The ultraviolet absorption curves for the synthetic and degradation hydrocarbon were determined with absolute alcohol solutions using concentrations of 2.4×10^{-5} M in the 210–315 mμ region and 3.4×10^{-4} M in the 315–360 mμ region. The earlier ultraviolet absorption curve was determined using petroleum ether solutions.[19]

BOSTON, MASS.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

The Cholegenins. II. Structure of Cholegenin, Isocholegenin and Dihydrocholegenin

BY MALCOLM J. THOMPSON, IRVING SCHEER AND ERICH MOSETTIG

RECEIVED MARCH 13, 1959

It is shown that cholegenin is a 22,25-epoxy-5β-furostane-3α,26-diol (I). Isocholegenin is formulated as spirostane-3α,25-diol (VI). Sodium metaperiodate oxidative cleavage of dihydrocholegenin (II) to a 16,22-epoxynorcoprostan-3α-ol-25-one (III) establishes the structure of II as 16,22-epoxycoprostane-3α,25,26-triol. The partial synthesis of II and III is described.

It was shown in the preceding paper[1] that the catalytic reduction of cholegenin and isocholegenin with platinum oxide in glacial acetic acid at 25° gave only one dihydrocholegenin which upon acetylation yielded a monohydroxy diacetate. The unacylable hydroxyl group was formed by the reductive opening of an epoxide ring of cholegenin and was tentatively placed at C-25 of dihydrocholegenin. It had been established previously[2] that the primary hydroxyl group of cholegenin was in the side chain. If the primary and tertiary hydroxyl groups of dihydrocholegenin are at C-26 and C-25, respectively, then dihydrocholegenin possesses a 1,2-glycol linkage. Oxidation of dihydrocholegenin with sodium metaperiodate in aqueous dioxane gave a precipitate of sodium iodate within five minutes. From the reaction mixture a compound was obtained, in quantitative yield, whose infrared spectrum exhibited strong hydroxyl absorption at 3571 cm.$^{-1}$ (unassociated) and a very strong carbonyl band at 1716 cm.$^{-1}$. Elemental analysis agreed with the empirical formula $C_{26}H_{42}O_8$, showing the loss of one carbon atom. Since the structural formula of cholegenin up to C-22 appears to be well established[2] the oxidation product III must be a 16,22-epoxynorcoprostan-3α-ol-25-one, and dihydrocholegenin a 16,22-epoxycoprostane-3α,25,26-triol (II).

In order to confirm further the structure of II and III, these compounds were synthesized from 16,22-epoxycoprost-25-en-3α-ol (IVa).[1] Acetylation of IVa and subsequent hydroxylation of the acetate IVb with osmium tetroxide in ether and a two-step hydrolysis (aqueous ethanolic sodium sulfite, 2% potassium hydroxide solution) gave II in a yield of 75%. Hydroxylation of IVa with Woodward and Brutcher's[3] more convenient reagent (iodine, silver acetate and wet acetic acid) gave II in a yield of 68%. Compound II obtained from I and from IV proved to be identical. Also the infrared spectra of the diacetates in chloroform and carbon disulfide solution were indistinguishable. In either procedure only one of the C-25 epimers of II was formed. Oxidation of IVb[1] and subsequent hydrolysis of the 3α-acetoxy group gave the 16,22-epoxynorcoprostan-3α-ol-25-one III in a yield of 85%. The compound III samples obtained from II and IVa were identical.

We obtained by oxidation of cholegenin in acetone with chromic acid in dilute sulfuric acid a keto-acid VIII (containing 27 carbons) identical with that of Mazur and Spring[2] formed by oxidizing cholegenin with chromic acid in 80% acetic acid. An identical keto-acid also was formed when isocholegenin was oxidized under our conditions. When this keto-acid (derived from cholegenin or isocholegenin) was reduced with lithium aluminum hydride, cholegenin was obtained in nearly quantitative yields, when no acid was used in the working up process.[4] We found, on the other hand, that cholegenin is very readily and nearly quantitatively isomerized to isocholegenin with dilute ethanolic hydrochloric acid at room temperature in 30 minutes, *i.e.*, under considerably milder conditions than previously had been employed for cholegenin[5] or for C-25 epimerizations.[6]

