[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Facile Synthesis of 19-Nortestosterone and 19-Norandrostenedione from Estrone

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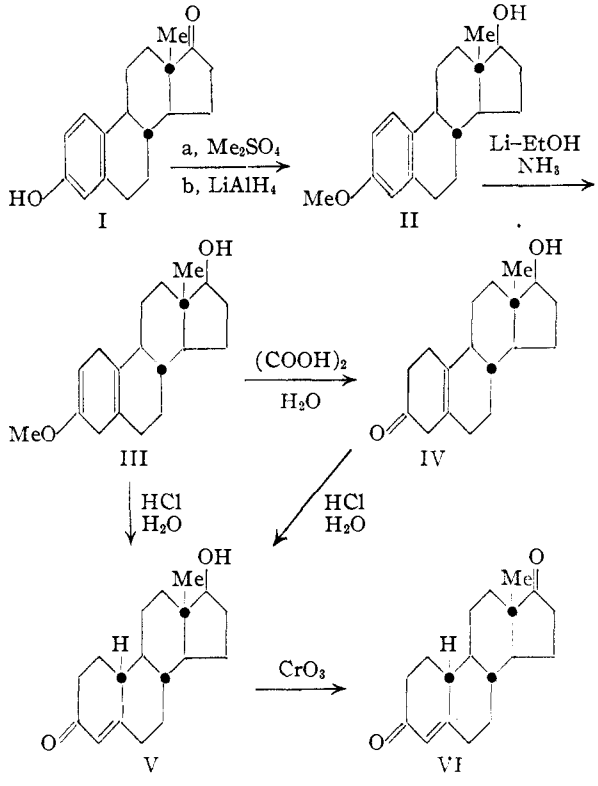
Using the improved lithium–ammonium procedure, excellent yields have been obtained for the reduction of estradiol methyl ether (II) to the dihydro derivative III, for conversion of the latter to 19-nortestosterone (V) (70–77% over-all yield) and to its β,γ-isomer IV. Oxidation of nortestosterone gave 19-nor-4-androstene-3,17-dione (VI) in excellent yield. From the molecular rotational differences of these compounds, their C-10 methyl homologs and the aromatic methyl ethers, it was concluded that they and the 19-norprogesterone of Miramontes, *et al.*,[9] all have the same configurations and probably with a β-C-10 hydrogen. The 19-norandrogens were less active than testosterone propionate. Isomer B of hexahydro-*meso*-hexestrol has been reduced and cleaved to the β,γ-unsaturated ketone, isolated as the dinitrophenylhydrazone.

The question of how important the angular methyl groups of the non-aromatic steroidal hormones testosterone, progesterone, etc., are for their physiological activity has for many years interested chemists and biologists concerned with the relation between structure and activity of these hormones. The classical experiments of Dirscherl and coworkers[2] on hydrogenation products of estrone clearly demonstrated, in 1936, that compounds lacking the methyl group between rings A and B possess some androgenic activity. Marker and Rohrmann[3] reported preparing 19-nor compounds related to androstenedione and testosterone.

The reduction of phenol ethers by sodium and alcohol in liquid ammonia to dihydro derivatives readily convertible to α,β-unsaturated ketones, extensively investigated by Birch,[4] provides a short route to such 19-nor derivatives from the corresponding estrone derivatives. Birch and Mukherji[5] were able to reduce the 3-glyceryl ether of estradiol to the dihydro derivative with sodium or potassium,[6] and hydrolyzed this compound to 19-nortestosterone (V) in about 12% over-all yield from estradiol.

Independently we have been concerned with the general problem of the synthesis and potency of 19-nor derivatives, in connection with our analogs of the non-aromatic steroid hormones lacking ring C, which also lack the 19-methyl group.[7] With the development of the improved lithium technique for such reductions now described in the previous paper,[8] we have been able to prepare 19-nortestosterone in over-all yields of 70–77% from estrone.

While Birch and Mukherji were forced to use the more soluble, but less available, glyceryl ether, our new procedure permits using the easily prepared 3-methyl ether of estradiol (II). Excellent yields (over 90%) of the crystalline dihydro derivative III were obtained. This was cleaved and rearranged in as high as 88% yield to the α,β-unsaturated ketone 19-nortestosterone (V), using dilute methanolic hydrochloric acid. This facile shift of



the double bond into conjugation was easily avoided however, with aqueous oxalic acid which gave the β,γ-unsaturated ketone IV in 83% yield. The cleaner nature of these reduction and cleavage reactions led to purer products than those obtained by Birch and without the need for chromatographic purification.

