tone (50 ml.) was then added and the mixture filtered with the aid of Celite. The filtrate, which still contained colloidal silver *p*-nitrobenzoate, was repeatedly treated with Darco until a clear solution was obtained. This was evaporated to dryness *in vacuo* at 40° and the solid residue crystallized three times from acetone–ether, giving 47.2 mg. (16% based on 0.5 × 1.0 mmole of Ic) of 1,3,4-tri-*O*-*p*-nitrobenzoyl-2,6-dideoxy-β-D-*ribo*-hexose (Ic), m.p. 178–179°, and giving no depression in the melting point when admixed with an authentic specimen of Ic, $[\alpha]^{22}D$ +37° (*c* 0.43, acetone).

The second portion of the chloro compound Va was hydrolyzed (*cf.* hydrolysis of V) by dissolving in 17.5 ml. of acetone, followed by the addition of 200 mg. of silver carbonate and 2.5 ml. of water. Two crystallizations of the purified filtrate gave material which, on drying, yielded 120 mg. (54% based on 0.5 × 1.0 mmole of Ic) of pure 3,4-di-*O*-*p*-nitrobenzoyl-2,6-dideoxy-D-*ribo*-hexose (VI), m.p. 141–142°, and which did not depress the melting point when admixed with a sample prepared *via* the bromide V.

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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XLVII. Preparation of 16α,17α-Epoxy-11α-hydroxypregnane-3,20-dione from 5β-Spirostanes[2]

BY HAROLD E. KENNEY, ELMER A. WEAVER AND MONROE E. WALL

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Sarsasapogenin (Ia) or smilagenin (Ib) acetates have been converted to a useful cortisone intermediate by the following route. Standard degradation of Ia or Ib gave 3β-acetoxy-16-pregnen-20-one (II) which on epoxidation with alkaline hydrogen peroxide yielded 3β-acetoxy-16α,17α-epoxypregnan-20-one (IIIa). Alkaline hydrolysis of IIIa gave the corresponding 3β-hydroxy compound IIIb. Oxidation of the latter with chromic oxide gave 16α,17α-epoxypregnane-3,20-dione (IV). Microbiological hydroxylation of IV with *Rhizopus nigricans* gave 16α,17α-epoxy-11α-hydroxypregnane-3,20-dione (Va). Oxidation of Va with chromium trioxide gave 16α,17α-epoxypregnane-3,11,20-trione (VI). Treatment of VI with hydrobromic acid followed by reduction in the presence of a palladium catalyst gave 17α-hydroxypregnane-3,11,20-trione (VIII), a known cortisone precursor.

This Laboratory has been investigating sources of steroidal sapogenins with emphasis on plants that can be grown in the United States.[3a,b,c] In the course of these surveys we have found some promising sources of 5β-sapogenins, in particular *Yucca schidigera*, a source of sarsasapogenin and *Agave lecheguilla*, a source of smilagenin. The latter species occurs in very high concentrations in the "Big Bend" region of southwestern Texas. Since the pioneer investigations of Marker and his associates,[4] there has been relatively little attention paid to hormonal derivatives which can be prepared from 5β-sapogenins. The present report deals with the preparation of 16α,17α-epoxy-11α-hydroxypregnane-3,20-dione. The route is shown in Fig. 1.

Sarsasapogenin (Ia) or smilagenin acetates (Ib) were converted to 3β-acetoxy-16-pregnen-20-one (II) by previously published procedures.[5] Treatment of II with alkaline hydrogen peroxide at 4° by Julian's procedure,[6,7] gave 3β-acetoxy-16α,17α-epoxy-pregnan-20-one (IIIa) which on alkaline hydrolysis gave the corresponding free hydroxy compound IIIb. Chromium trioxide oxidation of IIIb gave 16α,17α-epoxypregnane-3,20-dione (IV).[8] The over-all yield of IV from II was approximately 80%. Microbiological hydroxylation of IV with *Rhizopus nigricans*[9] or *Aspergillus ochraceus* gave 16α,17α-epoxy-11α-hydroxypregnane-3,20-dione (Va) in 30–40% yield. Unreacted IV accounted for most of the residual steroid and could be separated easily from V by chromatography.[10]

The structure of Va was proved as follows. Mild acetylation with acetic anhydride–pyridine gave 11α-acetoxy-16α,17α-epoxypregnane-3,20-dione (Vb). Oxidation of Va with chromium trioxide in acetic acid gave 16α,17α-epoxypregnane-3,11,20-trione (VI). Treatment of VI with hydrogen bromide in acetic acid gave the bromohydrin VII which was not isolated. Hydrogenation of crude VII with palladium–calcium carbonate catalyst[11] gave 17α-hydroxypregnane-3,11,20-trione (VIII) with melting point and optical rotation almost identical with that of VIII prepared from 3α-hydroxypregnane-11,20-dione[12] and infrared spectrum identical to that published by Dobriner, Katzenellenbogen and Jones.[13] After our re-

(1) A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture. Article not copyrighted. Paper presented at the 134th National Meeting of the American Chemical Society, Chicago, Ill., September 7–12, 1958.

