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The Cholegenins. I. 16,22-Epoxycoprostane-3α,26,27-triol and its Non-identity with Dihydrocholegenin

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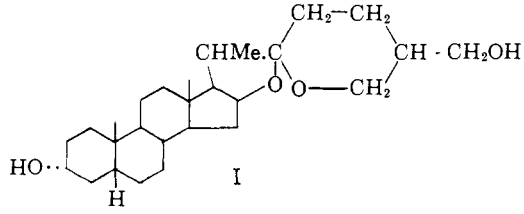
It is shown that the structure of cholegenin is not as reported in the literature. The partial synthesis of 16,22-epoxycoprostane-3α,26,27-triol and its 3β-epimer are described.

Our interest in the stereochemistry of the steroidal sapogenins prompted us to investigate the chemical reactivity of cholegenin and isocholegenin. These two compounds recently were isolated by Anita, Mazur, Wilson and Spring[1] from ox bile and their structures shown by Mazur and Spring[2] to be a spirostane-3α,27-diol differing in configuration at C-25 (I).



I

Structure I promised a ready means to resolve the question as to whether the normal and "iso" sapogenins were isomeric at C-22 as well as at C-25.[3] Dehydration of the hydroxyl function at C-27 and introduction of the C-25,27 double bond would eliminate the asymmetric center at C-25 giving identical products from cholegenin and isocholegenin if indeed they were isomeric only at C-25. However, our attempts to isolate a sapogenin from ox bile were unsuccessful.[4] We finally utilized the material obtained by Spring's group.[4] The controversial nature of the problem[5] we were attacking made it imperative that we deal with material of unequivocal structure.[6] Thus we set about to study further reactions of cholegenin and isocholegenin. The result of this work will be described below and in subsequent papers.

First an attempt was made to reduce cholegenin to 3-deoxysarsasapogenin thereby establishing both the location of the side-chain hydroxyl group on C-27 and the nature of the F-ring as proposed by Mazur and Spring.[2] Cholegenin ditosylate was readily prepared from cholegenin. Treatment of the ditosylate with lithium aluminum hydride did not yield the expected deoxysarsasapogenin but instead an 80% yield of cholegenin was obtained. A monohydroxy by-product was obtained in 20% yield but the total amount of material available was too small to identify. When the ditosylate was boiled for twenty hours with sodium iodide in acetone 80% of the starting material was recovered. The remainder consisted of a not clearly identifiable mixture of products (see Experimental).

Catalytic hydrogenation of C-25 epimeric steroidal sapogenins is known to yield C-25 isomeric dihydrosapogenins no longer containing a spiroketal structure.[7] If cholegenin had the assigned structure I and isocholegenin differed only in configuration at C-25 they would be expected to yield on hydrogenation the identical dihydrosapogenin, containing three hydroxyl groups. The experiment appeared to bear out this assumption, for hydrogenation (PtO_2, acetic acid, 25°) of either cholegenin or isocholegenin gave only one dihydrocholegenin. This compound no longer exhibited the characteristic bands in the infrared spectrum of a steroidal sapogenin and should be the hitherto unknown 16,22-epoxycoprostane-3α,26,27-triol (XIa).

Acetylation however of dihydrocholegenin (acetic anhydride–pyridine, 16 hours at room temperature or for one hour on steam-bath) yielded a monohydroxy-diacetate.

The partial synthesis of dihydrocholegenin appeared to be an available proof for the terminal position of the cholegenin side-chain. Episarsasapogenin (IIa) was chosen as starting material with the assumption that it does not differ from cholegenin at C-22. For model experiments we

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

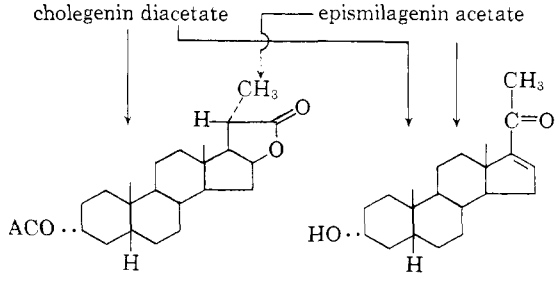
(2) Y. Mazur and F. S. Spring, *ibid.*, 1222 (1954).

(3) This question has been settled unequivocally by the recent elegant proof of R. K. Callow and P. N. Massy-Beresford, *ibid.*, 4482 (1957), showing isomerism existing only at C-25.

(4) We are indebted to Merck & Co., Inc., for supplying the neutral fraction from ox bile and to Dr. M. E. Wall of the Dept. of Agriculture, Eastern Regional Research Laboratory, for fractionating the material present therein. We wish also to acknowledge our debt to Professor F. S. Spring of the Royal Technical College, Glasgow, for his generous gift of all the cholegenin and isocholegenin left in his possession.