Establishment of the structure of II together with the finding of Mazur and Spring[2] that drastic oxidation of cholegenin diacetate leads to a 3α-acetoxy-16β-hydroxy bisnorcholanic lactone (VII) allows placement of the oxygen bridge of ring F at C-22 and C-25 and assignment of the structure of 22,25-epoxy-5β-furostane-3α,26-diol for cholegenin (I). According to Callow and Massy-Beresford[7] the C-22 oxygen of the F-ring of the steroidal sapogenins is α-oriented. Since ketone III can be prepared from cholegenin (*via* II) and from sarsasapogenin or smilagenin (*via* IV) it may be concluded that the C-22 oxygen of cholegenin is α-oriented.

Having assigned structure I to cholegenin, we

(1) Previous paper, Malcolm J. Thompson, Irving Scheer and Erich Mosettig, THIS JOURNAL, **81**, 5225 (1959).

(2) Y. Mazur and F. S. Spring, *J. Chem. Soc.*, 1223 (1954).

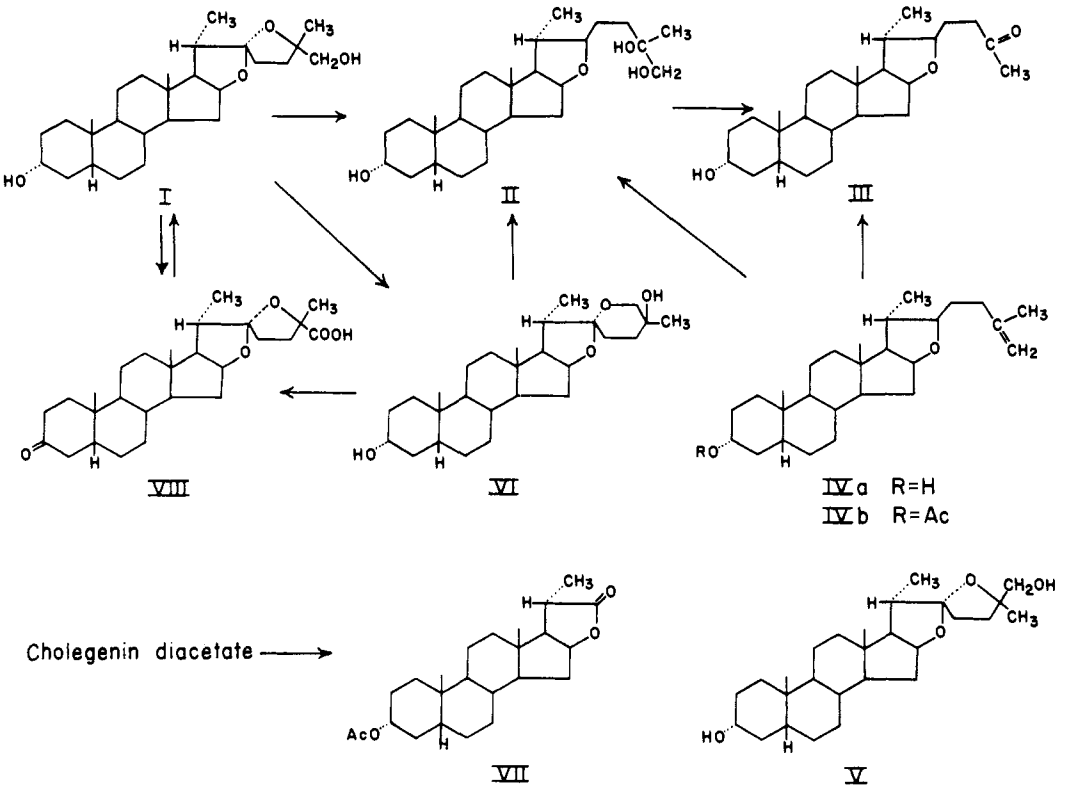
(3) R. B. Woodward and F. V. Brutcher, Jr., THIS JOURNAL, **80**, 209 (1958).

(4) A similar reduction had been carried out by Mazur and Spring[2] who used acetic acid for the decomposition of the reaction mixture. Because of the ease of isomerization of cholegenin to isocholegenin under acidic conditions we employed alkaline decomposition. We assured ourselves in separate experiments that neither cholegenin nor isocholegenin are affected by alkali or lithium aluminum hydride under conditions prevailing during the reduction or isolation.

