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Coprostane, Cholestane and their 16β-Hydroxy Derivatives from Steroidal Sapogenins

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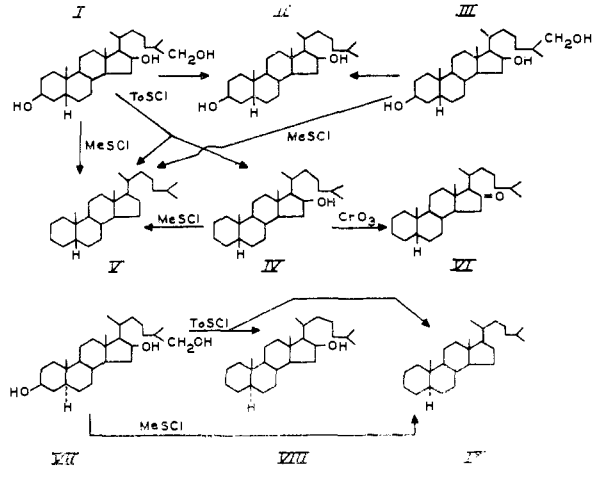
The deoxylation of tetrahydrosapogenins to coprostane, cholestane and their 16β-hydroxy derivatives is described.

In the course of a study of the stereochemistry of the D, E and F rings of steroidal sapogenins, we prepared various deoxy derivatives of these sapogenins by tosylation and subsequent reduction with lithium aluminum hydride, according to the procedure of Schmid and Karrer.[1] A part of this work was reported previously[2] and this communication deals with the deoxylation reactions of tetrahydrosapogenins (3,16,27-triols).

The reaction of tetrahydrosmilagenin (I) or tetrahydrosarsasapogenin (III) with 1 mole of *p*-toluenesulfonyl chloride resulted in selective esterification of the primary hydroxyl group (C-27). Reduction of the resultant oily tosylate with lithium aluminum hydride yielded coprostane-3β,16β-diol (II). Tosylation of I or III with an excess (6 moles) of *p*-toluenesulfonyl chloride (20°, overnight) followed by reduction of the crude ester with lithium aluminum hydride yielded an oil which was separated into two fractions by chromatography on alumina. The first fraction gave a strong Liebermann–Burchard reaction and a strong color with tetranitromethane and could not be crystallized. The second fraction spontaneously crystallized and gave negative tests for unsaturation. The crystalline material was found to be coprostan-16β-ol (IV). If the oil obtained from the lithium aluminum hydride reduction was hydrogenated in the presence of Raney nickel at 150° and 100 atmospheres for 18 hours it no longer gave a positive Liebermann–Burchard or tetranitromethane reaction and after chromatography afforded two crystalline products, IV and coprostane (V). The ratio of products formed was not significantly altered when the reaction time of I or III with *p*-toluenesulfonyl chloride was increased. The esterification of I with an excess of methanesulfonyl chloride followed by reduction of the crude oily mesylate with lithium aluminum hydride and then with Raney nickel afforded coprostane (V), in 65–75% yield, as the only crystalline product.

The structure of coprostan-16β-ol (IV) was established by the formation of a mono-3,5-dinitrobenzoate (on treatment with 3,5-dinitrobenzoyl chloride) and chromic acid oxidation which afforded a crystalline ketone [coprostan-16-one (VI)] whose infrared spectrum (CS_2) exhibited a strong band at 1736 cm.$^{-1}$ characteristic of a carbonyl group in a five-membered ring.[3] Tosylation and reduction of IV yielded a mixture of mainly starting material and some coprostane. However, mesylation and reduction of IV gave V in good yield. The difference in reactivity of the tetrahydrosapogenins and coprostan-16β-ol toward *p*-toluenesulfonyl chloride and methanesulfonyl chloride suggests that the 16-OH group is hindered in these compounds and is in the β-position.[4] The slight negative shift of rotation in going from IV to V is also indicative of a β-configuration at C-16.[5] While these facts strongly speak for a β-configuration this point remains to be proven.

Tetrahydrotigogenin[6] (VII), after tosylation and subsequent reduction, yielded a mixture of cholestan-16β-ol (VIII) and cholestane (IX). The reaction of VII with methanesulfonyl chloride and subsequent reductions yielded IX in very good yield. It should be noted that thirteen years ago Marker and Turner[7] had converted tetrahydrodiosgenin (cholest-5-ene-3,16,27-triol), *via* the bromide, into cholesterol and Δ^5-cholestene, thereby relating a sapogenin to a known sterol for the first time.



