Notes

A department for short papers of immediate interest.

Synthesis of Pyrazinoic Acid

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Received September 4, 1958

Considerable interest is attached to the synthesis of pyrazinoic acid since this compound is used in the preparation of the tuberculostat pyrazinamide.[1] The acid was formerly obtained in low yield by the oxidation of methylpyrazine with potassium permanganate[2] or by the monodecarboxylation of pyrazine-2,3-dicarboxylic acid.[3] The synthesis of the latter compound requires several steps. We wish to report two new simple syntheses of pyrazinoic acid which now make this acid a readily available material.

One method, consisting of the oxidation of methyl pyrazine with selenious acid in pyridine, gave the desired acid in 64% yield. There are several examples of the oxidation of activated methyl groups in a nitrogen heterocyclic compound to the corresponding carboxylic acid with selenium dioxide.[4,5]

The second synthesis is the oxidation of ethylpyrazine in water with potassium permanganate which gave a 48% yield of pyrazinoic acid. Ethylpyrazine was synthesized by a series of steps[6] similar to those used by Kitchen and Hanson[7] in the synthesis of methylpyrazine. Ethylenediamine was reacted with 1,2-butylene oxide to give *N*-(2-hydroxybutyl)ethylenediamine which was then cyclized to 2-ethylpiperazine by heating the substituted diamine at 105° for 2 hours in aqueous solution in the presence of Raney nickel.[8] Dehydrogenation of 2-ethylpiperazine to ethylpyrazine in 57% yield (and 19% recovery of starting material) was effected over copper chromite catalyst at 360° in aqueous rather than in a benzene solution.[7]

(1) E. P. Jordan, *Modern Drug Encyclopedia and Therapeutic Index*, 7th ed., Drug Publications Inc., New York, N. Y., 1958, p. 952.

(2) C. Stoehr, *J. prakt. Chem.*, (2) **51**, 468 (1895).

(3) W. L. McEwen, U. S. Patent **2,675,384** [*Chem. Abstr.*, **49**, 4730 (1955)].

(4) C. W. Larson, Ph.D. dissertation, Polytechnic Institute of Brooklyn, May 1949.

(5) D. Jerchel, J. Heider, and H. Wagner, *Ann.*, **613**, 153 (1958).

(6) These preparations were by Dr. Moses Cenker, Wyandotte Chemicals Corp.

(7) L. J. Kitchen and E. S. Hanson, *J. Am. Chem. Soc.*, **73**, 1838 (1951).

(8) W. K. Langdon, Canadian Patent **557,792**, May 20, 1958.

EXPERIMENTAL[9]

Ethylpyrazine, b.p. 152–153°/760 mm., had n_D^{25} 1.4969. *Anal.* Calcd. for $C_6H_8N_2$: C, 66.64; H, 7.46; N, 25.91. Found: C, 66.87; H, 7.56; N, 26.20.

Pyrazinoic acid. A. To 5 l. of pyridine was added a solution of 1.25 kg. (11.3 moles) of selenium dioxide in 500 ml. of water. Four hundred and forty grams (4.7 moles) of methylpyrazine was added and the mixture was refluxed with stirring for 10 hr. while selenium precipitated. The mixture was filtered and the filtrate evaporated *in vacuo*. The residue was dissolved in 3 l. of 2.5*N* sodium hydroxide. Decolorizing carbon, 50 g., was added and the mixture was stirred overnight. Acidification of the filtrate with one liter of 7.5*N* hydrochloric acid precipitated pyrazinoic acid which was filtered and washed well with water. The dissolution of the pyrazinoic acid in aqueous alkali, treatment with decolorizing carbon, and subsequent acidification was repeated and gave finally 372 g., (64%) of a light tan product, m.p. 219° dec. (m.p. 229–230° dec.)[2] and neut. equiv. 121.4 (calcd. 124.1). The infrared spectrum was identical with that of a sample prepared from methylpyrazine according to Stoehr.[2] The methyl ester, prepared by the Fischer method, melted at 59.5–60.5° (m.p. 61–62°).[10] For purposes of economy in large runs, 92% of the selenium was recovered (as selenium dioxide) from the precipitated selenium and the mother liquors containing selenious acid.[11]