(2) Paper XLVI, Wall and Walens, THIS JOURNAL, **80**, 1984 (1958).

(3) (a) M. E. Wall, *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1 (1954); (b) **43**, 503 (1954); (c) **44**, 438 (1955).

(4) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(5) M. E. Wall, H. E. Kenney and E. S. Rothman, *ibid.*, **77**, 5665 (1955).

(6) P. L. Julian, *et al.*, *ibid.*, **71**, 756 (1949); **71**, 3574 (1949); **72**, 5145 (1950).

(7) Under our experimental conditions there was only slight hydrolysis of the axial 3 β-acetate group.

(8) Rather surprisingly we have been unable to find prior references to compounds IIIa or IIIb.

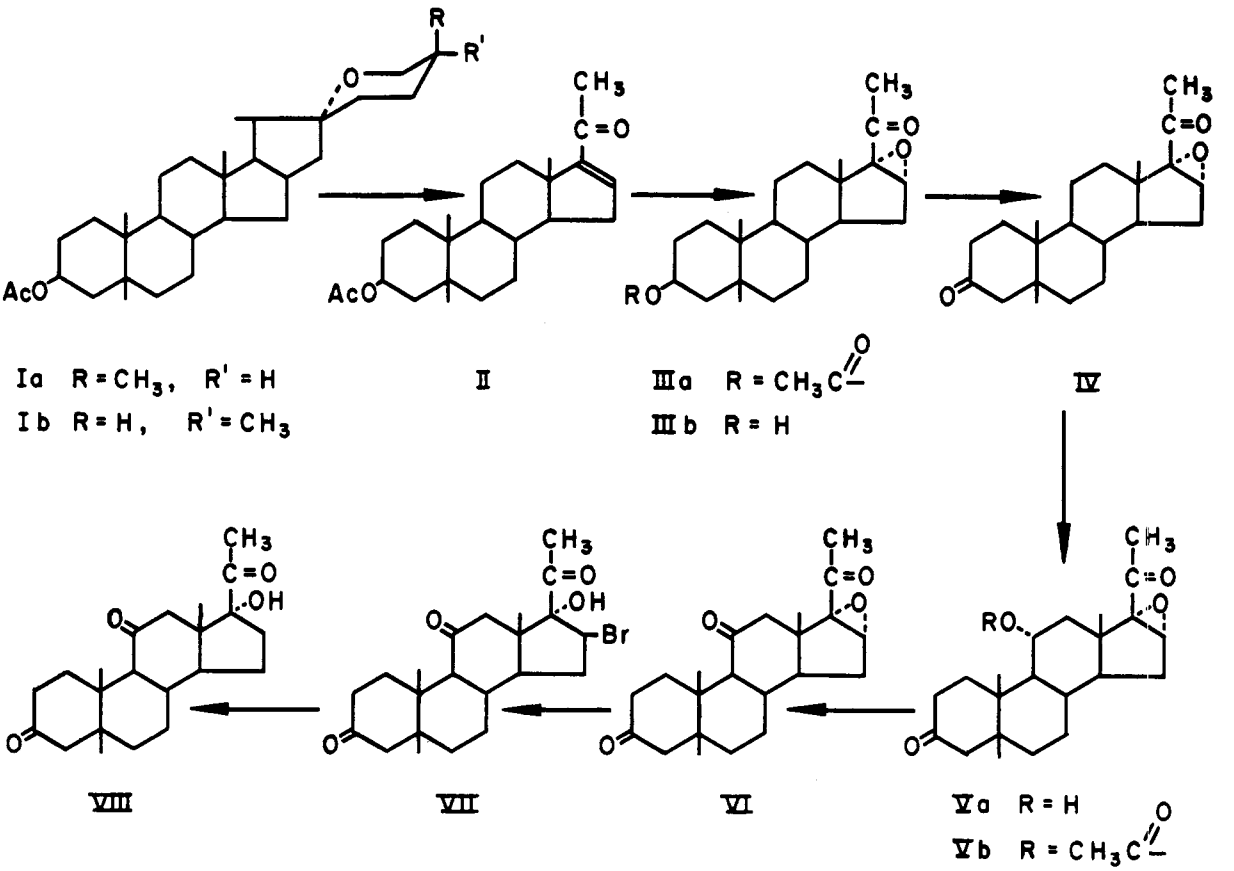
(9) *R. nigricans* has been utilized for 11α-hydroxylation of a large number of steroids; *cf.* S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, *Vitamins and Hormones*, **14**, 359 (1956), but has not been recorded as having been tested on IV.