Oxidation of nortestosterone with chromium trioxide smoothly gave 19-norandrostenedione (VI). This compound, m.p. 170–171°, evidently is different from that reported by Marker and Rohrmann, m.p. 146–148°,[3] although the possibility of dimorphism is not excluded. Their method of preparation, involving hydrogenation of estrone, oxidation to the diketone, bromination and dehydrobromination, makes possible a number of structures for their compound including the Δ^1- and Δ^{15}-structures, as well as Δ^4-, and perhaps epimeric at C-10 or C-9. The limited data reported do not permit reaching a definite conclusion about the structure of their compound at this time.

In each case only one of the C-10 epimers was isolated for our 19-nor derivatives. Recently

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(2) W. Dirscherl, *Z. physiol. Chem.*, **239**, 53 (1936); W. Dirscherl, J. Kraus and H. E. Voss, *ibid.*, **241**, 1 (1936).

(3) R. E. Marker and E. Rohrmann, THIS JOURNAL, **62**, 73 (1940).

(4) See A. J. Birch, *Quart. Revs*, **4**, 69 (1950), for a review of this reaction; also ref. 8.

(5) A. J. Birch and S. M. Mukherji, *J. Chem. Soc.*, 2531 (1949).

(6) A. J. Birch, *J. Chem. Soc.*, 367 (1950).

(7) A. L. Wilds and C. H. Shunk, THIS JOURNAL, **72**, 2388 (1950); A. L. Wilds, C. H. Hoffman and T. H. Pearson, to be published.

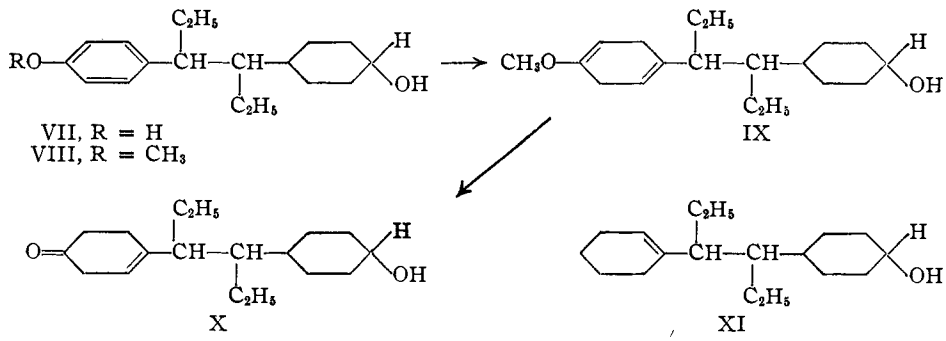
(8) A. L. Wilds and N. A. Nelson, *ibid.*, **75**, 5360 (1953).

Miramontes, Rosenkranz and Djerassi[9] have been able to use our lithium reduction procedure in modified form to prepare 19-norprogesterone from 17-acetyl-3-methoxy-1,3,5-estratriene. It was found to be more active in rabbits than progesterone itself.

Since these 19-nor compounds all are formed under conditions which would permit the C-10 hydrogen to shift to the more stable *anti*-configuration, it is reasonable to suppose that they have the same configuration at C-10 (and all other asymmetric centers) as their natural C-10 methyl homologs. The remarkably high activity of 19-norprogesterone seems to us reasonable support for this view until stronger evidence becomes available. It can be concluded from rotational differences, in any case, that these three 19-nor derivatives all have the same configuration at C-10. Miramontes, *et al.*,[9] found the molecular rotation difference between the aromatic methyl ether and norprogesterone ($M_{Ar} - M_H$) to be +59 to 68°; the difference between progesterone and norprogesterone ($M_{Me} - M_H$) was +187°. In our examples $M_{Ar} - M_H$ was +71° for nortestosterone and $M_{Me} - M_H$ +165 to 185°.[10] For norandrostenedione $M_{Me} - M_H$ was +195°. These values are in good agreement with those for norprogesterone, especially considering the large effect which changes near the C-10 carbon have on the rotation. Thus the dihydro compound was 105° and the β,γ-ketone VI was 299° higher in molecular rotation than estradiol methyl ether.

