed. About one mole of NBS was consumed in 40 minutes and further reaction did not occur on prolonged reflux (1 hr.). The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the residual oil was chromatographed through a column containing 30 g. of alumina (Brockmann Grade II/III) as a petroleum ether–benzene (1:1) solution. Crystallization of the oily residue from acetone yielded VI as colorless plates, m.p. 175°, the temperature recorded in the literature.[8]

Bromination of 7-Ketocholestenyl Acetate with NBS.—To a solution of 6 g. of VII in 50 cc. of CCl_4, 3 g. (1 mole) of NBS and 200 mg. of dibenzoyl peroxide were added, and the mixture was irradiated with a 375-watt infrared lamp at a distance of 40 cm. The reaction ended in about 10 minutes. The mixture was treated in the usual manner, and the red oil obtained thereby was crystallized from acetone; colorless needles, m.p. 147–150°. Further recrystallization from acetone raised the m.p. to 152° (IX), $\alpha^{17}D$ −130° (c 1.01 $CHCl_3$), $\lambda_{max}^{Et_2O}$ 233 mμ (E 14,800), yield 2.2 g.

Anal. Calcd. for $C_{29}H_{45}O_3Br$: C, 66.79; H, 8.63. Found: C, 67.32; H, 8.88.

When the bromide IX (1.7 g.) was purified by chromatography through 40 g. of alumina (Brockmann Grace II) with a 400-cc. mixture (1:2) of petroleum ether and benzene, the eluate yielded colorless needles which melted at 115–116° after two crystallizations from the same solvent mixture, $\lambda_{max}^{Et_2O}$ 279 mμ (E 17,100), $\alpha^{17}D$ −180° (c 1.2 $CHCl_3$), yield 240 mg., ν_{max} 1670, 1620 cm.$^{-1}$ in CCl_4, no (hydroxy) band.

Anal. Calcd. for $C_{27}H_{41}OBr$: C, 70.28; H, 8.89; Br, 17.35. Found: C, 70.54; H, 9.02; Br, 16.87.

Dehydrobromination of 8-Bromo-$\Delta^{3,5}$-cholestadiene-7-one (VIII).—A mixture of 1 g. of VIII and 10 cc. of pyridine was refluxed for 7 hours, cooled and poured into cold 10% HCl. The mixture was extracted with ether, and the ethereal extract washed with 10% HCl and water, and dried. Removal of the solvent under reduced pressure gave a reddish brown oil, which after crystallization from a mixture of acetone and alcohol gave crystals melting at 110–115°. Repeated recrystallization from alcohol raised the m.p. to 119–120°, identical with that of the trienone V, yield 130 mg.

A solution of 300 mg. of VIII in 20 cc. of ethyl acetate containing 50 mg. of PtO_2 was allowed to absorb about 2 moles of hydrogen. After removal of the catalyst, the filtrate was evaporated under reduced pressure, and the colorless solid residue was recrystallized three times from alcohol;

(9) P. Karrer and A. R. Naik, *Helv. Chim. Acta*, **32**, 2393 (1949); *ibid.*, **36**, 1527 (1953).

(10) All m.p.'s not corrected. The authors are indebted to Miss C. Furukawa and Mr. T. Onoe of the Takamine Laboratory, Sankyo Co., Ltd., for microanalysis.

crystals, m.p. 125–126°, λ_{max}^{EtOH} 234 mμ (*E* 13,800), assumed to be Δ^5-cholestene-7-one.

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.37; H, 11.46. Found: C, 83.97; H, 11.44.

Dehydrobromination of 7-Keto-8-bromocholesteryl Acetate (IX).—A solution of 1 g. of IX and 30 cc. of pyridine was refluxed for 7 hours and the reaction mixture was treated in the usual manner; the reddish-brown oily product was chromatographed through a column of 50 g. of alumina (Brockmann Grade II) with a petroleum ether–benzene (1:1) solution (200 cc.). The eluate was fractionated into 40 cc. each.

Fractions 3 and 4 afforded a solid which after crystallization from alcohol gave 80 mg. of colorless crystals, m.p. 119–120°, identical with V.

Development of the column with a benzene–ether (1:1) mixture afforded 90 mg. of III, m.p. 152–153°.

Preparation of 7-Keto-$\Delta^{3,5,8(9)}$-cholestatriene (V) from 7-Keto-$\Delta^{3,5}$-cholestadiene (XI).—To a solution of 4 g. of XI in 25 cc. of carbon tetrachloride, 2 g. of NBS and 150 mg. of dibenzoyl peroxide were added, and the mixture was refluxed on a water-bath, with protection from moisture, with irradiation of a 375-watt infrared lamp at a distance of 40 cm. Most of the NBS was converted to succinimide within 30 minutes, and the reaction was complete in about 50 minutes. The cooled reaction mixture was filtered, and the filtrate was evaporated to a brown, oily residue which could not be crystallized. Therefore, 2 g. of this bromide was dissolved in 30 cc. of pyridine, the mixture was refluxed for 6 hours, and the cooled mixture was poured into ice-cooled dilute hydrochloric acid. This was extracted with ether, the ether layer was washed consecutively with 10% hydrochloric acid and water, dried, and the solvent was removed under reduced pressure.

The brown oily residue (1.2 g.) was dissolved in 200 cc. of petroleum ether–benzene (1:1) and passed through a column containing 40 g. of alumina (Brockmann Grade I/II). The column was eluted with 200 cc. of petroleum ether–benzene and the eluate was fractionated into 40 cc. each. The oily residue obtained from fractions 2 and 3 was recrystallized from ethanol; colorless needles (XV), m.p. 150–151°, λ_{max}^{EtOH} 294 mμ (*E* 10,700), yield 250 mg.