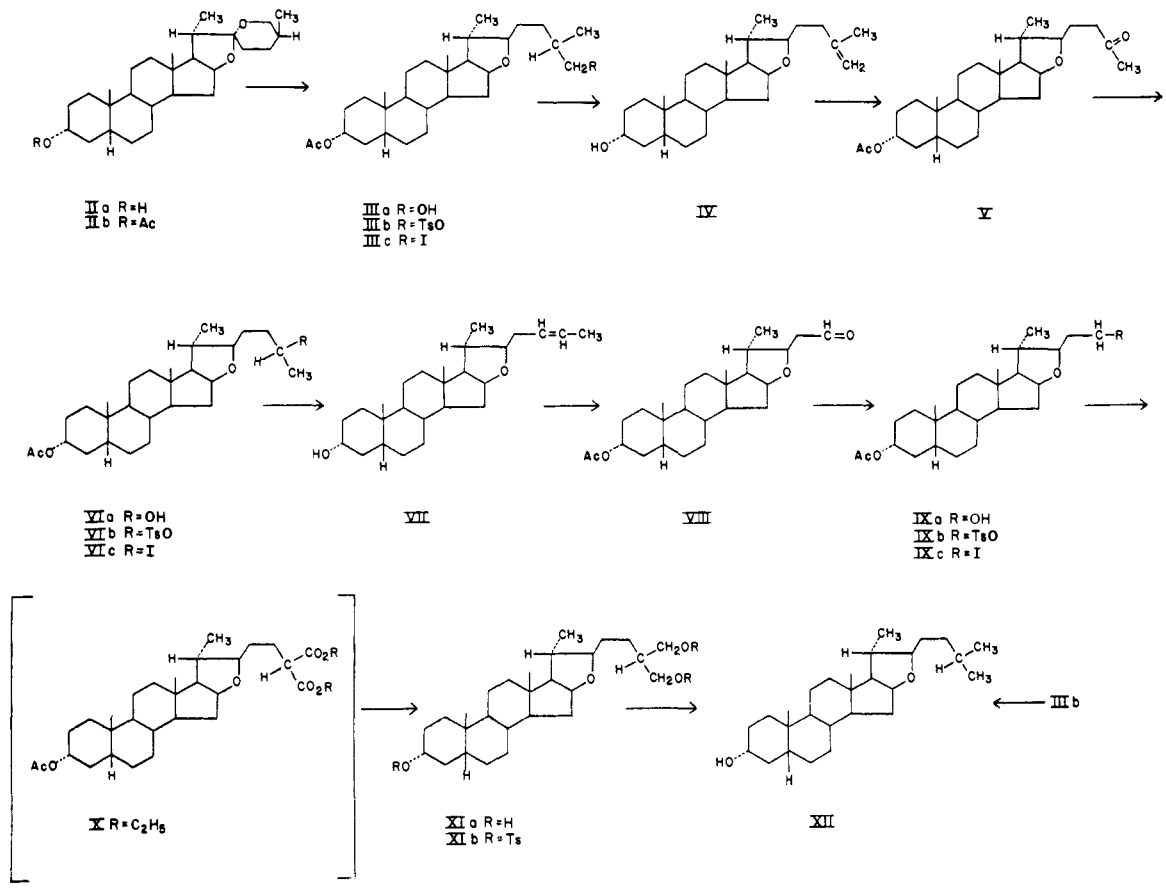
(5) I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **75**, 4871 (1953); M. E. Wall and S. Serota, *ibid.*, **76**, 2850 (1954); J. B. Ziegler, W. E. Rosen and A. C. Shabica, *ibid.*, **76**, 3865 (1954); M. E. Wall, S. Serota and C. R. Eddy, *ibid.*, **77**, 1230 (1955); R. K. Callow and V. H. T. James, *Chemistry & Industry*, 691 (1954); D. H. W. Dickson, J. Elks, R. M. Evans, I. F. Oughton and J. E. Page, *ibid.*, 692 (1954); D. A. H. Taylor, *ibid.*, 1066 (1954).

(6) Although Mazur and Spring's degradation studies related the C-22 moiety from cholegenin with 3α-acetoxy-16β-hydroxybisnorcholanic lactone obtained from epismilagenin acetate, the structure of the



F-ring and assignment of position of the hydroxyl group at C-27 was adduced from oxidation and infrared spectral studies and was not conclusive. The sapogenin nature of the side chain of cholegenin was apparent upon its behavior on treatment with acetic anhydride and subsequent oxidations to yield a Δ^{16}-pregnenolone compound identical with that obtained upon similar treatment of smilagenin.

(7) I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **77**, 641 (1955).



went through the planned synthesis with sarsasapogenin (3β-OH). The various intermediates of this series are mentioned in the Experimental only.

Hydrogenation of episarsasapogenin acetate (IIb) with platinum oxide in acetic acid at 25° gave 16,22-epoxycoprostane-3α,26-diol 3α-acetate (IIIa) in practically quantitative yield. Tosylation of IIIa afforded a crystalline tosylate IIIb which reacted readily with sodium iodide in acetone to give IIIc in an over-all yield of approximately 75% from IIb. Dehydrohalogenation of IIIc with a 25% methanolic potassium hydroxide solution[8] gave 16,22-epoxycoprost-25-en-3α-ol (IV). Its infrared spectrum showed hydroxyl absorption at 3550 and double bond absorption at 1653 cm.$^{-1}$. A very strong band at 887 cm.$^{-1}$ indicated the presence of a methylene group. Acetylation and oxidative cleavage of IV using the elegant procedure of Pappo, *et al.*,[9] gave 16,22-epoxynorcoprostan-3α-ol-25-one 3α-acetate (V) in 85% yield. The infrared spectrum of V exhibited strong absorption at 1736 and 1720 cm.$^{-1}$ (acetate and aliphatic ketone). This compound readily yielded a crystalline semicarbazone. Reduction of V with sodium borohydride in aqueous methanol gave 16,22-epoxynorcoprostane-3α-25-diol 3α-acetate (VIa) in quantitative yield. When the tosyl derivative VIb was treated with sodium iodide in acetone a 90% yield of VIc was obtained. Dehydrohalogenation of VIc readily gave 16,22-epoxynorcoprost-24-en-3α-ol (VII). The infrared absorption spectrum of VII showed hydroxyl absorption at 3570 cm.$^{-1}$ and a very strong band at 965 cm.$^{-1}$ which indicated *trans* ethylenic double bond. The presence of weak absorption at 910 and 995 cm.$^{-1}$ may be indicative of a vinyl type double bond due to the presence of some C - 25 ene - epimer. Recrystallization of VII had no effect on the intensity of these bands. Acetylation and oxidation of VII as of IV gave a 60% yield of the acetoxy-aldehyde VIII. The oily aldehyde was not further purified, but infrared analysis of the crude acetoxy-aldehyde indicated aldehyde absorption at 2725 cm.$^{-1}$ and in the carbonyl region at 1730 cm.$^{-1}$ as a shoulder due to some overlapping by the acetate absorption at 1736 cm.$^{-1}$. It was characterized as the crystalline semicarbazone. Reduction of VIII with sodium borohydride gave 16,22-epoxycholane-3α,24-diol 3α-acetate (IXa). It was obtained crystalline afterchromatography on alumina in a yield of 60% from VII. The crystalline tosylate IXb upon treatment as described above gave 16,22-epoxy-24-iodocholan-3α-ol acetate (IXc). Alkylation of IXc with diethyl malonate gave X which was not isolated but immediately reduced with lithium aluminum hydride in ether yielding 16,22-epoxycoprostane-3α,26-27-triol (XIa). Acetylation of XIa readily gave an oily triacetate. The tosylate XIb was prepared and without isolation reduced with lithium aluminum hydride to give 16,22-