Physiological tests have been carried out on these compounds by Dr. R. K. Meyer and associates of the Department of Zoology.[11] At a total dose of 1400 γ (given over 7 days to 21-day old rats), 19-nortestosterone gave about 0.3 the increase in ventral prostate weight as that produced by 1400 γ of testosterone propionate, but gave an equivalent increase in weight of levator ani muscle. At a total dose of 3500 γ the β,γ-isomer IV gave only 0.07 the increase in ventral prostate weight as that with an equal weight of the standard; 19-norandrostenedione (3500 γ) gave 0.2 the increase produced by the standard.

We have also carried out reduction of the more potent hexahydro-*meso*-hexestrol isomer B (VII) of Wilds and McCormack[12] as the methyl ether VIII by our lithium procedure. This compound proved to be one of the most difficultly reduced examples which we have encountered to date. Under the proper conditions, however, reduction proceeded to the extent of 97%, and from the resulting mixture the dinitrophenylhydrazone of the β,γ-unsaturated ketone **X** was isolated in 60–80% over-all yield from VII. In addition to the dihydro derivative **IX**, the reduction mixture contained demethoxylated material formulated as **XI**. The conversion of **X** and the related compound obtained from isomer A of VII to the α,β-unsaturated ketones, the analogs of testosterone, is being further investigated.



Experimental[13]

3,17β-Estradiol 3-Methyl Ether (II).—One gram of estrone was methylated at 35° in dilute methanolic potassium hydroxide, adding a total of 10 ml. of dimethyl sulfate during the first half of the two-hour period. The unrecrystallized methyl ether (1.04 g., sint. 159°, m.p. 163–167°) was reduced with 0.19 g. of lithium aluminum hydride in ether (Soxhlet extractor), and the product isolated by ether extraction with two Claisen alkali washes.[14]

Crystallization of the neutral product from 90% alcohol gave 0.87 g. (83%), m.p. 118–119°, and 0.07 g. (7%), m.p. 80.5–90°. The first crop was a higher melting polymorphic form of 3,17β-estradiol 3-methyl ether, which on further recrystallization from 90% ethanol melted at 120.5–121.5°; λ_{max} 278 mμ (1930), 286.5 mμ (1780); $[\alpha]^{22}D$ +77.4 ± 0.4°, MD +222°, $[\alpha]^{22}Hg$ +92.3 ± 0.3° (*c* 1.68 in chloroform). The second crop was mainly the lower melting form previously reported (m.p. 97–98°),[15] and on further recrystallization melted at 97–98.5°.

1,4-Dihydro-3,17β-estradiol 3-Methyl Ether (III).—The reduction was carried out in the apparatus and by the general procedure described by us for lithium–ammonia reductions.[8] To a solution of 1.38 g. of the methyl ether (m.p. 118–119°) in 110 ml. of anhydrous ether was added 140 ml. of liquid ammonia followed by 1.4 g. (42 equivalents per mole) of lithium wire in small pieces, and 10 minutes later 16 ml. of absolute alcohol was added dropwise over a 10- to 20-minute period. Occasionally frothing occurred during the last of this addition but was easily controlled by stopping the stirrer temporarily. After removing most of the ammonia and carefully adding cold water, the product was extracted with ether, washed with Claisen alkali,[16] water, saturated salt and dried over sodium sulfate. Crystallization of the product from 6 ml. of absolute alcohol and 25 ml. of petroleum ether (b.p. 60–68°) gave 1.00 g., m.p. 118–119.5° (vac., sint. 117.5°, capillary inserted at 90°),[17]

(9) L. Miramontes, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 3540 (1951).

(10) The values reported by A. J. Birch and H. Smith, *J. Chem. Soc.*, 1882 (1951), for V and IV suggest that each ketone was contaminated with its double bond isomer.

(11) L. G. Hershberger, E. G. Shipley and R. K. Meyer, to be published.

(12) A. L. Wilds and W. B. McCormack, THIS JOURNAL, **70**, 4127 (1948).

(13) All melting points are corrected unless otherwise indicated; those marked vac. were in Pyrex capillaries evacuated to at least 0.2 mm., micro m.p.'s were taken with a calibrated microscope hot-stage. Ultraviolet spectra were determined with Beckman DU or Cary Model 11 spectrophotometers in 95% alcohol solutions unless specified otherwise, and molecular extinction coefficients (ε) are reported. Infrared spectra run with Baird instrument. Microanalyses by John Belew, Edmund Eisenbraun, Gerald Gilbert and Gershen Winestock.

(14) From these 9% of 3,17β-estradiol, m.p. 174–176°, was recovered.