B. A solution of 54 g. (0.5 mole) of ethylpyrazine in 750 ml. of water was treated portionwise with 315 g. (2.0 moles) of solid potassium permanganate in 12 hr. while the mixture was kept at room temperature with slight cooling. After an additional 8 hr. of stirring, the precipitated manganese dioxide was removed by filtration and the filtrate was acidified with 60 ml. of concentrated hydrochloric acid. The precipitated pyrazinoic acid was filtered and washed well with water. The yield was 29.7 g. (48%) of pure white crystals, m.p. 218.5–219° dec., with neut. equiv. 123.8 (calcd. 124.1). There was no depression in the melting point when samples of pyrazinoic acid from both methods A and B were mixed. The methyl ester, m.p. 59.5–60.5° was prepared as noted in method A.

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(9) All melting points are uncorrected.

(10) T. I. Fand and P. E. Spoerri, *J. Am. Chem. Soc.*, **74**, 1345 (1952).

(11) N. Rabjohn, *Org. Reactions*, **5**, 344 (1949).

Microbiological Transformation of Steroids. VII. 15β-Hydroxylation

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Received October 8, 1958

Although hydroxylations of steroids by fungi have been demonstrated for a variety of positions

and substrates, few bacterium-induced hydroxylations have been disclosed so far.[1] We have now found that 4-pregnene-17α,21-diol-3,20-dione (I) (Reichstein's Compound S) and progesterone (II) are converted to 4-pregnene-15β,17α,21-triol-3,20-dione (III) and 4-pregnen-15β-ol-3,20-dione (IV),[1] respectively, by the action of *Bacillus megaterium* (Schering 41[1a]).[2] Bernstein and his coworkers,[3] in a preliminary communication, described the preparation of III by the action of a fungus of the *Spicaria* genus and provided evidence for this assignment of structure. We had reached similar conclusions about the structure of III on the basis of experiments which parallel in part those of Bernstein.

Reichstein's S (I), when incubated with *B. megaterium* in a yeast extract medium, afforded *ca.* 50% yield of III, isolated by chloroform extraction and purified by crystallization from acetone. Analysis indicated the presence of one additional hydroxyl group. Oxidation of III with sodium bismuthate[3a] gave a 17-ketosteroid (V) (carbonyl bands at 5.77 and 6.01μ in the infrared spectrum containing an hydroxyl group, which, upon further oxidation with chromic acid, gave an hydroxyl-free steroid (VI) with carbonyl bands at 5.68, 5.78, and 5.98 μ in the infrared spectrum. Since no absorption bands corresponding to a saturated, six-membered ring carbonyl could be demonstrated, and since study of the band at 5.78 μ indicated that two five-membered ring carbonyl groups were probably present (the D ring carbonyl band was more intense than the A ring carbonyl band), the entering hydroxyl group was placed at position 15 or 16.

From the literature[1,4] the changes in molecular rotation for hydroxylation at 15 and 16 are given as $\Delta M_D^{16\alpha OH-H}$ -64,[4a] $\Delta M_D^{16\beta OH-H}$ $+ 38$,[4b] $\Delta M_D^{15\alpha OH-H}$ $+ 87$ and $\Delta M_D^{15\beta OH-H}$ -114 (average of values for ΔM^{15} for 15α- and 15β-hydroxyprogesterone and 15α- and 15β-hydroxydesoxycortiscosterone). The change in molecular rotation from I to III was -138 units. From this it was inferred that the most likely possibilities for the position of the entering hydroxyl group were 15β and 16α.

The physical constants of V, m.p. 203–205°, $[\alpha]_D^{25}$ + 120° (ethanol) are notably different from those reported for 4-androstene-16α-ol-3,17-dione,[1] m.p. 185–187°, $[\alpha]_D$ + 194°. Microbiological reduction of V with *Saccharomyces cerevisiae*[5] afforded VIa, m.p. 220–222°, $[\alpha]_D^{25}$ + 57° (ethanol), which contrasts with 4-androstene-16α, 17β-diol-3-one,[1,6] m.p. 183–184°[1] or 191–192°.[6] $[\alpha]_D$ + 76°[1] or + 80°.[6] Consequently, 16α is rejected as a likely position for the entering hydroxyl group.