(8) Y. Sato, H. G. Latham, Jr., and I. Scheer, *J. Org. Chem.*, **21**, 689 (1956).

(9) R. Pappo, D. S. Allen, Jr., R. U. Lemieux and W. S. Johnson, *ibid.*, **21**, 478 (1956).

epoxycoprostan-3α-ol-(XII) in a 25% yield. No hydrocarbon was obtained. The structure of XII was established by comparison with authentic 16,22-epoxycoprostan-3α-ol obtained from IIIb in the usual manner.[7] The over-all yield of XIa from II was 25%. Also XIa was obtained in very poor yield on the Knoevengel reaction of the aldehyde VIII with diethyl malonate followed by hydrogenation of the alkylidene malonic ester and subsequent lithium aluminum hydride reduction.

There was a marked similarity between the infrared spectra of XIa and dihydrocholegenin although a distinct difference in the fingerprint region could be seen. Mixed melting point determination of XIa with the lower melting polymorphic form of dihydrocholegenin, m.p 155–157°, gave a slight depression in melting point accompanied by resolidification and remelting at 173–178° The optical rotation of XIa and dihydrocholegenin differed by about 5°.

The fact that the infrared spectra of XIa and dihydrocholegenin are different and that XIa readily forms a triacetate while dihydrocholegenin gives a monohydroxy-diacetate under similar conditions indicates that dihydrocholegenin is not a 16,22-epoxycoprostane-3α,26,27-triol as would be expected from the hydrogenation of I. Molecular model inspection of the C-22 epimers of XIa shows no indication of steric hindrance with regard to the ease of esterification of the hydroxyl groups. Therefore it is concluded that cholegenin (I) does not have the side-chain structure as reported by Mazur and Spring.[2] The unacylable hydroxyl group in dihydrocholegenin is most reasonably a tertiary hydroxyl group.

Experimental[10]

Cholegenin Ditosylate.—A solution of 13 mg. of cholegenin, 100 mg. of *p*-toluenesulfonyl chloride and 1 ml. of pyridine was allowed to stand overnight at room temperature. The mixture was poured into cold water and extracted with ether. The ethereal solution was washed with water, 2% sodium bicarbonate solution, water and dried over sodium sulfate. On evaporation *in vacuo* a crystalline residue was obtained m.p. 148–152°. Recrystallization from ether–petr. ether (30–60°) afforded 14.5 mg. of cholegenin ditosylate as fine needles, m.p. 160–160.5°, typical tosyl bands in infrared γ^{CS_2} 943, 813 and 668 cm.$^{-1}$, no hydroxyl absorption.

Reaction of Cholegenin Ditosylate with Lithium Aluminum Hydride.—To a solution of 9 mg. of cholegenin ditosylate in 5 ml. of dry ether was added a solution of 1 ml. of 1 *M* lithium aluminum hydride in ether plus 4 ml. of dry ether and the mixture was refluxed for 3 hr. The reaction mixture was cooled and after the addition of a few drops of ethyl acetate, treated with 4 *N* sodium hydroxide. The aqueous layer was separated, extracted with ether and the extract combined with the original ether layer. The combined extracts were washed with water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The crystalline residue, m.p. 154–183°, was chromatographed. The fraction eluted with benzene–$CHCl_3$ (9:1) gave 1 mg. of crystalline material, m.p. 148–152°. Infrared analysis indicated strong associated hydroxyl absorption at 3400 cm.$^{-1}$ in carbon disulfide. Its chromatographic behavior indicated that one hydroxyl group had been eliminated. Further elution of the column with benzene–chloroform 3:1 and 1:1 gave 4 mg. of cholegenin (identified by mixture melting point and infrared spectra) as needles from dilute alcohol, m.p. 189–192°.

Reaction of Cholegenin Ditosylate with Sodium Iodide.—A mixture of 14 mg. of cholegenin ditosylate, 0.1 g. of sodium iodide and 10 ml. of dry acetone was refluxed for 22 hr. The solution was diluted with water and extracted with ether. The ethereal solution was given the usual work-up and dried over sodium sulfate. The ether was evaporated to dryness *in vacuo*. The residue crystallized from light petroleum (30–60°) gave 9 mg. of starting material, namely, cholegenin ditosylate, m.p. 158–160°. The mother liquor yielded non-crystalline material that gave a positive Beilstein halogen test and exhibited the strong typical tosyl bands in the infrared.

