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-Δ^{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-Δ^{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-Δ^{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-Δ^{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-

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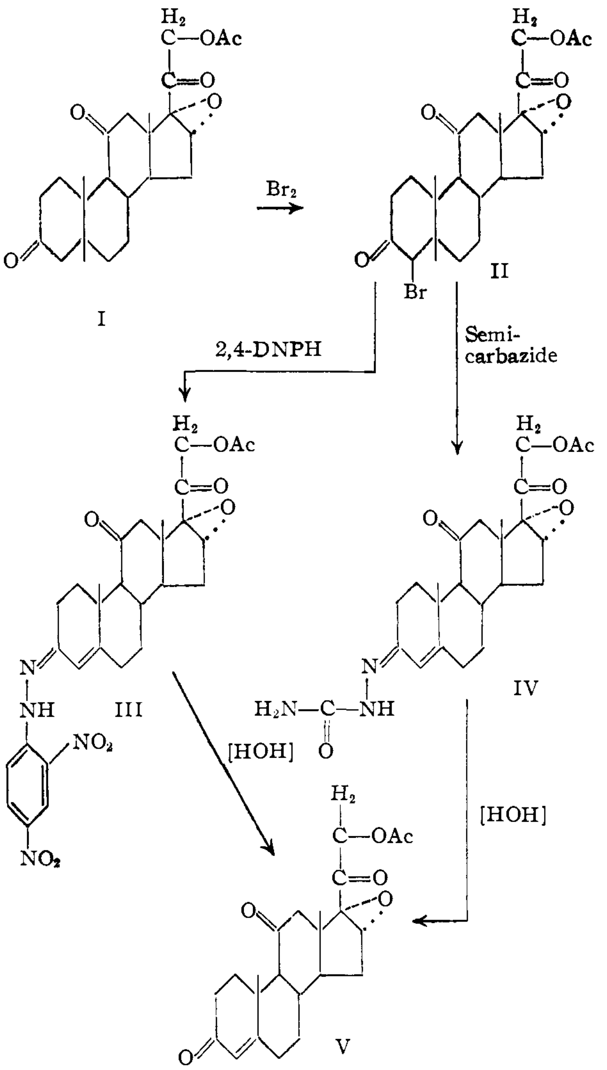
[1] Abridgment of portion of thesis submitted by W. F. McGuckin to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[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.