It was reasoned that the presence of a β-diketone structure in VI should permit ready titration with strong base. Potentiometric titration with 0.25*N* sodium hydroxide was carried out in 50% aqueous dimethyl sulfoxide, affording a molecular weight value of 303, in excellent agreement with the theory (300). Hence, the position of the entering hydroxyl in III is fixed as 15β.

Fried[1] has observed that the 15β-hydroxyl group in IV is not readily acylable. A mixture of acetic anhydride and pyridine did not acetylate V at room temperature in significant yield, only unreacted V being recovered from the reaction. Although III may be diacetylated with acetic anhydride in pyridine the second acetyl group enters with some reluctance. From a mixture of III with a large excess of acetic anhydride in pyridine after fifteen hours at room temperature some 21-monoacetate of III (VII) was isolated in addition to a major yield of 15β,21-diacetate (VIII). Selective acetylation of III with one mole of acetic anhydride was readily effected and the resulting VII was then oxidized to 4-pregnene-17α,21-diol-11,15,20-trione 21-acetate (IX) with chromic acid. Hence, III behaves in a manner consistent with its assigned structure.

Following an argument of Reichstein's,[7] we supposed that the degradation of III to the corresponding 17β-carboxylic acid by the action of potassium periodate should lead to lactone formation involving the 15β-hydroxyl group. We carried out this degradation and isolated a carboxylic acid (X), which was readily soluble in dilute sodium hydroxide, and which was esterified by diazomethane to a methyl ester. Hence, for compounds in this series, the argument is not valid. It is possible that the steric hindrance, which inhibits acetylation, is one of the factors operating here as well.

(1) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz, and A. Borman, *Rec. Prog. Hor. Res.*, XI, 149 (1955) describe the 14α-hydroxylation of progesterone with *B. cereus*.

(1a) Schering 41 now bears the ATCC number 13368.

(2) W. J. McAleer, T. H. Stoudt, *et al.*, *Arch. Biochem.*, **73**, 127 (1958) have reported independently the 15β-hydroxylation of progesterone with *B. megaterium;* in the same paper they also describe 11α-hydroxylation of progesterone with *B. cereus* strains. The strain of *B. megaterium* employed by us is not the same as that employed by the Merck group.

(3) S. Bernstein, L. I. Feldman, W. S. Allen, R. H. Blank, and C. E. Linden, *Chem. & Ind.* (*London*), 111 (1956).

(3a) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(4) (a) D. Perlman, E. Titus, and J. Fried, *J. Am. Chem. Soc.*, **74**, 2126 (1952); (b) A. Wettstein, *Experientia*, **XI**, 465 (1955). The configurational assignments for 15α- and 15β-hydroxydesoxycorticosterone, given originally by C. Meystre, E. Vischer, and A. Wettstein, *Helv. chim. Acta*, **38**, 381 (1955), were incorrect and are reversed in the *Experientia* article.