Dihydrocholegenin from Cholegenin.—A mixture of 50 mg. of cholegenin, 20 mg. of Adams catalyst and 10 ml. of acetic acid was shaken with hydrogen at room temperature and atmospheric pressure for 3 hr. The crystalline residue obtained from the mixture was chromatographed on benzene–chloroform 3:1 washed alumina. The fractions eluted with chloroform and chloroform–2% ethanol were hydrated, m.p. 78–83°, resolidification at 110° with remelting at 153–157°. Recrystallization from ether–light petroleum (60–70°) gave 38 mg. of needles, 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, 74.38; H, 10.85.

The dihydrocholegenin was obtained in a polymorphic form melting at 175–178°. Its identity was established by infrared analysis.

The monohydroxy-diacetate (acetic anhydride–pyridine, 18 hr., 25° or steam-bath, 1 hr.) was obtained as rectangular plates from dilute methanol, m.p. 114–116°, γ^{CS_2} 3400 cm.$^{-1}$ strong (associated hydroxyl) and 1736 cm.$^{-1}$ strong (acetate).

Anal. Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72. Found: C, 71.50; H, 9.80.

Dihydrocholegenin from Isocholegenin.—A mixture of 24 mg. of isocholegenin, 20 mg. of Adams catalyst and 10 ml. of acetic acid was shaken with hydrogen at room temperature and atmospheric pressure for 3 hr. The catalyst was removed by filtration and the solution was concentrated to dryness *in vacuo*. The residue recrystallized from dilute acetone gave the hydrate, m.p. 75–80°, resolidified about 110° and remelting at 150–157°. Recrystallization from ether–light petroleum (60–70°) yielded 16 mg. of needles, 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, 74.36; H, 10.63.

The monohydroxy-diacetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as rectangular plates from dilute methanol, m.p. 115–117°, γ^{CS_2} 3400 cm.$^{-1}$ strong (hydroxyl) and 1736 cm.$^{-1}$ strong (acetate).

The dihydrocholegenins obtained from cholegenin or isocholegenin gave no melting point depression on admixture. Their infrared spectra were found to be identical in every respect. The acetate derivatives behaved similarly.

Episarsasapogenin Acetate (IIb).—To a stirred solution of 50.0 g. of sarsasapogenin in 2.5 liters of acetone at 30° was added, within two minutes, 46 ml. of an 8 *N* solution of chromic acid in dilute sulfuric acid[11] (*ca.* 40%). A precipitate of the sarsasapogenone commenced coming out of solution immediately. The mixture was stirred for 30 minutes and after dilution with water the crude sarsasapogenone was collected and dried, 48.0 g., m.p. 218–222°, $[\alpha]^{20}D$ −69°, γ^{CS_2} 1710 cm.$^{-1}$ (lit.[12a] m.p. 226°,[12b] 222–223°, $[\alpha]^{26}D$ −70°).

To the crude sarsasapogenone, without further purification, in 500 ml. of benzene was added 1 liter of ether and 100 ml. of a 1 *M* solution of lithium aluminum hydride in ether and refluxed for 2 hr. After cautious decomposition with ethyl acetate and water, 6 *N* hydrochloric acid was added until two distinct clear layers were obtained. The aqueous layer was separated, extracted with ether and the extract combined with the benzene–ether layer. The combined extracts were washed with 10% sodium bicarbonate solution and with water, dried over sodium sulfate and evaporated to dryness

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

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

(12) (a) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 943 (1939); (b) M. E. Wall and S. Serota, *ibid.*, **78**, 1747 (1956).

in vacuo. The crystalline residue of episarsasapogenin (43.0 g., m.p. 194–198°) was treated with 270 ml. of pyridine and 135 ml. of acetic anhydride and allowed to stand for 18 hr. at room temperature. The solution was evaporated to dryness *in vacuo* and the resultant crystalline residue was triturated with methanol and filtered to give 44.0 g. of needles, m.p. 194–197°. A small sample of **episarsasapogenin acetate** (IIb), recrystallized from ether–methanol, gave needles, m.p. 195–197°, $[\alpha]^{20}D$ −46° (lit.[12a] m.p. 194°).

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.88; H, 10.06.

16,22-Epoxycoprostane-3α,26-diol 3α-Acetate (IIIa).—A mixture of 25.0 g. of episarsasapogenin acetate (IIb), 2.0 g. of Adams catalyst and 700 ml. of glacial acetic acid was shaken with hydrogen at room temperature and atmospheric pressure for 24 hr. The spent catalyst was removed by filtration and the solution concentrated to dryness *in vacuo* to give 25.0 g. of IIIa as needles, m.p. 125–129°. A sample recrystallized from dilute methanol gave needles, m.p. 131–133°, $[\alpha]^{20}D$ +16°.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.61; H, 10.50. Found: C, 75.39; H, 10.62.

16,22-Epoxycoprostane-3β-Acetate (Epimer of IIIa).—This compound[13] was obtained as needles from dilute methanol, m.p. 96–97°, $[\alpha]^{20}D$ +2° upon hydrogenation of sarsasapogenin acetate.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.61; H, 10.50. Found: C, 75.50; H, 10.55.

16,22-Epoxycoprostane-3α,26-diol 3α-acetate-26-tosylate (IIIb).—To a solution of 24.0 g. of crude 16,22-epoxycoprostane-3α,26-diol 3α-acetate (IIIa) in 180 ml. of dry pyridine was added 26.0 g. of *p*-toluenesulfonyl chloride and the mixture was allowed to stand overnight at room temperature. The solution was poured into ice and water and the oily precipitate was ether extracted. The ethereal solution was washed with cold 5% hydrochloric acid, water, 2% sodium bicarbonate solution, water and dried over sodium sulfate. The solution was concentrated to dryness *in vacuo* to leave 25.0 g. of a colorless oily tosylate (IIIb). For characterization and analysis a 0.2 g. portion of the oil was chromatographed on Florisil. Crystallization from petroleum ether (60–70°) of the fraction eluted with benzene–petroleum ether (60–70°) 1:1 yielded 150 mg. of rectangular plates, m.p. 118–120°, $[\alpha]^{20}D$ +21°.