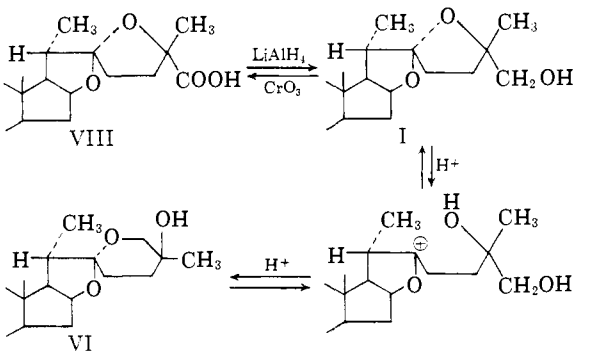
(5) N. J. Anita, Y. Mazur, R. R. Wilson and F. S. Spring, *J. Chem. Soc.*, 1218 (1954).

(6) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939).

(7) R. K. Callow and P. N. Massy-Beresford, *J. Chem. Soc.*, 4482 (1957).



thought isocholegenin may have the C-25 epimeric structure V analogous to the C-25 isomeric spirostanes. This would explain to a certain extent the fact that isocholegenin, in contrast to cholegenin, gives under ordinary conditions only a monoacetate (at C-3). Molecular models show the C-26 hydroxyl group is sterically hindered not only by the C-27 methyl but also by the 3,21α-methyl group. Such a formulation, however, would make it difficult to account for the reaction sequence isocholegenin → keto-acid → cholegenin. Furthermore, inspection of the infrared spectra of cholegenin and isocholegenin showed that only the latter exhibited the typical picture of the 25-D steroidal sapogenin side chain,[8] and led us to assign for isocholegenin structure spirostane-3α,25-diol VI, wherein the methyl group at C-25 is equatorial and the tertiary hydroxyl group is axial. This not only explains more readily the resistance of one of the hydroxyl groups to acetylation, but is also in agreement with the observation of Mazur and Spring[2] that the isocholegenin diacetate band at 1250 cm.$^{-1}$ is complex. This had been observed previously in a number of steroidal axial acetates.[9] Furthermore, we observed that the oxidation of isocholegenin to the keto-acid VIII proceeds considerably more slowly and with greater difficulty than that of cholegenin. In the acidic oxidizing medium an equilibrium between cholegenin and isocholegenin may be assumed which proceeds completely to the left as the cholegenin is oxidized and the oxidation and subsequent lithium aluminum hydride reduction may be pictured as



As further proof for this scheme it was observed that treatment of cholegenin diacetate under the acidic conditions required for the isomerization of cholegenin to isocholegenin gave only starting material.

The secured structure of isocholegenin as VI and the fact that isocholegenin and cholegenin yield upon catalytic reduction only one dihydrocholegenin establish the configuration at C-25 of cholegenin as shown in I.

It is noteworthy that cholegenin is the only known naturally occurring steroidal sapogenin containing a five-membered F-ring and being at the same time a neopentyl alcohol (primary hydroxyl at C-26).

Recently, Sato, *et al.*,[10] transformed N-nitrosotomatodine into 22,25-epoxy-5α-furostan-3β-ol, a

(8) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953); C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).

(9) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951); A. Fürst, H. H. Kuhn, R. Scontoni, Jr., and Hs. H. Gunthard, *Helv. Chim. Acta*, **35**, 951 (1952).

(10) Y. Sato, H. G. Latham, Jr., L. H. Briggs and R. N. Seelye, THIS JOURNAL, **79**, 6089 (1957).

sapogenin with a five-membered F-ring and a *gem*-dimethyl group at C-25.