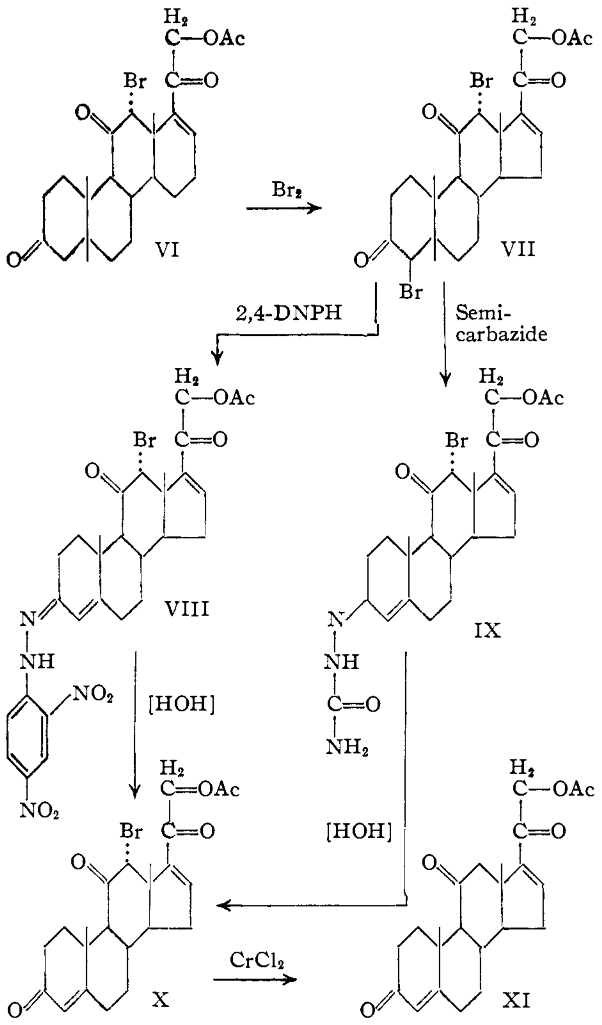


aration of XI, namely, removal of the 12α-bromine atom, was achieved by the use of chromous chloride in alcohol. Nes and Mason[6] reported previously that treatment of a 21-acetoxy-20-keto-Δ^{16}-pregnene with zinc and acetic acid resulted in loss of the 21-acetoxy group. The product XI had a molecular extinction coefficient of 25,300 at 237–238 mμ. An extinction coefficient of this magnitude would be expected from the additive effects of a Δ^4-3-keto and a Δ^{16}-20-keto group. XI also possessed the correct elementary composition and reducing properties characteristic of the α-ketol group.

Both of the Δ^4-3-keto compounds prepared were tested for ability to promote deposition of glycogen in the livers of fasted, adrenalectomized rats.[7] Neither compound had any effect on hepatic glycogen, nor did they enhance or inhibit the effect of cortisone acetate.

Experimental

Microanalyses were performed by Joseph F. Alicino. Melting points were determined with the Fisher–Johns electrically heated block and are reported as read. Optical rotations were determined with approximately 1% solutions and at a temperature of about 25°.



21-Acetoxy-16α,17α-epoxy-4β-bromopregnane-3,11,20-trione (II).—To 200 ml. of glacial acetic acid containing 1.4 ml. of 0.5 *N* hydrobromic acid was added 7.50 g. of 21-acetoxy-16α,17α-epoxypregnane-3,11,20-trione (I). At room temperature and with vigorous stirring, 48 ml. of 0.78 *N* bromine in acetic acid containing 1.53 g. of sodium acetate slowly was added dropwise. After all the solution of bromine had been added, the reaction mixture was slowly diluted to 1.5 liters with water and then allowed to stand for 2 hours. The granular, non-crystalline solid was filtered off, washed well with 15% acetic acid and water and dried at 60° to a constant weight of 7.045 g. (78%). This granular product was converted to the unsaturated 2,4-dinitrophenylhydrazone without further purification.

21-Acetoxy-16α,17α-epoxy-Δ^4-pregnene-3,11,20-trione 3-(2,4-Dinitrophenylhydrazone) (III).—The granular, 4-bromo compound II (7.045 g.) was dissolved in a mixture of 200 ml. of chloroform, 200 ml. of acetic acid and 20 ml. of water. The solution was cooled to 15° and carbon dioxide was passed through to eliminate oxygen. Sodium acetate (1.48 g.) and 3.60 g. (1.2 equiv.) of 2,4-dinitrophenylhydrazine were added simultaneously, and the mixture was agitated until all components were in solution. The mixture was allowed to stand for 40 minutes at 15° and the chloroform and part of the acetic acid were removed by distillation *in vacuo* until the volume was reduced to about 100 ml. After 1 hour the crystals were removed by filtration; the yield was 5.01 g. (59%), m.p. 261–262°, $\lambda_{\max}^{\text{chloroform}}$ 387 mμ, ε 29,900.

Anal. Calcd. for $C_{29}H_{32}O_9N_4$: C, 59.99; H, 5.56. Found: C, 59.90; H, 5.71.

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(6) W. R. Nes and H. L. Mason, This Journal, **73**, 4765 (1951).

(7) Details of the biologic studies with these compounds were given at the American Chemical Society Meeting, Kansas City, Missouri, March 25, 1954.

21-Acetoxy-16α,17α-epoxy-Δ^4-pregnene-3,11,20-trione 3-Semicarbazone (IV).—The 4-bromo compound II (1.9 g.) was dissolved in 60 ml. of *t*-butyl alcohol and 20 ml. of chloroform. Carbon dioxide was passed through the mixture for 5 minutes and then 600 mg. of semicarbazide was added. An orange color appeared within 10 to 15 minutes, then disappeared at 40 minutes. The mixture was allowed to stand for 2 hours, the solvents were removed *in vacuo* and 10 ml. of alcohol and 100 ml. of ether were added to the residue. After 30 minutes the crystals were filtered off and resuspended in 20 to 30% alcohol to dissolve any semicarbazide or its hydrobromide. After filtration the product was dried at 105°. Its weight was 1.42 g. (79%), m.p. 223–225°; $\lambda_{max}^{alc.}$ 270 mμ, ϵ 29,500.