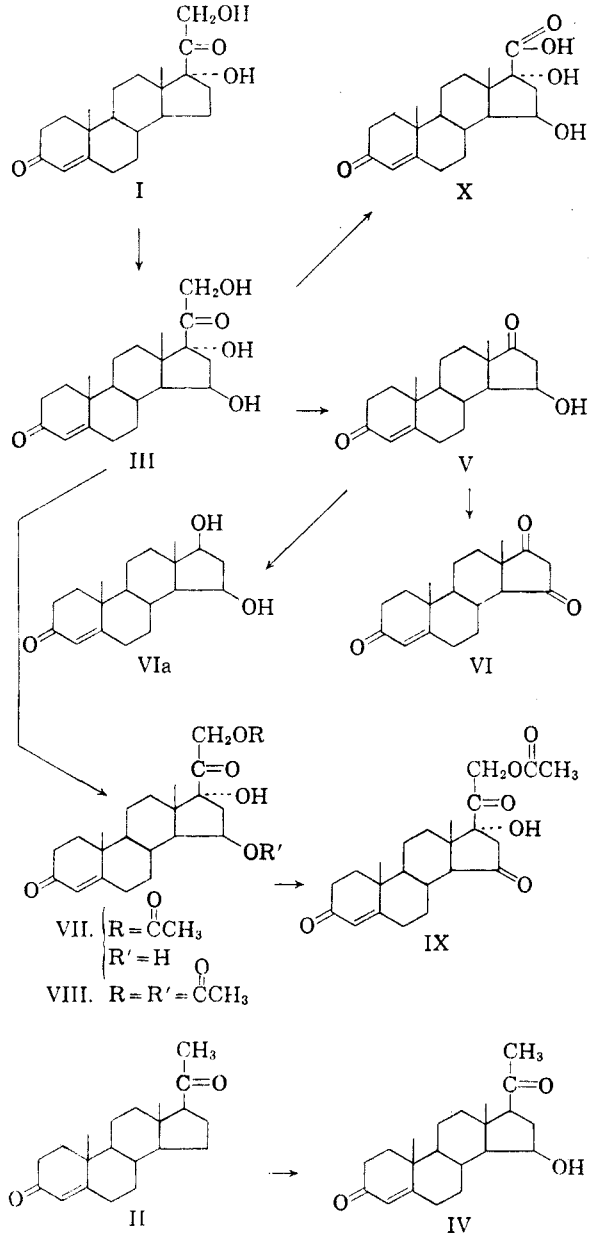
(5) For a number of examples, see the review of O. Hanc and E. Reidl-Tumova, *Die Pharmazie*, **11**, 877 (1954).

(6) W. J. Adams, D. K. Patel, V. Petrow, and I. A. Stuart-Webb, *J. Chem. Soc.*, 297 (1956).

(7) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. von Euw, O. Schindler, and T. Reichstein, *Helv. chim. Acta*, **37**, 1200 (1954).

Hydroxylation of II with *B. megaterium* (Schering 41) in the way described previously afforded a low yield of IV, whose constants were in reasonable agreement with those given by Fried.[1] At least nine other substances (ultraviolet-absorbing) were indicated as reaction products in a paper chromatogram.

On the other hand, 1-dehydrocortisol and 1-dehydrocortisone did not afford 15β-hydroxylated derivatives and were reduced in part to cortisol and cortisone respectively. The yields in these experiments were low and we were unable to isolate other crystalline products. We are not aware of any prior report of microbiological reduction of a 1,2-double bond in steroids.



EXPERIMENTAL[8]

4-Pregnene-15β,17α,21-triol-3,20-dione (III). A medium was prepared from 10 g. of yeast extract (Difco) and 10 g. of Cerelose made up to 1 l. with tap water and distributed equally among ten 300-ml. Erlenmeyer flasks. The contents of the flasks were sterilized and inoculated with a loopful of *Bacillus megaterium* (Schering 41) culture which had been maintained on nutrient agar. The culture was then incubated at 28°C. and shaken at 220 revolutions/minute for 16 hr. Thereupon, 25 mg. of I in 0.5 ml. of 80% aqueous ethanol was added to each flask, and incubation with shaking was continued for 24 hr. At the end of this time paper chromatography[9] by Shull's method of the chloroform extract of an aliquot indicated disappearance of the starting material and the formation of a single, new, ultraviolet-absorbing product which stained with red tetrazolium.[10] The reaction mixture was extracted thoroughly with chloroform, the extracts were washed with water, dried, concentrated, and the residue was crystallized from acetone-hexane and from acetone in turn. There resulted 0.128 g. of III, m.p. 240–241° dec., $[\alpha]_D^{25}$ + 103° (ethanol), $\lambda_{max}^{methanol}$ 242 mμ (ε = 16,600), λ_{max}^{Nujol} 2.91 μ (OH), 5.83 μ (20-carbonyl), 6.01 and 6.18 μ (Δ⁴-3-one).

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.51; H, 8.46.

The melting points of III varied from 216–220° dec. to 253–255° dec. At least two additional polymorphic varieties were observed. Bernstein[3] gives m.p. 240–242°, $[\alpha]_D^{24}$ + 96° (methanol).