(15) A. Butenandt and C. Georgens, *Z. physiol. Chem.*, **248**, 136 (1937).

(16) Acidification of the alkaline layers gave no estradiol showing demethylation to be insignificant.

(17) In an open capillary inserted at room temperature the m.p. was 105–113°,

$[\alpha]^{25}D$ +112.7 ± 0.6° ($CHCl_3$); the ultraviolet absorption showed less than 1% of unreduced material to be present. Additional crops of 0.20 g., m.p. 116.5–118° (vac.), and containing less than 2% unreduced material (ultraviolet), and 0.06 g., m.p. 89–96° (vac.), containing less than 5% unreduced material (ultraviolet), brought the total solid material to 90%. The remaining oil (0.18 g.) contained less than 8% unreduced material (ultraviolet). In other runs the yield of solid melting above 114° was 86–95%, the lower yield resulting from use of 1,2-dimethoxyethane instead of ether.

The pure dihydro derivative was obtained by recrystallization from benzene and then from petroleum ether (b.p. 60–68°), and was dried at 40° (0.05 mm.) for 20–36 hours to remove last traces of solvent, m.p. 118–119.5° (vac. inserted at 90°). There was no appreciable absorption of light in the region 275–290 mμ; the infrared spectrum in carbon disulfide gave a hydroxyl band at 2.74 μ and sharp bands at 5.94 and 6.03 μ characteristic of the unconjugated dihydroanisole ring,[18] $[\alpha]^{22}D$ +113.4 ± 0.4°, MD +327, $[\alpha]^{22}Hg$ +138.6 ± 0.4° (*c* 1.13 in chloroform).

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.1; H, 9.79. Found: C, 79.2; H, 9.99.

In one run by the general procedure of Birch, except using 15 ml. of ether, 2.6 ml. of isopropyl alcohol, 45 ml. of liquid ammonia, 0.50 g. of estradiol methyl ether and adding 0.69 g. of sodium last over a five-minute interval, the solid product obtained in 84% yield, m.p. 97–107°, contained as much as 28% unreduced material (ultraviolet).

17β-Hydroxy-5(10)-estrene-3-one (IV).—A solution of 175 mg. of dihydroestradiol methyl ether (m.p. 116.5–118°) in 15 ml. of methanol and 0.23 g. of oxalic acid dihydrate in 3 ml. of water were mixed (solution *ca.* 0.1 *M* in oxalic acid) and allowed to stand at 25° for 40 minutes. Ether was then added, the extract washed thoroughly with bicarbonate solution, water and dried and the product was crystallized from ethyl acetate to give 124 mg., m.p. 197.5–199.5° (dec., vac., inserted at 180°) and 14 mg., m.p. 193–196.5° dec., for a total yield of 83%. The remaining oil (23 mg.) appeared to contain approximately 18% of estradiol methyl ether, corresponding to 2% in the original dihydro derivative.

Further crystallization from ethyl acetate gave the pure β,γ-unsaturated ketone IV as colorless elongated plates, m.p. 199.8–201° (dec., vac., sint. 196°),[19] $[\alpha]^{22}D$ +189.8 ± 0.7°, MD 521, $[\alpha]^{22}Hg$ +228.8 ± 0.6° (*c* 1.02 in chloroform); λ_{max}^{Nujol} 2.92 μ (OH) and 5.91 μ (unconj. C=O); λ_{max}^{EtOH} 282–289 mμ (36), and with low absorption in the 240 mμ region. Birch, *et al.*, reported the m.p.'s 181–182°[5] and 187–188°,[6] MD +460° (chloroform).[10] This low value for rotation suggests that their sample may have been contaminated with as much as 16% of the α,β-isomer.

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 78.8; H, 9.55. Found: C, 78.7; H, 9.57.

17β-Hydroxy-4-estrene-3-one (19-Nortestosterone) (V).—To a solution of 500 mg. of dihydroestradiol methyl ether (m.p. 116.5–118°) in 25 ml. of methanol at 60° was added 15 ml. of 3 *N* hydrochloric acid and heating continued at 60° for 15 minutes. Then the clear solution was cooled, ether and water added and the ether extracts washed thoroughly with bicarbonate solution, saturated salt solution, counter extracting the aqueous layers with additional ether, and dried over sodium sulfate. The product was crystallized from ether (seeding with the higher melting form is helpful) to give 346 mg., m.p. 123.2–124.5°, 29 mg., m.p. 120–122°, and 43 mg., m.p. 106–116.5° (λ_{max} 240 mμ 13,400), totalling 88% of crystalline nortestosterone. The spectrum of the residual oil (7%) indicated the presence of about 40% nortestosterone. The yield was lower in other runs using less pure material, chromatographic purification being helpful in some cases, but the over-all yields of crystalline nortestosterone from estradiol methyl ether were 70–77%.