Experimental[8]

Coprostane-3β,16β-diol (II).—A solution of 2 g. of tetrahydrosarsasapogenin[9] (III) in 20 ml. of dry pyridine was cooled to −10°, and a solution of 1.0 g. of *p*-toluenesulfonyl chloride in 10 ml. of dry pyridine was added over a one-hour period, maintaining the temperature at −6 to −10°. The mixture was allowed to stand overnight at room tempera-

(1) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949).

(2) I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **75**, 4871 (1953).

(3) R. Norman Jones and K. Dobriner, *Fed. Proceed.*, **6**, #1 (1947).

(4) H. Hirschmann, F. B. Hirschmann and M. A. Daus, *J. Biol. Chem.*, **178**, 75 (1949).

(5) D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **73**, 196 (1951).

(6) As reported by R. E. Marker and E. Rohrmann [*ibid.*, **61**, 1516 (1939)] VII could not be prepared from tigogenin by the usual Clemmensen reduction. It was prepared by the reduction of tetrahydrodiosgenin (cholest-5-ene-3,16,27-triol) as described by R. E. Marker and D. L. Turner (ref. 7).

(7) R. E. Marker and D. L. Turner, *ibid.*, **63**, 767 (1941).

(8) Melting points were determined on the Kofler block. Unless otherwise noted, rotations were determined in approximately 1% solutions in chloroform. Infrared spectra were obtained with a Perkin-Elmer Model 21 double beam spectrophotometer with sodium chloride prism and cells.

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

ture. The pale yellow solution was poured on 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. On evaporation *in vacuo* a clear, yellow, oily residue was obtained which could not be crystallized, and was reduced without further purification. The oil was dissolved in 65 ml. of benzene and the solution concentrated to a volume of 45 ml. After the addition of 45 ml. of dry ether and 13 ml. of a 1.6 *M* solution of lithium aluminum hydride in ether the reaction mixture was refluxed overnight. The reaction mixture was cooled, and after the addition of a few drops of ethyl acetate, treated with 40 ml. of 6 *N* hydrochloric acid. The aqueous layer was separated, extracted with ether and the extracts 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 *in vacuo*. When crystalline material began to separate out, the distillation was interrupted and the crystalline material filtered. It proved to be starting material (infrared analysis, m.p.), wt. 0.27 g. The oily residue was chromatographed on ethyl acetate–washed alumina. The fraction eluted with benzene was crystallized from acetone–water to yield 0.54 g. (28.4%) of white plates, m.p. 155–157°, $[\alpha]^{20}D$ +24.0°.

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.13; H, 11.96. Found: C, 80.14; H, 11.99.

Tosylation and reduction of tetrahydrosmilagenin[2] (I) in the same manner gave similar results. The product obtained was identical with the one described above in every respect (melting point, rotation, infrared spectra, derivatives).

The **diacetate** (acetic anhydride–pyridine, 18 hours, 25°) was obtained as a clear colorless oil, $[\alpha]^{20}D$ +41.2°.

Anal. Calcd. for $C_{31}H_{52}O_4$: C, 76.18; H, 10.73. Found: C, 76.21; H, 10.93.

The **dibenzoate** (benzoyl chloride–pyridine, 18 hours, 25°) crystallized from ether–methanol, m.p. 131–137° (partial melt at 131–133°, resolidifies and melts at 136–137°), $[\alpha]^{20}D$ +43.4°.

Anal. Calcd. for $C_{41}H_{56}O_4$: C, 80.34; H, 9.21. Found: C, 80.45; H, 9.35.