4-Pregnene-15β,17α,21-triol-3,20-dione 21-acetate (VII). To a solution of 130 mg. of III in 4 ml. of pyridine was added 44 mg. of acetic anhydride. After 2.5 hr. the reaction mixture was poured into water and 100 mg. of precipitate, m.p. 226–231° was recovered by filtration. Recrystallization from acetone-hexane afforded 80 mg. of fine needles, m.p. 244–246° dec., $[\alpha]_D^{25}$ +92° (ethanol), $\lambda_{max}^{ethanol}$ 242 mμ (ε = 17,700), λ_{max}^{Nujol} 2.86 and 2.96 μ (OH), 5.72, 5.77 and 5.82 μ (combined 20-carbonyl 21-acetate), 6.06 and 6.20 μ (Δ⁴-3-ketone) and 8.10 μ (C—O—C of acetate). Bernstein[3] reports VII, m.p. 245.5–247°, $[\alpha]_D^{24}$ + 98° ($CHCl_3$).

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.38; H, 8.08.

4-Pregnene-15β,17α,21-triol-3,20-dione 15,21-diacetate (VIII). A solution of 260 mg. of III in 6 ml. of pyridine was treated with 3 ml. of acetic anhydride and allowed to stand overnight at room temperature. Dilution of the reaction mixture with water afforded a precipitate which was removed by filtration and recrystallized from acetone-hexane. If concentration of the acetone-hexane mixture was carried out slowly, in two of three experiments, it was possible to cause the crystallization from dilute solution of about 20 mg. of VII, m.p. 239–242°, as prisms, with an infrared spectrum identical with that from authentic VII. Further concentration of the filtrate afforded 180 mg. of soft needles of VIII, m.p. 193–197°. Chromatography on Florisil and recrystallization from acetone-hexane gave VIII, m.p. 211–214°, $[\alpha]_D^{25}$ + 56.8° (ethanol), $\lambda_{max}^{ethanol}$ 239 mμ (ε = 16,800), λ_{max}^{Nujol} 3.15 μ (OH), 5.78, 5.80, and 5.84 μ (20-carbonyl and acetate carbonyls), 6.06 μ and 6.20 μ (Δ⁴-3-ketone), 8.20 μ (C—O—C of acetate). Bernstein[3] gives m.p. 252–254°, $[\alpha]_D^{25}$ + 109° ($CHCl_3$). Our sample was homogeneous (by paper chromatography), free of 21-monoacetate, and non-identical with Dr. Bernstein's (infrared).

(8) All m.p.'s are corrected. Analyses and optical data were obtained by the Physical Chemistry Department of these laboratories and by Galbraith Laboratories, Knoxville, Tenn.

(9) G. M. Shull, Abstracts of Papers, 126th meeting of the American Chemical Society, Sept. 12–17, 1954, New York, p. 9A.

(10) W. J. Mader and R. R. Buck, *Anal. Chem.*, 24, 666 (1952).

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 66.93; 66.99; H, 7.46; 7.40.

4-Pregnene-17α,21-diol-3,15,20-trione 21-acetate (IX). A solution of 150 mg. of VII in 2 ml. of pyridine was added to slurry of 75 mg. of chromic acid in 5 ml. of pyridine[11] at 0° with mechanical stirring, and the mixture was allowed to warm slowly to room temperature. Stirring was continued overnight, and then a solution of 0.5 g. of sodium sulfite in 10 ml. of water was added. After stirring for an hour, the reaction mixture was poured into 200 ml. of water, and the resulting precipitate (120 mg., m.p. 240–247°) was removed by filtration. Recrystallization from acetone-hexane afforded IX, m.p. 258–260° dec., $[\alpha]_D^{25}$ + 129° (acetone), $\lambda_{max}^{ethanol}$ 240 mμ (ε = 17,300), λ_{max}^{Nujol} 3.05 μ (OH), 5.75 μ (acetate and 15-carbonyl), 5.83 μ (20-carbonyl), 6.10 and 6.20 μ (Δ⁴-3-one) and 8.15 μ (C—O—C of acetate). Bernstein[3] reports IX, m.p. 254–255.5°, $[\alpha]_D^{24}$ + 143° ($CHCl_3$).

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.60; H, 7.78.