Anal. Calcd. for $C_{27}H_{40}O$: C, 85.20; H, 10.59. Found: C, 85.36; H, 10.49.

Fractions 5–8 afforded an oil which crystallized from ethanol as colorless needles (V), 600 mg., m.p. 119–120°, λ_{max}^{EtOH} 281 mμ (*E* 25,100).

Anal. Calcd. for $C_{27}H_{40}O$: C, 85.20; H, 10.59. Found: C, 85.48; H, 10.73.

Hydrogenation of 7-Keto-$\Delta^{5,8(9)}$-cholesteryl Acetate (III) with Raney Nickel.—A solution of 500 mg. of III dissolved in 20 cc. of ethyl acetate, with Raney nickel as catalyst, was hydrogenated until about 1 mole of H_2 had been absorbed. After removal of the catalyst, the filtrate was evaporated under reduced pressure; 400 mg. of a colorless solid, m.p. 135–145°, was obtained. This was purified by chromatography through 30 g. of acid-treated alumina by dissolving 450 mg. of the crude crystals in 400 cc. of a 1:4 mixture of petroleum ether and benzene. Development with the same solvent and fractionation of the eluate into 40-cc. portions gave, from fractions 4–5, crystals, m.p. 138–146°, λ_{max}^{EtOH} 250–251 and 258–260 mμ. From the results of ultraviolet absorption data, the crude crystals are the mixture of about 15% of a product showing maximal absorption at 250–251 mμ and 85% of a substance with λ_{max} at 258–260 mμ. Recrystallization from methyl alcohol failed to provide a pure substance but a solution of the crystals in 80% methyl alcohol afforded after standing plate crystals which were recrystallized three times from methyl alcohol; colorless plates, m.p. 154.5–155.5°, λ_{max}^{EtOH} 253 mμ (*E* 15,000), yield 25 mg.

The crystals that precipitated from the solution in later stages melted at 143–147°; after three crystallizations from methyl alcohol the colorless scaly crystals melted at 143–144°, λ_{max}^{EtOH} 261 mμ (*E* 9,400), yield 165 mg.

The material of m.p. 143–144° is probably 7-keto-$\Delta^{8(14)}$-cholestenyl acetate, and that of m.p. 154.5–155.5° is probably 7-keto-$\Delta^{8(9)}$-cholestenyl acetate. Recrystallization of fraction 10–12 from ethyl alcohol gave colorless prismatic crystals, m.p. 153–154°, λ_{max}^{EtOH} 239.5 mμ (*E* 26,500), yield 70 mg. This substance is 7-keto-$\Delta^{5,8(9)}$-cholestenyl acetate (III).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF CALIFORNIA, BERKELEY]

The Preparation of Colchicide[1] (Demethoxycolchicine)

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The reaction of colchicine (I) with methyl mercaptan in the presence of zinc chloride resulted in replacement of the methoxyl group by methyl thio to give methylthiocolchicide (II). Partial desulfurization with nickel afforded the tropone, colchicide (III), which formed a hydrochloride and was hydrogenated to tetrahydrodemethoxycolchicine (IV).

Although colchicine has a dramatic activity as a mitotic poison, its use chemotherapeutically has been severely limited by its marked toxicity, and much effort has been expended in attempts to prepare less toxic derivatives which still are mitotic poisons. Within the group of derivatives in which ring C has remained tropoloid, the structural variations have for the most part consisted in replacement of the methoxy group by various alkoxyls, replacement of the methoxyl group by various aminos, and variations in the substituents on the amino group at C_7.[3] The preparation of two further derivatives of this type, methylthiocolchicide[1] (II) and colchicide (III), is described in the present report.

Alkylthiotropones have been prepared by several methods,[4] none of which appeared particularly attractive for the preparation of methylthiocolchi-

(1) H. Rapoport, A. R. Williams, J. E. Campion and D. E. Pack, THIS JOURNAL, **76**, 3693 (1954). A rational basis for this nomenclature has been proposed in footnote 13. Colchicide serves as the name for the compound in which the methoxyl (C_{10}) has been replaced by hydrogen, and as the root name for those compounds in which replacement has been by groups other than hydrogen.

(2) Supported in part from a generous grant by Smith, Kline and French Laboratories.

(3) An excellent review of the physiological activity of colchicine and its derivatives is provided by J. W. Cook and J. D. Loudon in Manske and Holmes, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 291. See also (a) R. M. Horowitz and G. E. Ullyot, THIS JOURNAL, **74**, 587 (1952); (b) J. L. Hartwell, M. V. Nadkarni and J. Leiter, *ibid.*, **74**, 3180 (1952); (c) R. F. Rauffauf, A. L. Farren and G. E. Ullyot, *ibid.*, **75**, 2576 (1953); (d) F. Šantavý, *Chem. Listy*, **46**, 280 (1952); (e) A. Uffer, *Helv. Chim. Acta*, **35**, 2135 (1952); (f) A. Uffer, O. Schindler, F. Šantavý and T. Reichstein, *ibid.*, **37**, 18 (1954).

(4) (a) B. D. Abadir, J. K. Cook, J. D. Loudon and D. K. V. Steel, *J. Chem. Soc.*, 2350 (1952); (b) T. Nozoe, M. Sato and K. Matsui, *Proc. Japan Acad.*, **28**, 407, 410 (1952); *ibid.*, **29**, 22 (1953).