Anal. Calcd. for $C_{36}H_{54}O_6S$: C, 70.32; H, 8.85. Found: C, 70.05; H, 8.99.

16,22-Epoxycoprostane-3β,26-diol 3β-Acetate-26-tosylate (Epimer of IIIb), plates from petroleum ether (60–70°), m.p. 94–96°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{36}H_{54}O_6S$: C, 70.32; H, 8.85. Found: C, 70.61; H, 8.76.

16,22-Epoxy-26-iodo-coprostan-3α-ol 3α-Acetate (IIIc).—A solution of 27.5 g. of 16,22-epoxycoprostane-3α,26-diol 3α-acetate-26-tosylate (IIIb) in 100 ml. of acetone was added to a solution of 40.0 g. of sodium iodide in 150 ml. of acetone and refluxed for 16 hr. The precipitate of sodium *p*-toluenesulfonate was collected and the acetone solution was evaporated to dryness *in vacuo*. The residue was treated with water and extracted with ether. The ethereal extract was washed with 5% sodium thiosulfate solution, water, dried over sodium sulfate and concentrated to dryness *in vacuo* to yield 24.0 g. of crystalline residue IIIc, m.p. 106–109°. A 0.2-g. sample crystallized from ether–ethanol gave 0.17 g. of rods, m.p. 111–112°, $[\alpha]^{20}D$ +24°.

Anal. Calcd. for $C_{29}H_{47}O_3I$: C, 61.04; H, 8.30. Found: C, 61.30; H, 8.50.

16,22-Epoxy-26-iodo-coprostan-3β-ol acetate (epimer of IIIc), rods from ether–ethanol, m.p. 123–125°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{29}H_{47}O_3I$: C, 61.04; H, 8.30. Found: C, 61.00; H, 8.40.

16,22-Epoxycoprost-25-en-3α-ol (IV).—A mixture of 23.8 g. of 16,22-epoxy-26-iodo-coprostan-3α-ol acetate (IIIc) and 200 ml. of a 25% methanolic potassium hydroxide solution[8] was refluxed for 3.5 hr. The mixture was poured into 2 liters of water and the crude precipitate of IV collected. Crude IV (16.0 g., m.p. 110–116°) was dissolved in benzene and chromatographed on benzene washed neutral alumina. The fractions eluted with benzene–chloroform 9:1 and 6:1 were recrystallized from petroleum ether to yield 14.5 g. of needles, m.p. 121–122°, $[\alpha]^{20}D$ +4°; γ^{CHCl_3} 3550 and 3400 cm.$^{-1}$ (hydroxyl), 1653, 887 cm.$^{-1}$ ($R_1R_2C{=}CH_2$).

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 80.65; H, 11.25.

The acetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as thin needles from dilute methanol, m.p. 92–93.5°, $[\alpha]^{20}D$ +20°.

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.91; H, 10.74.

The benzoate (benzoyl chloride–pyridine, 2 hr., steam-bath) was obtained as plates from methanol, m.p. 126–128°, $[\alpha]^{20}D$ +17°.

Anal. Calcd. for $C_{34}H_{48}O_3$: C, 80.90; H, 9.59. Found: C, 80.88; H, 9.67.

16,22-Epoxycoprost-25-en-3β-ol (epimer of IV), needles from dilute methanol, m.p. 111–113°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 81.01; H, 11.10.

The acetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as thin plates from dilute methanol, m.p. 72–74°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.37; H, 10.42.

The benzoate (benzoyl chloride–pyridine, 18 hr. 25°) was obtained as needles from methanol, m.p. 120–122°, $[\alpha]^{20}D$ +1°.

Anal. Calcd. for $C_{34}H_{48}O_3$: C, 80.90; H, 9.59. Found: C, 80.96; H, 9.79.

16,22-Epoxynorcoprostan-3α-ol-25-one Acetate (V).—A mixture of 10.5 g. of 16,22-epoxycoprost-25-en-3α-ol (IV), 70 ml. of pyridine and 30 ml. of acetic anhydride was allowed to stand overnight at room temperature. The solution was concentrated to dryness *in vacuo* and the residue dissolved in 220 ml. of dioxane. To the solution at room temperature was added 55 ml. of water, 150 mg. of osmium tetroxide and, after stirring for about 10 minutes, 11.0 g. of sodium metaperiodate[9] was added over a period of 30 minutes. The mixture was stirred for an additional 3 hr. at which time the solution had become colorless and contained a thick white precipitate of sodium iodate. The solution was filtered and the filtrate was poured into 2 liters of cracked ice and water. The crystalline precipitate was collected and dried to give 10.0 g. of V, m.p. 103–107°. A 200-mg. sample recrystallized twice from dilute acetone gave 160 mg. of rods, m.p. 114–116°, $[\alpha]^{20}D$ +26°; γ^{CS_2} 1736, 1720 cm.$^{-1}$ (acetate, 25-ketone, respectively).

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.83; H, 10.14.

The semicarbazone (semicarbazide hydrochloride, ethanol–pyridine steam-bath, 2 hr.) was obtained as needles, m.p. 209–212°.

Anal. Calcd. for $C_{29}H_{47}O_4N_3$: C, 69.42; H, 9.44; N, 8.38. Found: C, 69.28; H, 9.42; N, 8.31.