4-Androstene-15β-ol-3,17-dione (V). A solution of 250 mg. of III in 40 ml. of glacial acetic acid was diluted with 40 ml. of water and 5 g. of sodium bismuthate was added. The resulting mixture was stirred overnight at room temperature, whereupon the solids were removed by filtration. Both the filtrate and the solids were extracted with methylene chloride; then the extracts were combined, and washed free of acetic acid with water. Concentration of the resulting solution and chromatography of the residue over Florisil afforded a series of fractions, eluted with ether, m.p. 203–205°. After recrystallization from methylene chloride-hexane, there was isolated 60 mg. of V, m.p. 203–205°, $\lambda_{max}^{methanol}$ 240 mμ (ε = 16,900), $[\alpha]_D^{25}$ + 120° (ethanol), λ_{max}^{Nujol} 2.99 μ (OH), 5.77 μ (17-carbonyl), 6.01 and 6.20 μ (Δ⁴-3-one). Bernstein[3] reports m.p. 199.5–201°, $[\alpha]_D^{25}$ + 147° (methanol).

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.31; H, 8.58.

A solution of 200 mg. of V in 4 ml. of pyridine and 1 ml. of acetic anhydride was allowed to stand overnight at room temperature. The reaction mixture was then diluted with water and extracted with methylene chloride. The extracts were washed with 10% sulfuric acid and with water, and then dried. After concentration of the solution and crystallization of the residue from ether-hexane there resulted 60 mg. of V, m.p. 195–205°, with an infrared spectrum identical with that of starting material. The mother liquors from the crystallization were not examined further. While some 15-acetate may have been present, it is clear that conditions which are adequate for complete acetylation of most secondary hydroxyl groups do not suffice here.

4-Androstene-3,15,17-trione (VI). To a solution of 540 mg. of V in 40 ml. of glacial acetic acid was added 170 mg. of chromic acid dissolved in 4 ml. of water and 16 ml. of glacial acetic acid. After the reaction had proceeded for 3 hr. at room temperature, water was added, and the mixture was extracted with methylene chloride. The extracts were washed with water, dried, concentrated, and chromatographed over Florisil. Elution with 50% ether-in-hexane afforded 70 mg. of VI, m.p. 192–197°. Recrystallization from acetone-hexane raised the m.p. to 194–197°, $[\alpha]_D^{25}$ + 117.5° (methanol), $\lambda_{max}^{methanol}$ 241 mμ (ε = 17,300), 275 mμ (ε = 7,700), $\lambda_{minimum}^{methanol}$ 268 mμ (ε = 7,600), λ_{max}^{Nujol} 5.67 and 5.78 μ (D-ring carbonyl), 5.98 and 6.22 μ (Δ⁴-3-ketone).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.53, H, 8.56.

The equivalent weight of VI was determined by titrating potentiometrically with sodium hydroxide. The automatic recording titration assembly consisted of a motor-driven Gilmont microburet, capacity 0.1 ml., Beckman glass and calomel electrodes, a Leeds and Northrup Model 7664 line operated *p*H Indicator with recording attachment and a Brown recording potentiometer. A solution of 3.062 mg. of VI in 6.0 ml. of 50% by volume dimethylsulfoxide and water required 0.03954 ml. of 0.2558*N* sodium hydroxide for neutralization yielding an equivalent weight of 303 (MW of VI is 300). The apparent pK_a is 6.17.

4-Androstene-15β,17β-diol-3-one (VIa). To a sterile medium composed of 600 g. of cerelose and 100 g. of yeast extract (Difco) made up to 1 l. with tap water and buffered at *p*H 6.8 with phosphate buffer was added 300 ml. of an inoculum of *Saccharomyces cerevisiae*, prepared in shake flasks with the same medium, and the mixture was incubated at 28°, with aeration at one-half volume of air/volume of medium/minute for 42 hr. At the end of this time 0.5 g. of V in 10 ml. of methanol was added, the air rate was increased to 1.5 vol. of air/vol. of medium/minute and incubation was continued for 3 days. At the end of that period paper chromatography of the chloroform extract of an aliquot indicated that the reaction was essentially complete. The mixture was extracted with chloroform, the extracts were washed with water, dried, and concentrated. Crystallization of the residue from methylene chloride-hexane afforded 0.2 g. of VIa, m.p. 208–210°. Recrystallization raised the m.p. to 220–222°, $[\alpha]_D^{25}$ + 57° (ethanol), $\lambda_{max}^{methanol}$ 242 mμ (ε = 15,000), λ_{max}^{Nujol} 2.94 and 3.12 μ (OH), 6.05 and 6.21 μ (Δ⁴-3-one).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 74.96; H, 9.27. Found: C, 74.92; H, 9.72.