Anal. Calcd. for $C_{24}H_{31}O_6N_3$: C, 62.95; H, 6.82; N, 9.16. Found: C, 62.89; H, 6.72; N, 8.97.

21-Acetoxy-16α,17α-epoxy-Δ^4-pregnene-3,11,20-trione (V). A. From the 2,4-Dinitrophenylhydrazone.—The unsaturated hydrazone III (4.35 g.) was dissolved in a mixture of 50 ml. of glacial acetic acid and 150 ml. of chloroform; 70 ml. of a 70% aqueous solution of pyruvic acid was then added. After evacuation and partial filling of the flask with carbon dioxide, the solution was allowed to stand for 17 hours at 45°. The chloroform and acetic acid were removed under reduced pressure and most of the pyruvic acid was removed by distillation at a pressure of 0.1 mm. The residue was then taken up in chloroform; the extract was washed with water and a saturated solution of sodium bicarbonate; it was then dried and the solvent was removed. The residue was dissolved in 10 ml. of acetone and when 50 ml. of ether was added, 2.65 g. of the ketone crystallized, m.p. 188–190°. The yellowish crystals were redissolved in methanol and the solution was treated with charcoal, filtered and diluted with an equal volume of water. The crystals that separated still retained some color that could not be removed by further treatment. The yield was 2.04 g. (68%), m.p. 193–194°; $\lambda_{max}^{methanol}$ 238 mμ, ϵ 16,550; $[\alpha]_D$ +206 ± 4° (1% in alc.). The over-all yield of the Δ^4-3-keto compound from I (the 16α,17α-epoxy-3-keto-4,5β-dihydro compound) was 31%.

Anal. Calcd. for $C_{23}H_{28}O_6$: C, 68.90; H, 7.10. Found: C, 68.46; H, 6.98.

B. From the Semicarbazone (IV).—To 35 ml. of acetic acid, 10 ml. of water and 3.3 ml. of 1.66 *N* pyruvic acid, was added 1.27 g. of IV. The mixture was warmed briefly to 40° to effect complete solution and then was allowed to stand at room temperature for 8 hours; after addition of 50 ml. of water, the solution was extracted with chloroform. The extract was washed with water, a solution of sodium bicarbonate, and water; it was then dried and concentrated. On addition of ether, 0.99 g. of V (88%) separated, m.p. 185–188°. The substance was recrystallized to a constant m.p. of 193–194°; $\lambda_{max}^{alc.}$ 238 mμ, ϵ 15,800.

21-Acetoxy-4β,12α-dibromo-Δ^{16}-pregnene-3,11,20-trione (VII).—Fourteen grams of 21-acetoxy-12α-bromo-Δ^{16}-pregnene-3,11,20-trione (VI) was brominated under the same conditions as used for the preparation of II. After addition of the bromine, the mixture was diluted slowly with water to 3 liters and allowed to stand for 1 hour. The crystalline precipitate was washed on the filter with 15% acetic acid and dried at room temperature in air to a constant weight of 15.63 g. (96% yield). The m.p. was 177–178°, $[\alpha]_D$ +67 ± 2° (1% in acetone); $\lambda_{max}^{alc.}$ 235 mμ, ϵ 9,600.

Anal. Calcd. for $C_{23}H_{28}Br_2O_5$: C, 50.76; H, 5.19; Br, 29.36. Found: C, 50.84; H, 5.49; Br, 29.26.

21-Acetoxy-12α-bromo-$\Delta^{4,16}$-pregnadiene-3,11,20-trione 3-(2,4-Dinitrophenylhydrazone) (VIII).—Sixteen grams of VII was treated with 7.0 g. of 2,4-dinitrophenylhydrazine under the same conditions as used for the preparation of III. The reaction mixture was allowed to stand for 30 minutes with agitation at frequent intervals, and then the chloroform was removed slowly by vacuum distillation; 150 ml. of water was added and after 1 hour the crystals of hydrazone were filtered off, washed well with 70% acetic acid, dried at 60° for 3 hours and recrystallized from 300 ml. of acetic acid. The yield was 11.01 g. (58%), m.p. 264–265°; $\lambda_{max}^{alc.}$ 385 mμ, ϵ 31,500.