Nortestosterone was obtained in two crystallographic forms: colorless needles from methylcyclohexane, containing a small amount of ethyl acetate, m.p. 111–112.3° retaining solvent removed by drying at 55–70° (0.05 mm.) for 22 hours, and from ether, m.p. 123.8–124.6°, mixed m.p. 123.6–124.5°,[20] $[\alpha]^{22}D$ +55.0 ± 0.6°, MD +151°, $[\alpha]^{22}Hg$ + 68.9 ± 0.8° (*c* 0.93 in chloroform); λ_{max}^{EtOH} 240.5 mμ (17,000), 307–310 mμ (92); $\lambda_{max}^{CS_2}$ 2.77 μ (OH), 6.02 μ (conj. C=O). Reported by Birch, *et al.*, m.p. 111°,[6] MD +184° (chloroform),[10] λ_{max}^{EtOH} 240–241.5 mμ (17,000).[6] The high value for rotation suggests that their sample may have been contaminated with as much as 9% of the β,γ-isomer.

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 78.8; H, 9.55. Found C, 78.5; H, 9.58.

Preliminary attempts to prepare nortestosterone propionate with propionyl chloride or propionic anhydride gave mixtures of mono- and di-(enol) propionates even at room temperature.

4-Estrene-3,17-dione (19-Norandrostenedione) (VI).—A solution of 200 mg. of nortestosterone (m.p. 123–124.5°) in 10 ml. of glacial acetic acid was treated with 56 mg. of chromium trioxide in 5 ml. of 95% acetic acid at 16° over a 30-minute period with stirring. After an additional 2 hours at 21°, 1 ml. of methanol was added, most of the solvent removed under reduced pressure, ether and dilute hydrochloric acid added and the product isolated as usual. Crystallization of the neutral product from cyclohexane gave 156 mg., m.p. 169–170.5° (vac., inserted 150°), and 19 mg., m.p. 160.5–166°, for a total of 88%. Further recrystallization from cyclohexane gave the diketone as colorless, elongated plates, m.p. 170–171° (vac., sint. 168.5° and inserted at 150°), $[\alpha]^{27}D$ +137 ± 1°, MD 374°, $[\alpha]^{27}Hg$ +162 ± 2° (*c* 0.88 in chloroform), λ_{max}^{EtOH} 239 mμ (16,900) and 298 mμ (130), $\lambda_{max}^{CS_2}$ 5.77 μ (unconj. C=O in five-membered ring) and 5.99 μ (conj. C=O).

Anal. Calcd. for $C_{18}H_{24}O_2$: C, 79.4; H, 8.88. Found: C, 79.0; H, 9.00.

Marker and Rohrmann[3] have reported a norandrostenedione of uncertain structure, m.p. 146–148°, no rotation given.

Reduction of 3-(*p*-Methoxyphenyl)-4-(4′-hydroxycyclohexyl)-hexane (Hexahydrohexestrol Monomethyl Ether (VIII)).—To 600 mg. of hexahydro-*meso*-hexestrol isomer B (m.p. 132.5–134°)[12] in 10 ml. of methanol and 0.8 g. of potassium hydroxide in 10 ml. of water were added concurrently over 30 minutes and with stirring at 30–35° 3 ml. of dimethyl sulfate and 2 g. of potassium hydroxide in 5 ml. of water. After another 60 minutes ammonia was added, the product was extracted with three portions of ether, the latter washed with Claisen alkali, water and dried. After evaporation of the ether, the oil was dried to constant weight (610 mg., 97%) at 50° and 0.05 mm.; λ_{max}^{EtOH} 225 mμ (10,800), 277.5 mμ (1590) and 284 mμ (1350). In some runs the oil crystallized, micro m.p. 44–53°, but was used without purification.