Coprostan-16β-ol (IV).—A solution of 3.0 g. of tetrahydrosmilagenin (I), 8.2 g. of *p*-toluenesulfonyl chloride and 60 ml. of dry pyridine was allowed to stand overnight at room temperature. An oily product was obtained after treating the solution as described above. The oil was dissolved in 300 ml. of benzene and the solution was concentrated to a volume of 180 ml. After the addition of 180 ml. of dry ether and 70 ml. of a 1.5 *M* solution of lithium aluminum hydride in ether the reaction mixture was refluxed overnight. A yellow oil was obtained on treatment of the reduction mixture as described above for the preparation of II. The oil gave a strong positive test with tetranitromethane. It was dissolved in 50 ml. of dioxane and shaken under hydrogen in a stainless steel bomb for 24 hours at 150° and 1500 p.s.i. in the presence of 10 ml. of Raney nickel in ethanol. The mixture was filtered and concentrated *in vacuo* to a colorless oil which gave a negative test with tetranitromethane. The oil was chromatographed on ethyl acetate–washed alumina. The first two fractions eluted with hexane yielded a colorless oil which slowly formed crystals melting at 52–56°, wt. 0.75 g. Rechromatography of this material on alumina afforded 0.45 g. of crystals, m.p. 65–66°, from the fraction eluted with hexane. The infrared spectrum of this material was identical with that of coprostane (V). The fraction eluted with chloroform afforded 0.25 g. of white crystals, m.p. 75–78°. Further elution of the original chromatogram with hexane and with benzene afforded an oil which slowly crystallized and then melted at 73–75°. This material was combined with the 0.25 g. of material obtained from the chloroform eluate of the second chromatogram, combined wt., 1.45 g. Crystallization from ether–ethanol gave 1.13 g. (41.3%) of coprostan-16β-ol (IV), m.p. 75–77°, $[\alpha]^{20}D$ +29°.

Anal. Calcd. for $C_{27}H_{48}O$: C, 83.43; H, 12.44. Found: C, 83.27; H, 12.46.

Reaction of tetrahydrosarsasapogenin (III) in the same manner gave similar results. Allowing the tosylation mixture to stand for 5 days did not significantly alter the ratio of products formed.

The **di-3,5-dinitrobenzoate** of IV (3,5-dinitrobenzoyl chloride–pyridine, 4 hours, steam-bath) was obtained crystalline from acetone–methanol, m.p. 145–146°, $[\alpha]^{20}D$ +60.4°.

Anal. Calcd. for $C_{34}H_{50}O_6N_2$: C, 70.07; H, 8.91; N, 4.80. Found: C, 70.18; H, 8.91; N, 4.84.

Coprostan-16-one (VI).—To a solution of 1.0 g. of coprostan-16β-ol (IV) in 40 ml. of chloroform and 10 ml. of glacial acetic acid at 0° was added, with rapid stirring, a solution of 0.6 g. of chromic acid in 0.6 ml. of water. The mixture was vigorously stirred for 15 min. at 0° and 0.6 ml. of concd. sulfuric acid was added. The mixture was then kept at 0° and vigorously stirred for 1 hour, diluted with water, and extracted with ether. The ethereal solution was washed with 2% aqueous sodium bicarbonate, then with water, dried over sodium sulfate and concentrated to an orange oil. The oil was chromatographed on ethyl acetate–washed alumina and the fraction eluted with hexane was crystallized from ether–methanol to yield 0.41 g. (41.2%) of coprostan-16-one (VI), m.p. 49–51°, $[\alpha]^{20}D$ −121°, The fraction eluted with benzene yielded 0.2 g. of starting material.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.73; H, 11.87.

The **semicarbazone** (sodium acetate, semicarbazide hydrochloride, methanol, steam-bath, 2 hours) was obtained as white needles from hexane, m.p. 150–155°, resolidifies and remelts at 204–219°. A similar behavior was noted upon melting a sample in a capillary tube. At a temperature rise of 3°/min. the compound melted at 204–208° (cor.). At a temperature rise of 10°/min. a sample melted at 160–163° (cor.), resolidified, and remelted at 216–220° (cor.). These melting points were reproducible.

Anal. Calcd. for $C_{28}H_{49}ON_3$: C, 75.79; H, 11.13; N, 9.47. Found: C, 75.99; H, 11.15; N, 9.52.

Coprostane (V). A. From I.—To a solution of 1.0 g. of tetrahydrosmilagenin (I) in 10 ml. of dry pyridine at 0° was added an ice-cold solution of 2.0 ml. of methanesulfonyl chloride in 10 ml. of dry pyridine and the mixture was kept at 3° overnight. The red 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 a yellow oil which was dissolved in 100 ml. of benzene, distilled to a volume of 60 ml., and 60 ml. of dry ether and 15 ml. of a 2.1 *M* solution of lithium aluminum hydride in ether were added and the mixture was refluxed overnight. The reaction mixture was treated in the usual manner to yield a yellow oil which gave a strong positive test with tetranitromethane. A solution of the oil in 100 ml. of dioxane in a stainless steel bomb was shaken under hydrogen at 150° and 1500 p.s.i. for 24 hours with 20 ml. of Raney nickel in ethanol. The mixture was filtered and concentrated to dryness *in vacuo* to yield a colorless oil which gave a negative test with tetranitromethane. Crystallization from ether–methanol gave 0.68 g. (76.1%) of coprostane (V), m.p. 69–70°, $[\alpha]^{20}D$ +22°, (lit.[10] m.p. 70°, $[\alpha]^{20}D$ +25°).