Anal. Calcd. for $C_{29}H_{31}O_8N_4Br$: C, 54.12; H, 4.86; Br, 12.42. Found: C, 53.99; H, 4.97; Br, 12.77.

21-Acetoxy-12α-bromo-$\Delta^{4,16}$-pregnadiene-3,11,20-trione 3-Semicarbazone (IX).—The dibromo compound VII (9.4 g.) was dissolved in 300 ml. of *t*-butyl alcohol and 50 ml. of chloroform. Semicarbazide (2.6 g.) was added and carbon dioxide was passed through the solution for 5 minutes. After 3 hours the solvent was removed *in vacuo* and 300 ml. of water was added slowly. The crystals were filtered off, dried at 60°, weight 7.74 g. (86%); $\lambda_{max}^{alc.}$ 270 mμ, ϵ 30,400; m.p. 228–229° dec., $[\alpha]_D$ +147 ± 2° (0.3% in *t*-butyl alcohol), $[\alpha]_D$ + 119 ± 2° (1% in dioxane).

Anal. Calcd. for $C_{24}H_{30}O_5N_3Br$: C, 55.29; H, 5.82; N, 8.12. Found: C, 55.02; H, 5.92; N, 8.26.

21-Acetoxy-12α-bromo-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (X). A. From VIII.—The dinitrophenylhydrazone VIII (10.0 g.) was dissolved in a mixture of 400 ml. of chloroform, 150 ml. of acetic acid, 150 ml. of 88% pyruvic acid and 50 ml. of water. The mixture was kept at 45° for 36 hours, then worked up as previously described for V. The product was crystallized from acetone–ether to yield 5.5 g. (76%) of 21-acetoxy-12α-bromo-$\Delta^{4,16}$-pregnadiene-3,11,20-trione, m.p. 172–173°, $[\alpha]_D$ +94 ± 2° (1% in acetone); $\lambda_{max}^{alc.}$ 237–238 mμ, ϵ 24,950.

Anal. Calcd. for $C_{23}H_{27}O_5Br$: C, 59.61; H, 5.87. Found: C, 59.63; H, 6.03.

B. From the Semicarbazone (IX).—Compound IX (6.6 g.) was hydrolyzed with 2 equivalents of pyruvic acid in 70% acetic acid (as previously described) to give X, yield 5.4 g. (91%), m.p. 172–173°.

21-Acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione (XI).—A solution of 5 g. of chromic chloride ($CrCl_3 \cdot 6H_2O$) in 35 ml. of absolute alcohol containing 2.0 ml. of concentrated hydrochloric acid was allowed to percolate through a Jones reductor[8] (2 by 10 cm. containing 30-mesh zinc amalgam) at a rate of 2 ml. per minute. The filtrate was protected from oxygen by allowing the tip of the column outlet tube to project well inside the receiving flask and by passing carbon dioxide continuously through the collected solution. The solution of chromous chloride was stored over 1 g. of zinc amalgam in a closed flask. Its strength was estimated by addition under carbon dioxide of an excess of 0.1 *N* chromic acid to an aliquot followed by addition of an excess of potassium iodide and titration of the liberated iodine with sodium thiosulfate. This standardized solution was used for removal of the 12α-bromine atom. Air was removed from a solution of 2.3 g. of X (5 millimoles) in 100 ml. of 95% alcohol by means of a stream of carbon dioxide. Then 44 ml. of 0.3 *N* chromous chloride in alcohol (protected from air) was added dropwise over a period of 5 to 10 minutes. The solution was allowed to stand for 5 minutes; 400 ml. of water was added and the mixture was extracted three times with chloroform. After the extract was washed with water to rid it of chromic salts and halogen, it was dried and the solvent was removed. The residue was dissolved in 2 ml. of acetone, and 50 ml. of ether was added. The crystals that separated were dried at 105°; weight 1.5 g. (78%); m.p. 183–184°; $\lambda_{max}^{ethanol}$ 237–238 mμ, ϵ 25,300; $[\alpha]_D$ +220 ± 2° (1% alcohol).

Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 72.07; H, 7.21.

ROCHESTER, MINNESOTA

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[8] I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," Rev. Ed., The Macmillan Co., New York, N. Y., 1948, pp. 597–599.