(10) We have not attempted to develop further the microbiological conversion of IV to Va. It would seem from the excellent review of Eppstein, *et al.*, ref. 9, that a detailed study of this conversion would almost certainly lead to higher yields.

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

(12) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, THIS JOURNAL, **74**, 483 (1952).

(13) K. Dobriner, E. R. Katzenellenbogen and R. N. Jones, "In-



searches were completed we noted that compounds V, VI, VII and VIII had been prepared by Ercoli and deRuggieri.[14] They reduced 16α,17α-epoxy-11α-hydroxyprogesterone to give Va, which was then converted to VIII in a manner quite similar to that described here.[15] Since Ercoli and de Ruggieri have converted VIII to cortisone,[14] it is apparent that conversion of smilagenin or sarsasapogenin to VIII constitutes a formal synthesis of cortisone from these compounds.

Experimental[16]

3β-Acetoxy-16α,17α-epoxypregnan-20-one (IIIa).—A solution of 25 g. of 3β-acetoxy-16-pregnen-20-one[5] (II) was dissolved at room temperature in 2400 ml. of absolute methanol and cooled to 5°. To the cooled solution was added 72 ml. of 4 *N* sodium hydroxide solution followed by 136 ml. of 30% hydrogen peroxide. The solution was kept in the refrigerator at 5° for 23 hours. Then 3.2 liters of saturated sodium chloride solution was added, followed by 18 ml. of glacial acetic acid. The crystalline precipitate was filtered, washed repeatedly with water and dried. The product weighed 25.0 g., m.p. 180–185°. The analytical sample was crystallized three times from acetone to give plates, m.p. 185°, $[\alpha]^{25}D$ +61.2°. Infrared spectrum shows strong bands at 1735 (acetate) and 1707 cm.$^{-1}$ (20-ketone).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.73; H, 9.21.

3β-Hydroxy-16α,17α-epoxy-pregnan-20-one (IIIb).—To a warm solution of 25.0 g. of IIIa in 350 ml. of *t*-butyl alcohol were added 25 g. of potassium hydroxide in 20 ml. of water. The water solution was flushed with nitrogen and stirred for three hours at 50°. To the butanol solution was then added 1200 ml. of water and product allowed to stand overnight. It next was extracted repeatedly with ether and the ether washed well with dilute hydrochloric acid and then water. The dried ethereal solution was concentrated to 250 ml.; crystallization occurred and a crop of 14.4 g. of IIIb, m.p. 224–226°, was obtained. From the mother liquors 6.7 g. of similar product was isolated. The analytical sample was crystallized from ethyl acetate as irregular plates, m.p. 228°, $[\alpha]^{25}D$ −66.3°. The infrared spectrum shows a band at 3620 cm.$^{-1}$ (hydroxyl) and a strong band at 1705 cm.$^{-1}$ (20-ketone).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.72; H, 9.91.

16α,17α-Epoxypregnane-3,20-dione (IV).—A solution of 10.0 g. of IIIb in 300 ml. of glacial acetic acid was cooled to 14°. A solution of 4.0 g. of chromium trioxide in 60 ml. of aqueous acetic acid 1:1 was cooled to 14° and added dropwise with stirring to the steroid solution over a period of 5 minutes. The temperature was kept below 15° during the addition of the oxidant and was then brought to room temperature in 15 minutes. The solution was poured into 2000 ml. of water, neutralized with potassium hydroxide and filtered. The light green crystalline product was passed

frared Absorption Spectra of Steroids," Interscience Publishers, Inc., New York, N. Y., 1953.

(14) A. Ercoli and P. deRuggieri, *Gazz. chim. ital.*, **85**, 628 (1955).