Anal. Calcd. for $C_{27}H_{48}$: C, 87.02; H, 12.98. Found: C, 86.90; H, 12.81.

The melting point, rotation and infrared spectrum of V were identical with those of an authentic sample of coprostane prepared by the reduction of coprostan-3-one.[11]

B. From IV *via* Mesylation.—As in A above, 0.8 g. of coprostan-16β-ol (IV) in 5 ml. of dry pyridine was treated with 0.5 ml. of methanesulfonyl chloride in 2.5 ml. of dry pyridine at 3°. The pale yellow oil thus obtained was dissolved in 50 ml. of benzene and distilled to a volume of 30 ml. To the solution was added 30 ml. of dry ether and 10 ml. of a 2.4 *M* solution of lithium aluminum hydride in ether, and the mixture was refluxed overnight. The yellow oil, obtained after treating the reaction mixture in the usual manner, gave a weak positive test with tetranitromethane. It was chromatographed on ethyl acetate–washed alumina and the fraction eluted with hexane was crystallized from ether–methanol to yield 0.47 g. (61.3%) of coprostane (V), m.p. 66–67°.

(10) Mauthner, *Monatsh.*, **30**, 635 (1909).

(11) We wish to express our appreciation to Dr. B. Armbrecht, Georgetown University Medical School, Wash., D. C., for a generous sample of this material.

The mother liquor from the crystallized material was evaporated to an oil which gave a positive test with tetranitromethane. Reduction with Raney nickel as described above and crystallization from ether–methanol yielded an additional 0.11 g. of V, m.p. 64–67°.

C. From IV *via* Tosylation.—When 0.35 g. of coprostan-16β-ol (IV) was treated with excess *p*-toluenesulfonyl chloride in the usual manner for 7 days and the resultant oil reduced first with lithium aluminum hydride and then with Raney nickel, in the manner already described above, 0.11 g. (32.8%) of crystalline V, m.p. 66–68°, was obtained.

When 0.47 g. of IV was tosylated for 2 days and then reduced, 0.22 g. of starting material was recovered. The yield of coprostane was 0.05 g. (11.1%), m.p. 67–68°.

Cholestan-16β-ol (VIII).—Three grams of tetrahydrotigogenin (VII)[6] was tosylated and reduced exactly as described above for the preparation of IV from tetrahydrosmilagenin. The final oily product was chromatographed on ethyl acetate–washed alumina. The fraction eluted with hexane crystallized from ether–methanol as white plates of cholestane (IX), 0.22 g., m.p. 75–78°. Crystallization from ether–methanol of the fraction eluted with benzene gave 0.73 g. (26.3%) of cholestan-16β-ol (VIII), m.p. 109–111°, $[\alpha]^{20}D$ +25°. A second crop weighing 0.27 g., m.p. 100–105°, was obtained.

Anal. Calcd. for $C_{27}H_{48}O$: C, 83.43; H, 12.44. Found: C, 83.68; H, 12.50.

The di-3,5-dinitrobenzoate (3,5-dinitrobenzoyl chloride, pyridine, steam-bath, 4 hours) crystallized as white plates from acetone–methanol, m.p. 159–161°, 165–166°, $[\alpha]^{20}D$ +57.3°.

Anal. Calcd. for $C_{34}H_{50}O_6N_2$: C, 70.07; H, 8.65. Found: C, 70.18; H, 8.93.

Cholestane (IX).—From 1.0 g. of tetrahydrotigogenin (VII), treated as described above for the preparation of V by procedure A, was obtained 0.60 g. (67.1%) of cholestane (IX), m.p. 78–79°, $[\alpha]^{20}D$ +23.5° (lit.[10] m.p. 80°, $[\alpha]^{20}D$ +24.4°). The product was identical (m.p., rotation, infrared spectrum) with an authentic sample of cholestane.