Experimental[11]

Oxidation of Cholegenin to 22,25-Epoxy-5β-furostan-3-one-26-carboxylic Acid (VIII).—To a stirred solution of 50 mg. of cholegenin in 50 ml. of acetone at 25° was added dropwise an 8 *N* solution of chromic acid in sulfuric acid and water until a persistent orange-brown coloration indicated oxidation was complete.[12] The addition required 10 minutes and the mixture was stirred for an additional 35 minutes. The mixture was diluted with water and the precipitate was collected and dissolved in ether. The ethereal solution was extracted with 5% sodium bicarbonate solution. The aqueous alkaline solution was acidified with 6 *N* hydrochloric acid and the resultant white precipitate was collected and dried to yield 27 mg. of needles, m.p. 202–206°. The ethereal extract was dried over sodium sulfate and concentrated to dryness *in vacuo* to give 17 mg. of neutral material, m.p. 194–197°. Infrared analysis of this neutral material showed strong absorption at 3400 (associated hydroxyl) and 1709 cm.$^{-1}$ (carbonyl). This neutral material was subjected further to oxidation as above except that the reaction was allowed to continue for an additional 3 hours after endpoint was reached and excess of chromic acid reagent had been added. Working up as the above gave 13 mg. of acidic material, m.p. 203–206°. Recrystallization of each acidic fraction from ether–light petroleum (60–70°) gave a total yield of 38 mg. of plates, m.p. 204–207°, $[\alpha]^{20}D$ −37° (lit.[2] m.p. 197–201°, $[\alpha]^{15}D$ −36°).

The two acidic fractions were identical in the infrared and showed γ^{CS_2} 2700–2500 (weak broad adsorption), 1777 and 1709 cm.$^{-1}$ (strong carbonyl absorption).

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.06. Found: C, 72.94; H, 9.35.

Oxidation of Isocholegenin to 22,25-Epoxy-5β-furostan-3-one-26-carboxylic Acid (VIII).—The oxidation of isocholegenin (25 mg.) was carried out under similar conditions as the oxidation of cholegenin except that the oxidation mixture which contained an excess of chromic acid reagent was stirred for 4 hours at 25°. The reaction mixture was worked up as above and 12 mg. of acidic material was obtained, m.p. 203–206°, $[\alpha]^{20}D$ −36°. The neutral fraction recrystallized from dilute acetone gave 8 mg. of needles, m.p. 194–198°, showing an identical infrared spectrum to the one of the neutral fraction obtained above from cholegenin.

Reduction of VIII.—To the keto-acid VIII (10 mg.) in 10 ml. of benzene and 5 ml. of ether was added 1 ml. of 1 *M* solution of lithium aluminum hydride in ether and refluxed for 1 hr. After the addition of a few drops of acetone, 5 ml. of water and 1 ml. of 4 *N* sodium hydroxide, the product was isolated with ether. A solution of the product in benzene was chromatographed on alumina. The fractions eluted with benzene–chloroform 6:1 were recrystallized from dilute ethanol to give 7 mg. of **cholegenin** as needles, m.p. and mixed m.p. 191–193°. Its infrared spectrum was identical with that of cholegenin. The results were the same with either sample of VIII, whether derived from a cholegenin oxidation or an isocholegenin oxidation.

Isocholegenin from Cholegenin.—A solution of 10 ml. of 95% ethanol, 1 ml. of 6 *N* hydrochloric acid and 29 mg. of cholegenin (I) was allowed to stand at room temperature for 30 minutes. The mixture was diluted with water and the resultant precipitate was recrystallized from dilute ethanol to give 25 mg. of isocholegenin (VI) as needles, m.p. 255–258°, $[\alpha]^{20}D$ −68°.

The treatment of cholegenin diacetate under the acidic conditions required for the isomerization of cholegenin to isocholegenin gave only starting material.

Sodium Metaperiodate Oxidation of Dihydrocholegenin (II).—To a solution of 7.4 mg. of dihydrocholegenin[1] in 1 ml. of dioxane and 0.2 ml. of water was added 5 mg. of sodium metaperiodate and the mixture was allowed to stand for three hours at room temperature. Sodium iodate began to come out of solution within 10 minutes after the addition of sodium metaperiodate. The mixture was diluted with 9 ml. of water and the white crystalline precipitate was collected, washed with water and dried to yield 6 mg. of **16,22-epoxynorcoprostan-3α-ol-25-one (III)**, m.p. 133–136°. Recrystallization from dilute acetone gave 5 mg. of thin plates, m.p. 137–139°, γ^{CS_2} 1716 strong (ketone) and 3571 cm.$^{-1}$ strong (unassociated hydroxyl).

Anal. Calcd. for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.38; H, 10.42.