3-Keto-15β,17α-dihydroxy-4-etienic acid. To a solution of 40 mg. of III in 4 ml. of methanol was added 14.5 mg. of a solution of potassium periodate in water (1.18 g. per 200 ml. of water). The reaction mixture was allowed to stand overnight in the dark. The solution was then made acid with a few drops of 10% sulfuric acid. After 0.5 hour at room temperature the *p*H of the solution was adjusted to 5.0–6.0 (*p*H paper) with methanolic sodium hydroxide, 1 drop of glycerine was added, and the solution was concentrated in a stream of air. There followed crystallization of 26 mg. of the acid as needles, m.p. 265–270° dec., $\lambda_{max}^{methanol}$ 241 mμ (ε = 16,400), λ_{max}^{Nujol} 2.89, 3.01 and 3.14 μ (OH), 5.75 μ (?) 5.89 μ (carboxyl carbonyl), 6.14 and 6.22 μ (Δ⁴-3-ketone). The band at 5.75 μ was anomalous as was the absence of a band at 3.75 μ.

Anal. Calcd. for $C_{20}H_{28}O_5 \cdot H_2O$: C, 65.55; H, 8.25. Found: C, 65.55, 65.72; H, 8.47, 8.59.

The apparent *p*H of the acid was 4.4 in dimethylsulfoxide-water (apparent *p*H 7.3).

The water of crystallization could not be driven off by heating at 140° over phosphorus pentoxide *in vacuo*. Recrystallization from acetone-hexane afforded another crystalline variety of the acid, m.p. 261–263°, with variations in the infrared spectrum.

Methyl 3-keto-15β,17α,-dihydroxy-4-etienate. The carboxylic acid (50 mg.) from the preceding experiment was esterified in methanol solution by the addition of ethereal diazomethane in excess. The mixture was allowed to stand overnight at room temperature and was then concentrated and crystallized from methanol-water. Recrystallization from the same solvents afforded the methyl ester (22.5 mg.), m.p. 199–201°, $[\alpha]_D^{25}$ + 50° (dioxane), $\lambda_{max}^{methanol}$ 242 mμ (ε = 15,500) λ_{max}^{Nujol} 2.96 μ (OH), 5.79 μ (ester carbonyl), 6.01 and 6.21 μ (Δ⁴-3-one) and 8.33 μ (C—O—C of ester).

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.30; H, 8.34.

4-Pregnene-15β-ol-3,20-dione (IV) *from progesterone* (II). A gram of II was transformed in 48 hr. according to the procedure described earlier, with *B. megaterium*. Paper chromatography indicated that at least ten substances absorbing in the ultraviolet were present. Chromatography of the residue from chloroform extraction over Florisil afforded a series of crystalline fractions (total weight 140 mg.) eluted with ether, which were pooled and crystallized from acetone–hexane. There resulted 90 mg. of needles of IV, m.p. 195–199°, $[\alpha]_D^{25}$ + 158° ($CHCl_3$), λ_{max}^{Nujol} 2.92 μ (OH), 5.88 μ (20-carbonyl), 6.02 and 6.19 μ (Δ⁴-3-one).

(11) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.49; H, 9.34.

Fried[1] gives IV, m.p. 204–205°, $[\alpha]_D$ + 151°.