(15) In general the melting points and rotations obtained by Ercoli and deRuggieri were in rough agreement with those of our compounds. However, the melting points of most of our compounds were 10–20° higher than theirs. It is possible that some of the products obtained by Ercoli and deRuggieri may have contained small quantities of 5α-isomers. It has been shown (THIS JOURNAL, **75**, 1286 (1953)) that the catalytic reduction of 11α-hydroxy-Δ^4-3-ketosteroids does not give exclusively 5β-reduction products but also a minor but appreciable 5α-fraction.

(16) We wish to thank S. Serota for optical rotation data, C. S. Fenske for infrared spectra and R. B. Kelley for carbon and hydrogen analyses. Optical rotations were obtained in chloroform solution using 2 decimeter tubes, at concentrations of approximately 16.7 mg./ml.; infrared spectra, unless otherwise specified, were measured in carbon bisulfide solution, concentration 10 g./liter.

through a short, thick column of Florisil in chloroform solution. The crystalline product, 9.8 g., had a m.p. 164–168°. Crystallization from methanol gave irregular plates subliming to needles on heating, m.p. 174°, $[\alpha]^{25}D$ +81.6° (lit.[17] gives m.p. 168–170°, $[\alpha]^{20}D$ +79°). Infrared spectrum shows strong band at 1705–1720 cm.$^{-1}$ (3- and 20-ketone).

16α,17α-Epoxy-11α-hydroxypregnane-3,20-dione (Va).—To a 5-gallon stainless steel fermentor equipped with an agitator was added 12 liters of a medium consisting of 240 g. of Edamine, 600 g. of dextrose and 60 ml. of corn steep liquor. The fermentor and contents were sterilized in an autoclave at 15 lb. per sq. in. pressure (121°) for one hour. The medium was allowed to cool and inoculated with a spore culture (6 days old) of *Rhizopus nigricans* ATCC 6227b. After 24 hours at 28° with rapid stirring (500 r.p.m.) and 1.2 liters of air per minute being added a vigorous growth of mycelium was noted. At this time 3.0 g. of IV in 120 ml. of a 1:1 mixture of acetone–ethanol was added and the fermentation continued for 48 hours. The mycelium was recovered by filtration and extracted three times with a total of 5 liters of acetone and three times with 5 liters of chloroform. The filtrate was extracted eleven times with a total of 24 liters of chloroform. The extracts were combined and concentrated. The foregoing procedure differs slightly in detail from, but is essentially based on, the techniques described by the Upjohn group.[9,18] The residue from the concentrated solvent extracts was resinous. It was saponified with hot methanolic potassium hydroxide solution. After addition of water and the usual ethereal extraction and work up, the product in benzene solution was passed through a Florisil column. Elution with benzene gave 1.3 g. of unreacted IV. Elution with benzene-containing 20% chloroform gave 1.4 g. of Va. The analytical sample was crystallized from ether as rods, m.p. 170–172°, $[\alpha]^{25}D$ +57.3° (lit.[14] gives m.p. 158–160°, $[\alpha]^{21}D$ +68°). Infrared spectrum shows bands at 3640 cm.$^{-1}$ (hydroxyl) and strong, broad band at 1705–1720 cm.$^{-1}$ (3- and 20-ketone).

(17) O. Mancera, H. J. Ringold, C. Djerassi, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **75**, 1286 (1953).

(18) D. H. Peterson, *et al.*, *ibid.*, **74**, 5933 (1952), and subsequent papers in THIS JOURNAL.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.55; H, 8.82.

11α-Acetoxy-16α,17α-oxido-pregnane-3,20-dione (Vb).—Compound Va (0.1 g.) was refluxed 0.5 hour in 1 ml. of acetic anhydride. The solvent was removed *in vacuo*. Crystallization from methanol gave plates, m.p. 222–224°, $[\alpha]^{25}D$ +24.4° (lit.[14] gives m.p. 215–216°, $[\alpha]^{19}D$ +25°). Infrared spectrum shows strong band at 1735 cm.$^{-1}$ (11-acetate) and strong, broad band at 1705–1720 cm.$^{-1}$ (3- and 20-ketone).

16α,17α-Oxidopregnane-3,11,20-trione (VI).—A solution of 0.7 g. of Va in 15 ml. of glacial acetic acid was oxidized with 0.3 g. of chromium trioxide in 50% aqueous acetic acid as described under the preparation of IV; yield 0.6 g., m.p. 194–196°. The analytical sample was crystallized from ether as plates, m.p. 199–201°, $[\alpha]^{25}D$ +115° (lit.[14] gives m.p. 181°, $[\alpha]^{21}D$ +105°). Infrared spectrum shows a strong, broad band at 1705–1720 cm.$^{-1}$ (3-, 11- and 20-ketone).