This compound was directly compared with an authentic sample of 16,22-epoxynorcoprostan-3α-ol-25-one (III) by mixture melting point and infrared spectra, and found to be identical in every respect.

16,22-Epoxynorcoprostan-3α-ol-25-one (III) was prepared by the osmium tetroxide–sodium metaperiodate oxidation of IVa.[1] Hydrolysis with 2% potassium hydroxide in methanol afforded 16,22-epoxynorcoprostan-3α-ol-25-one (III) in 85% yield, plates from dilute acetone, m.p. 138–140°, $[\alpha]^{20}D$ +9°, γ^{CS_2} 3571 cm.$^{-1}$ (unassociated hydroxyl), 1716 cm.$^{-1}$ strong (ketone).

Anal. Calcd. for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.28; H, 10.62.

The semicarbazone (semicarbazide hydrochloride, methanol–pyridine–water, steam-bath, 4 hr.) was obtained as white needles from dilute methanol, m.p. 220–224° with slight decomposition.

Anal. Calcd. for $C_{27}H_{45}O_3N_3$: C, 70.55; H, 9.87; N, 9.14. Found: C, 70.36; H, 9.66; N, 9.28.

16,22-Epoxycoprostane-3α,25,26-triol (Dihydrocholegenin (II) from IVa).—A mixture of 2.0 g. of 16,22-epoxycoprost-25-en-3α-ol (IVa), 40 ml. of pyridine and 10 ml. of acetic anhydride was heated on a steam-cone for 1 hr. and concentrated to dryness *in vacuo*. The crystalline residue was dissolved in 150 ml. of dry ether. To the solution was added 2 ml. of pyridine and 1.6 g. of osmium tetroxide. The mixture was allowed to stand overnight at room temperature. The ether was removed *in vacuo* at 30°. To the black residue was added 200 ml. of ethanol and 100 ml. of 10% aqueous sodium sulfite and the mixture was refluxed for 1.5 hr. The black precipitate of osmium was collected and 6.0 g. of solid potassium hydroxide was added to the filtrate and refluxed for 2 hr. on a steam-cone. The solution was concentrated to 100 ml. *in vacuo* and extracted with ether. The ethereal solution was washed with water and dried over sodium sulfate. The ether was evaporated *in vacuo* to a crystalline residue which was dissolved in 50 ml. of benzene–chloroform (3:1) and chromatographed over neutral alumina. The fraction eluted with chloroform and chloroform–2% ethanol was obtained as a hydrate melting at 82–86° which resolidified at 110° and remelted at 153–158°. Recrystallization from ether–petr. ether (60–70°) gave 1.6 g. of needles, m.p. 149–152°. One recrystallization from ethyl acetate gave analytically pure material, m.p. 155–157°, $[\alpha]^{20}D$ +15°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.40; H, 10.88.

The diacetate (acetic anhydride–pyridine, 1 hr., steam-bath) was obtained as white rectangular plates, m.p. 116–118°, $[\alpha]^{20}D$ +22°, γ^{CS_2} 1736 strong (acetate) and 3400 cm.$^{-1}$ strong (associated hydroxyl).

Anal. Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72. Found: C, 71.52; H, 9.83.

Hydroxylation of IVa with iodine, silver acetate and wet acetic acid[3] gave II in yield of approximately 68%.

Dihydrocholegenin (II) obtained from the catalytic reduction of cholegenin[1] and 16,22-epoxycoprostane-3α,25,26-triol (II) obtained from IV and their respective acetates were identical (mixed melting point, infrared spectra and optical rotations).

Acknowledgments.—We are deeply indebted to Professor F. S. Spring of the Royal Technical College, for his generous permission to continue the work with the cholegenins. Microanalyses are by the Analytical Service Laboratory of this Institute under the direction of Dr. William C. Alford. Infrared spectra were determined by Mr. H. K. Miller of this Laboratory.

BETHESDA 14, MD.

(11) All melting points were determined on the Kofler block. Unless otherwise noted, rotations were determined in approximately 1% solutions in chloroform and activity grade I alumina (Woelm) was used for chromatography. Infrared spectra were obtained with a Perkin–Elmer model 21 double beam spectrophotometer with sodium chloride prism and cells.

(12) A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, 2548 (1953).