Cortisone from 1-dehydrocortisone. Incubation of 2.0 g. of 1-dehydrocortisone with *B. megaterium* for 48 hr. followed by isolation of the steroidal products in the usual way afforded 1.6 g. of crude solids. Paper chromatography[10] indicated that a substance with the same mobility as cortisone was present. Chromatography on 15 g. of Florisil and elution with 50% ether-in-hexane afforded small amounts of crystalline solids, which were pooled and recrystallized from acetone-hexane. There resulted 15 mg. of cortisone, m.p. 215–220° dec., whose infrared spectrum was identical with that of an authentic sample. No additional crystalline products other than some starting material were obtained on completing the chromatogram.

Cortisol from 1-dehydrocortisol. From 2 g. of 1-dehydrocortisol by incubation with *B. megaterium*, a crude mixture of 1.5 g. of oily steroids was obtained. Initial chromatography on 15 g. of Florisil and elution with 5% methanol in methylene chloride afforded a series of crystalline fractions of m.p. > 200°, which were pooled (420 mg.) and rechromatographed on 15 g. of Florisil. Elution with 1% methanol in methylene chloride afforded a series of fractions which had the same mobility as cortisol in a paper chromatogram. Recrystallization from acetone-hexane gave 45 mg. of cortisol, m.p. 210–215°, whose infrared spectrum was identical with an authentic sample.

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Microbiological Transformation of Steroids. VI. Stereospecific Reductions of the 20-Carbonyl Group

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Received October 8, 1958

Reduction of the 20-carbonyl to 20β-hydroxyl by microbiological means was first noted by Fried, Thoma, and Klingsberg[1] from the action of *Streptomyces lavendulae* on progesterone. Szpilfogel, Van Hemert, and DeWinter[2] have described the simultaneous reduction at 20- and 1,2-dehydrogenation of cortisone with *Fusarium* and *Calonectria* strains to give 1-dehydro Reichstein's U. None of these organisms is suitable for the generalized reduction of 4-pregnene-3,20-diketosteroids to the corresponding 4-pregnene-3-keto-20β-hydroxysteroids because of the other chemical transformations promoted simultaneously by these organisms.

We have found that various species of *Streptomyces*, in particular *Streptomyces griseus* (Schering FC No. 103), *Streptomyces sp.* (FC No. B222), and *Streptomyces sp.* (QM No. 1086), and an unidentified bacterium (FC No. C78) are capable of reducing the 20-carbonyl to 20β-hydroxyl in a variety of corticosteroids in good yield without producing other chemical alterations in the molecule. In this way we have transformed 4-pregnene-17α,21-diol-3,20-dione (I) (Reichstein's Compound S) into 4-pregnene-17α,20β,21-triol-3-one (II),[3a,3b] cortisone (III) into 4-pregnene-17α,20β,-21-triol-3,11-dione (IV),[4] cortisol (V) into 4-pregnene-11β,17α,20β,21-tetrol-3-one (VI),[5] 1-dehydrocortisone (VII)[6] into 1,4-pregnadiene-17α,-20β,21-triol-3,11-dione (VIII),[7] and 1-dehydrocortisol (IX)[6] into 1,4-pregnadiene-11β,17α,20β,21-tetrol-3-one (X).

Formation of the 20β-carbinols was carried out by aerobic incubation of the appropriate steroid with the *Streptomyces* strain in a yeast extract–dextrose–corn steep liquor medium. Progress of the reaction was measured by the disappearance of the substrate as estimated by paper chromatography according to Shull,[8] and by appearance of a more polar spot which did not stain with "red tetrazolium".[9] When the starting material had been consumed (usually 1–3 days), the reaction mixture was extracted with chloroform, and the product was isolated by concentration of the extract and crystallization from a suitable solvent (usually acetone-hexane). Yields of the reduced product varied between 20% and 75%. The poorest results occurred in the 11 β-hydroxyl series.

The structure of II was assigned on the basis of the absence of the 20-carbonyl band in the infrared spectrum (and the presence of the other appropriate bands), the correspondence of physical constants with those reported by Julian,[3b] and the preparation of the known diacetate.[3b] The structure of IV was confirmed by comparison of its melting point with that given by Reichstein and von Euw,[4] and by preparation of the diacetate.[4] The structure of VI was assigned by similar techniques. Compound VIII was characterized by preparation of the previously described diacetate[6] and by the changes in the molecular rotation accompanying this reaction[10] (see Table I). Compound

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