Anal. Calcd. for $C_{27}H_{48}$: C, 87.02; H, 12.98. Found: C, 87.06; H, 12.84.

Acknowledgment.—We wish to thank Mrs. Alma L. Hayden and Mr. Harold K. Miller for determining the infrared spectra. Microanalyses are by the Analytical Service Laboratory of this Institute under the direction of Dr. William C. Alford.

BETHESDA, MD.

[CONTRIBUTION FROM THE SECTION OF BIOCHEMISTRY, MAYO CLINIC]

Adrenal Hormone Analogs: 16α,17α-Epoxy-11-dehydrocorticosterone Acetate and Δ^{16}-11-Dehydrocorticosterone Acetate[1]

BY WARREN F. McGUCKIN AND HAROLD L. MASON

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21-Acetoxy-16α,17α-epoxy-Δ^4-pregnene-3,11,20-trione (16α,17α-epoxy-11-dehydrocorticosterone acetate) (V) and 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (Δ^{16}-11-dehydrocorticosterone acetate) (XI) both possess three characteristics in common with the known glycogenic compounds of the adrenal cortex, namely, the 11-keto group, the 3-keto group conjugated with a 4,5-double bond, and an α-ketol side chain. The partial synthesis of these compounds as a preliminary to the testing of their physiologic activity is described here.

16α,17α-Epoxy-11-dehydrocorticosterone acetate (V) and 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (XI) are structurally very similar to the glycogenic steroids of the adrenal cortex. It was, therefore, of interest to prepare these compounds for investigation.

The desired intermediate, 21-acetoxy-16α,17α-epoxypregnane-3,11,20-trione (I), for the preparation of V is also an intermediate in the partial synthesis of cortisone as developed in this Laboratory.[2]

Compound I was brominated at room temperature in acetic acid containing a small amount of hydrogen bromide to give the 4β-bromo derivative[3] (II). This substance was difficult to purify by recrystallization and the crude material was used for the next step of dehydrobromination by the Mattox–Kendall or McGuckin–Kendall procedure.[4] Treatment of II with dinitrophenylhydrazine in acetic acid or with semicarbazide in *t*-butyl alcohol and chloroform gave the respective Δ^4-3-(2,4-dinitrophenylhydrazone) (III) or Δ^4-3-semicarbazone (IV).

Hydrolysis of III at 45° in the presence of pyruvic acid gave 21-acetoxy-16α,17α-epoxy-Δ^4-pregnene-3,11,20-trione (V) in a yield of 68%. Similar hydrolysis of IV, but at room temperature and for a shorter period, gave 88% of V.[5]

21-Acetoxy-12α-bromo-Δ^{16}-pregnene-3,11,20-trione[2] (VI) served as the starting material for the preparation of 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (XI). Bromination of VI in acetic acid containing hydrogen bromide proceeded smoothly to give a good yield of the 4β-bromo derivative (VII). Compound VII was converted to the Δ^4-3-(2,4-dinitrophenylhydrazone) (VIII) and also to the Δ^4-3-(semicarbazone) (IX). In agreement with the structure as shown for IX the compound exhibited a molecular extinction coefficient of 18,600 at 240 mμ and an absorption maximum at 270 mμ with a molecular extinction coefficient of 30,400. Hydrolysis of the hydrazone and the semicarbazone gave, in both instances, 21-acetoxy-12α-bromo-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (X); a better yield was obtained from the semicarbazone than from the dinitrophenylhydrazone. The final step in the prep-

(1) Abridgment of portion of thesis submitted by W. F. McGuckin to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) F. B. Colton, W. R. Nes, D. A. Van Dorp, H. L. Mason and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

(3) The bromine atom is assigned the 4β-configuration in accord with E. J. Corey, *Experientia*, **9**, 329 (1953).

(4) W. F. McGuckin and E. C. Kendall, THIS JOURNAL, **74**, 5811 (1952).

(5) The possibility was considered that attack on the epoxy group might occur. In a preliminary experiment, 100 mg. of I (m.p. 234–235°) was dissolved in 5 ml. of chloroform containing 2 ml. of glacial acetic acid and 2 ml. of 50% pyruvic acid. The mixture was heated at 45° for 15 hours. Since 75 mg. (75%) of I was recovered, it was concluded that these conditions would be suitable for the hydrolysis of the hydrazone (III). The greater yield of V from IV may have been due to the use of lower temperature and shorter time for the hydrolysis of IV.