16,22-Epoxynorcoprostan-3β-ol-25-one acetate (Epimer of V) yielded needles from dilute acetone, m.p. 157–159°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.60; H, 10.15.

16,22-Epoxynorcoprostane-3α,25-diol 3α-Acetate (VIa).—A mixture of 9.8 g. of crude 16,22-epoxynorcoprostan-3α-ol-25-one acetate (V), 3.0 g. of sodium borohydride, 150 ml. of methanol and 5 ml. of water was allowed to stand overnight at 0°. The mixture was acidified with dilute acetic acid and poured into 1.5 liters of cracked ice and water. The crystalline residue was collected, dried and chromatographed on benzene-washed alumina. The fractions eluted with benzene–chloroform 9:1 and 6:1 were combined and crystallized from petroleum ether (60–70°) to give 9.0 g. of VIa as white rods, m.p. 115–118°. A sample recrystallized only once from petroleum ether (60–70°) gave analytically pure material, m.p. 117–119°, $[\alpha]^{20}D$ +21°.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: 75.54; H, 10.09.

(13) Since the experimental conditions for the 3β-epimers were dentical to that of the 3α-series, only their physical properties will be reported. The parent compound in this synthetic series was, of course, sarsasapogenin. We regret, however, that the physical properties of VIc and VIII, and IXb of the 3β-epimers were not obtained.

16,22-Epoxynorcoprostane-3β,25-acetate (epimer of VIa) was obtained as plates from petroleum ether (60–70°), m.p. 148–150°, $[\alpha]^{20}D$ +2°.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.23; H, 10.47.

16,22-Epoxynorcoprostane-3α,25-diol 3α-Acetate-25-tosylate (VIb).—A mixture of 9.6 g. of crude 16,22-epoxynorcoprostan-3α,25-diol 3α-acetate (VIa), 50 ml. of pyridine and 10.0 g. of *p*-toluenesulfonyl chloride treated in the same manner as described for the preparation of IIIb yielded 12.0 g. of crude tosylate VIb, m.p. 115–120°. For characterization and analysis a portion was chromatographed on Florisil. Crystallization from petroleum ether (60–70°) of the fraction eluted with benzene–petroleum ether (9:1) yielded needles, m.p. 122–124°, $[\alpha]^{20}D$ +24°.

Anal. Calcd. for $C_{55}H_{52}O_6S$: C, 69.96; H, 8.72. Found: C, 69.87; H, 8.45.

16,22-Epoxynorcoprostane-3β,25-diol 3β-acetate-25-tosylate (epimer of VIb) was obtained as needles from petroleum ether (60–70°), m.p. 112–114°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{35}H_{52}O_6S$: C, 69.96; H, 8.72. Found: C, 69.75; H, 8.50.

16,22-Epoxy-25-iodonorcoprostan-3α-ol Acetate (VIc).—A mixture of 11.8 g. of crude 16,22-epoxynorcoprostane-3α,25-diol 3α-acetate-25-tosylate (VIb), 20.0 g. of sodium iodide and 140 ml. of dry acetone was refluxed overnight. The mixture, worked up in exactly the same manner as described above for the preparation of IIIc, gave 10.0 of the 25-iodo compound VIc, m.p. 120–124°. A sample recrystallized from ether–ethanol yielded needles, m.p. 122–124°.

Anal. Calcd. for $C_{28}H_{45}O_3I$: C, 60.42; H, 8.15. Found: C, 60.30; H, 8.30.

16,22-Epoxynorcoprost-24-en-3α-ol (VII).—Ten grams of the 25-iodo compound VIc was disssolved in 100 ml. of methanolic potassium hydroxide (25%) and refluxed for 18 hr. The solution was poured into 1 liter of cold water and the resultant precipitate collected. The dried material was chromatographed over benzene-washed alumina. The fractions eluted with benzene–chloroform 9:1 and 6:1 were combined and recrystallized from petroleum ether to yield 7.0 g. of elongated needles of VII, m.p. 94.5–95.5, $[\alpha]^{20}D$ +4°, γ^{CS_2} 3540 cm.$^{-1}$ (hydroxyl) and strong band at 965 cm.$^{-1}$ (*trans* ethylenic double bond).

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.86; H, 10.83.

The benzoate (benzoyl chloride–pyridine, 2 hr., steam-bath) was obtained as needles, m.p. 108–109.5°, $[\alpha]^{20}D$ +22°.

Anal. Calcd. for $C_{33}H_{46}O_3$: C, 80.77; H, 9.45. Found: C, 80.83; H, 9.38.

16,22-Epoxynorcoprost-24-en-3β-ol (epimer of VII) was obtained as needles from dilute acetone, m.p. 119–121°, $[\alpha]^{20}D$ +2°.

Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.50; H, 10.90.

The benzoate (benzoyl chloride–pyridine, 18 hr., 25°) was obtained as plates from methanol, m.p. 101–102.5°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{33}H_{46}O_3$: C, 80.77; H, 9.45. Found C, 80.62; H, 9.31.

16,22-Epoxycholan-3α-ol-24-al 3α-Acetate (VIII).—A solution of 5.0 g. of 16,22-epoxynorcoprost-24-en-3α-ol (VII), 30 ml. of pyridine and 15 ml. of acetic anhydride was allowed to stand overnight at room temperature. The mixture was concentrated to dryness *in vacuo* and the residue was dissolved in 110 ml. of dioxane (free of peroxide). To the stirred solution at room temperature was added 27 ml. of water and 50 mg. of osmium tetroxide. After solution was complete 6.0 g. of sodium metaperiodate was added over a 15-minute period and the mixture was stirred for an additional 3.5 hr. The light yellow solution was filtered and the precipitate of sodium iodate was washed with dioxane. The filtrate was poured into 1 liter of cracked ice and water. The oily precipitate was collected by decantation. Infrared analysis obtained of an immediately dried sample showed aldehyde absorption at 2725 cm.$^{-1}$ and in the carbonyl region at 1730 cm.$^{-1}$ as a shoulder due to over-lapping of acetate absorption at 1736 cm.$^{-1}$. A sample of acetoxy-aldehyde was characterized further as the semicarbazone and the remainder immediately used in the preparation of IXa.