17α-Hydroxypregnane-3,11,20-trione (VIII).—A solution of 0.6 g. of compound VI in 18 ml. of acetic acid was cooled to 15° and mixed with 6 ml. of acetic acid containing 8% v./v. hydrobromic acid. The mixture was allowed to stand overnight at 15°. It was then diluted with water and given the usual ethereal extraction and work up. The ether was removed *in vacuo* at room temperature. The crystalline residue which we believe was 16β-bromo-17α-hydroxypregnane-3,11,20-trione (VII) was not further characterized. The crude VII was dissolved at once in 100 ml. of 90% methanol and hydrogenated in the presence of 0.3 g. of palladium on calcium carbonate for five hours at 4 atmospheres pressure. The solution then was filtered and concentrated. A crystalline product, weight 0.6 g., m.p. 180–188°, was obtained. Crystallization from ether gave 0.4 g. of rods, m.p. 198–201°, $[\alpha]^{25}D$ +41° (lit.[12] gives m.p. 203–204°, $[\alpha]^{25}D$ +41°). Infrared spectrum (chloroform) shows a strong band at 3500 cm.$^{-1}$ (bonded 17-hydroxyl), and a strong, broad band at 1700–1720 cm.$^{-1}$ (3-, 11- and 20-ketone). The infrared spectrum was identical to authentic VIII.[12]

INDIANAPOLIS, IND.

COMMUNICATIONS TO THE EDITOR

π-TROPENIUM-MOLYBDENUM-TRICARBONYL FLUOROBORATE

Sir:

Even though "sandwich" complexes of transition metals or their carbonyls with 5- or 6-membered aromatic systems, *e.g.*, $(\pi\text{-}C_5H_5)Fe(\pi\text{-}C_5H_5)$, $(\pi\text{-}C_5H_5)Mn(CO)_3$, $(\pi\text{-}C_6H_6)Cr(\pi\text{-}C_6H_6)$, $(\pi\text{-}C_6H_6)Cr(CO)_3$, are well-known[1] and similar complexes with the unknown 4-membered aromatic system, cyclobutadiene, have been postulated on theoretical grounds as reaction intermediates,[2] no report of the preparation of an analogous metal-π-molecular orbital complex with the 7-membered aromatic system, tropenium ion ($C_7H_7^+$), has appeared. In a recent attempt to prepare such a complex, Abel, Bennett and Wilkinson[3] found that cycloheptatriene reacted with molybdenum hexacarbonyl to give only the cycloheptatriene complex, $(\pi\text{-}C_7H_8)Mo(CO)_3$ (I). Application of our previously reported general preparative method for tropenium ions,[4] involving hydride ion abstraction from cycloheptatrienes by trityl carbonium ions, to the cycloheptatriene complex (I) has resulted in the first synthesis of a metal-tropenium ion complex, $(\pi\text{-}C_7H_7^+)Mo(CO)_3$, BF_4^- (II).

π-Cycloheptatriene-molybdenum-tricarbonyl (I), prepared in low yield by the passage of cyclohepta-

(1) (a) P. L. Pauson, *Quart. Revs.*, **9**, 391 (1955); (b) T. S. Piper, F. A. Cotton and G. Wilkinson, *J. Inorg. Nucl. Chem.*, **1**, 165 (1955); (c) E. O. Fischer and W. Hafner, *Z. anorg. Chem.*, **286**, 146 (1956); H. H. Zeiss and W. Herwig, THIS JOURNAL, **78**, 5959 (1956); (d) E. O. Fischer and K. Öfele, *Chem. Ber.*, **90**, 2532 (1957).

(2) H. C. Longuet-Higgins and L. E. Orgel, *J. Chem. Soc.*, 1969 (1956). *Cf.*, J. C. Sauer and T. L. Cairns, THIS JOURNAL, **79**, 2660 (1957), and M. Tsutsui and H. H. Zeiss, Abstracts, 134th Meeting, American Chemical Society, Chicago, Ill., Sept. 7–12, 1958, p. 59-P, for experimental evidence bearing on this postulate.

(3) E. W. Abel, M. A. Bennett and G. Wilkinson, *Proc. Chem. Soc.*, 152 (1958).

(4) H. J. Dauben, Jr., F. A. Gadecki, K. M. Harmon and D. L. Pearson, THIS JOURNAL, **79**, 4557 (1957); K. M. Harmon, Ph.D. Thesis, University of Washington, 1958.