The **semicarbazone** (semicarbazide hydrochloride methanol–pyridine, 4 hr., steam-bath) was obtained as rods from methanol, m.p. 217–220°.

Anal. Calcd. for $C_{27}H_{43}O_4N_3$: C, 68.47; H, 9.15; N, 8.87. Found: C, 68.20; H, 9.43; N, 8.70.

16,22-Epoxycholane-3α,24-diol 3α-Acetate (IXa).—To a mixture of approximately 5.0 g. of the crude acetoxy-aldehyde VIII dissolved in 150 ml. of methanol at 0° was added 3.0 g. of sodium borohydride in 15 ml. of water and allowed to stand overnight at 0°. The solution was diluted with water and extracted with ether. The ethereal extract was washed with water, dried over sodium sulfate and concentrated to dryness *in vacuo*. The oily residue was chromatographed over benzene-washed alumina. The fraction eluted with benzene–chloroform (6:1) upon recrystallization from petroleum ether (60–70°) yielded 4.0 g. of needles, m.p. 101–102.5°, $[\alpha]^{20}D$ +20°.

Anal. Calcd. for $C_{26}H_{42}O_4$: C, 74.65; H, 10.05. Found: C, 74.53; H, 10.22.

16,22-Epoxycholane-3β,24-diol 3β-acetate (epimer of IXa) was obtained as needles from dilute methanol, m.p. 120–122°, $[\alpha]^{20}D$ +2°.

Anal. Calcd. for $C_{26}H_{42}O_4$: C, 74.65; H, 10.05. Found: C, 74.47; H, 10.19.

16,22-Epoxycholane-3β,24-diol 3α-Acetate-24-tosylate (IXb).—As in the preparation of IIIb above, 2.0 g. of 16,22-epoxycholane-3α,24-diol 3α-acetate (IXa), 15 ml. of pyridine and 2.5 g. of *p*-toluenesulfonyl chloride was allowed to stand at room temperature overnight. The oily tosylate thus obtained was crystallized from petroleum ether (60–70°) to give 2.5 g. of needles, m.p. 127–132°. A portion recrystallized a second time from petroleum ether gave analytically pure material, m.p. 134–136°, $[\alpha]^{20}D$ +23°.

Anal. Calcd. for $C_{33}H_{48}O_6S$: C, 69.20; H, 8.45. Found: C, 68.97; H, 8.38.

16,22-Epoxy-24-iodocholan-3α-ol Acetate (IXc).—A mixture of 2.0 g. of crude 16,22-epoxycholane-3α,24-diol 3α-acetate-24-tosylate (IXb), 20 ml. of dry acetone and 4.0 g. of sodium iodide was refluxed overnight. The mixture worked up in exactly the same manner as described above for the preparation of IIIc, gave 1.7 g. of the 24-iodo compound IXc, m.p. 167–171°. A sample recrystallized from ether–ethanol yielded analytically pure material as rods, m.p. 174–176°, $[\alpha]^{20}D$ +26°.

Anal. Calcd. for $C_{26}H_{42}IO_3$: C, 58.97; H, 7.99. Found: C, 59.05; H, 7.93.

16,22-Epoxy-24-iodocholan-3β-ol acetate (epimer of IXc) was obtained as rods from ether–ethanol, m.p. 137–139°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{26}H_{42}IO_3$: C, 58.97; H, 7.99. Found: C, 58.80; H, 8.20.

16,22-Epoxycoprostane-3α,26,27-triol (XIa).—To a solution of 1.5 g. of 16,22-epoxy-24-iodocholan-3α-ol acetate (IXc) in 60 ml. of absolute ethanol (freshly distilled after being dried with sodium) was added 10 ml. of absolute ethanol which contained 74 mg. of sodium as the sodium ethoxide and 1 ml. of dry diethyl malonate. The reaction mixture was refluxed under anhydrous conditions for 5 hr. and was concentrated nearly to dryness *in vacuo*, diluted with water and extracted with ether. The ethereal solution was washed with water and dried over sodium sulfate. To the ethereal solution (300 ml.) was added 10 ml. of 1 *M* lithium aluminum hydride in ether and the mixture refluxed for 18 hr. The mixture was cooled and the excess lithium aluminum hydride was destroyed with ethyl acetate and then treated with water and 50 ml. of 6 *N* hydrochloric acid. The aqueous layer was separated, extracted with ether and the extracts combined. The ethereal extract was washed with 2% bicarbonate solution, water, dried over sodium sulfate and concentrated to dryness *in vacuo*. The oily residue was chromatographed over benzene–chloroform 1:1 washed alumina. The fractions eluted with chloroform and chloroform–2% ethanol were obtained as hydrates, m.p. 131–135°. Recrystallization from ether gave 0.9 g. of thin spears, m.p. 130–135°. By allowing the material to crystallize from hot ether–petroleum ether (60–70°) it was obtained in the anhydrous state as fine needles, m.p. 152–155°, $[\alpha]^{20}D$ +9°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.32; H, 10.88.

A mixed melting point determination of XIa with the lower melting polymorphic form of dihydrocholegenin, m.p. 155–157°, gave a m.p. of 148–153° accompanied by resolidification and remelting at 173–178°. Their infrared spectra were completely different.

The triacetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as a colorless oil, $[\alpha]^{20}D$ +23°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.67; H, 9.35. Found: C, 70.49; H, 9.39.

16,22-Epoxycoprostane-3β,26-27-triol (epimer of XIa) was obtained as needles from ether–petroleum ether, m.p. 155–157°, $[\alpha]^{20}D$ +2°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.72; H, 10.79.

The triacetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as needles from dilute methanol, m.p. 93–95°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.67; H, 9.35. Found: C, 70.56; H, 9.45.

16,22-Epoxycoprostan-3α-ol (XII). a. From XIb.—A mixture of 100 mg. of 16,22-epoxycoprostane-3α,26,27-triol (XIa), 5 ml. of pyridine and 160 mg. of *p*-toluenesulfonyl chloride was allowed to stand overnight at room temperature. The light yellow solution was poured into ice and water, and the oily precipitate was extracted with ether. The ethereal solution was washed with cold 5% hydrochloric acid, water, 2% sodium bicarbonate solution, water and dried over sodium sulfate. The solution was evaporated *in vacuo* to an oil which was dissolved in 30 ml. of ether and 2 ml. of 1 *M* solution of lithium aluminum hydride in ether was added and mixture was refluxed for 2 hr. The reaction mixture was cooled and after addition of several drops of ethyl acetate, followed by water, it was treated with 10 ml. of 6 *N* hydrochloric acid. The ether layer was separated and washed with 10% bicarbonate solution, water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The oily residue was chromatographed over benzene–petroleum ether (60–70°) 9:1 washed alumina. The fraction eluted with benzene–chloroform 9:1 crystallized from methanol to give 26 mg. of needles, m.p. 143–145°, $[\alpha]^{20}D$ +6°, γ^{CS_2} 3590 cm.$^{-1}$ (hydroxyl). No hydrocarbon was obtained.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.60; H, 11.65.

b. From IIIb.—A mixture of 110 mg. of 16,22-epoxycoprostane-3α,26-diol 3α-acetate-26 tosylate (IIIb), 50 ml. of ether and 3 ml. of 1 *M* solution of lithium aluminum hydride in ether was refluxed for 1.5 hr. The lithium aluminum hydride mixture worked up as in **a** above yielded 80 mg. of XII as needles from dilute methanol, m.p. 146–147.5°. $[\alpha]^{20}D$ +7°, identical with the material obtained from procedure **a** above.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.44; H, 11.60.

The acetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as needles from dilute methanol, m.p. 91–93°, $[\alpha]^{20}D$ +22°.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.05; N, 10.88.

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BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATURAL PRODUCTS AND INDUSTRIAL MICROBIOLOGY DEPARTMENTS, SCHERING CORPORATION]

Some Substances Derived from Ruscogenin

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The conversion of ruscogenin into 1β-hydroxylated pregnane derivatives is described. An analog corresponding to Compound S is found to be identical with a product obtained by microbiological hydroxylation of the latter.

Ruscogenin, a steroidal sapogenin, was isolated from *Ruscus aculeatus* L. by Sannié and Lapin and found to possess the structure of diosgenin plus an additional hydroxyl group.[1a-d] The latter was at first assigned to C-19,[1b-c] thus making the genin an interesting potential starting material for the synthesis of 19-norsteroids, but subsequent work showed the hydroxyl group in question to be located at C-1. Burn, Ellis and Petrow[2] submitted a mixture of ruscogenin and neoruscogenin[3] to Oppenauer oxidation, and isolated a ring A dienone having the 25L stereochemistry. Nussbaum, *et al.*,[4] starting with ruscogenin diacetate proper,[5] found a corresponding oxidation product to be identical with a dienone prepared from diosgenone (25D). Analogous dienones were prepared in the pregnane[2] and androstane[6] series, so that assignment of the hydroxyl group to C-1 became quite certain.[7]

The configuration of the substituent in question was established to be β, as suggested by its rotatory contribution[8] and perhaps on biogenetic grounds.[8] This was proved by the work of the Searle group who found that Δ^5-androstene-1ζ,3β,17β-triol derived from ruscogenin differed from an authentic 1α-isomer, prepared from a 1α,2α-epoxide by reduction with lithium aluminum hydride in the manner of Tamm,[9] but was identical with 1β-

(1) (a) Ch. Sannié and H. Lapin, *Compt. rend.*, **241**, 1498 (1955); (b) H. Lapin and Ch. Sannié, *Bull. soc. chim. France*, **22**, 1552 (1955); (c) Ch. Sannié and H. Lapin, *ibid.*, **22**, 1556 (1955); (d) Ch. Sannié, H. Lapin, F. Eloy and L. Cogolludo Sanchez, *Bull. soc. chim. biol.*, **39**, 301 (1957).

(2) D. Burn, B. Ellis and V. Petrow, *Proc. Chem. Soc.*, **119** (1957).

(3) The 25D and 25L isomers, respectively; see ref. 2 and H. Lapin, *Compt. rend.*, **244**, 3065 (1957).

(4) A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore and W. Charney, THIS JOURNAL, **79**, 4814 (1957).

(5) Again we wish to thank Dr. Lapin for supplying us with both sapogenins.

(6) W. R. Bonn, F. Colton and R. Pappo, THIS JOURNAL, **79**, 3920 (1957).

(7) Dr. Lapin concurs with this assignment; see his article quoted in ref. 3, and H. Lapin, *Bull. soc. chim. France*, **24**, 1237 (1957).

(8) D. Burn, B. Ellis and V. Petrow, *J. Chem. Soc.*, 795 (1958).

(9) See ref. 6, footnote 11.