To the oily methyl ether as prepared above (600 mg.) dissolved in 60 ml. of 1,2-dimethoxyethane (dried over potassium hydroxide and distilled from sodium) were added 100 ml. of liquid ammonia and 0.6 g. (42 equivalents per mole) of lithium wire in portions. After 15 minutes stirring, enough absolute alcohol (5 ml.) was added (dropwise over 15 minutes) to just discharge the blue color, 15 ml. of ammonia and 0.4 g. (28 equivalents per mole) of lithium were added and after another 10 minutes 3.5 ml. of absolute alcohol was added over a 10-minute period. Finally the ammonia was evaporated, ether and cold water added and the product isolated as above. The neutral oil amounted to 580 mg. (constant weight at 50° and 0.1 mm.), negligible absorption at 227 and 284 mμ (less than 3% of unreduced material).

To a solution of 50 mg. of dinitrophenylhydrazine in 2 ml. of 95% alcohol and 0.3 ml. of concentrated hydrochloric acid (heated to dissolve, cooled and filtered) was added 50 mg. of the above reduction product dissolved in 1.5 ml. of alcohol. After two hours at 0°, 1 ml. of 3 *N* hydrochloric acid was added, and the product which had crystallized was filtered and washed with dilute acid, water and bicarbonate solution, giving 65 mg. (80%) of yellow solid, m.p. 126–134°.

(18) G. Stork, THIS JOURNAL, **73**, 504 (1951).

(19) Reproducible melting points were obtained using Pyrex capillaries made from tubing soaked in dilute ammonia and water, evacuating the capillaries to 0.05 mm. and inserting in a bath preheated to 180–190°. By the usual technique there was an appreciable conversion to α,β-unsaturated ketone with lowering of the m.p.

(20) This higher melting form was first obtained by Dr. André Dreiding (private communication), to whom we are indebted for a sample.

Recrystallization from alcohol containing a drop of pyridine gave 52 mg. (80% recovery) of yellow plates, m.p. 144.5–145.5°. The analytical sample of the **2,4-dinitrophenylhydrazone of 3-(4′-keto-1-cyclohexenyl)-4-(4′-hydroxycyclohexyl)-hexane** melted at 145.3–146.5° (sint. 144°), $\lambda_{max}^{CHCl_3}$ 263.5 mμ (24,600).

Anal. Calcd. for $C_{24}H_{34}O_5N_4$: C, 62.9; H, 7.47. Found: C, 62.6; H, 7.45.

Similar reductions in ether gave less reduction (22–34% starting material present from ultraviolet spectrum). After hydrolysis of such a reduction product (550 mg.) in 30 ml. of methanol and 15 ml. of 3 *N* hydrochloric acid kept for three hours at 25°, chromatography on acid-washed alumina gave 18% of material micro m.p. 77–86°, in the first eluates. This proved to be reduced and demethoxylated material, probably **3-(1′-cyclohexenyl)-4-(4′-hydroxycyclohexyl)-hexane (XI)**, colorless needles from dilute acetone, m.p. 94–94.5° (sint. 93°), no selective absorption in the ultraviolet.

Anal. Calcd. for $C_{18}H_{32}O$: C, 81.7; H, 12.20. Found: C, 81.8; H, 11.95.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. VIII. Preparation of 17α-Hydroxycorticosterone

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Incubation of Reichstein's Compound S with *Cunninghamella blakesleeana* strain H-334 yielded 17α-hydroxycorticosterone.

Discussion

Previous papers in this series have reported the microbiological oxygenation of steroids by fungi of the order *Mucorales*.[1] In an earlier paper[2] details were given for the conversion of Reichstein's Compound S (11-desoxy-17α-hydroxycorticosterone) by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) to 11α,-17α,21-trihydroxy-4-pregnene-3,20-dione (epi-Compound F). We have isolated a fungus, identified as *Cunninghamella blakesleeana* of the order *Mucorales* and designated strain H-334, which converts Compound S directly to the biologically active steroid, 17α-hydroxycorticosterone (Kendall's Compound F, hydrocortisone, Reichstein's Compound M). The description of this one-step fermentation is the subject of the present paper. A similar conversion with an Actinomycete, *Streptomyces fradiae* Waksman 3535, has been reported previously.[3]

Experimental[4]

Fermentation.—Ten liters of a soybean–dextrose medium[5] was added to a 5-gallon glass bottle fitted with a revolving stainless steel paddle and an aluminum tube which delivered sterile air to the bottom of the vessel. After autoclaving at 120° for 45 minutes, the medium was inoculated with 500 ml. of vegetative mycelium[6] of *C. blakesleeana* H-334 and the mixture was incubated at constant temperature (26°), aeration (1 l. air/min.), and agitation (84 r.p.m.) for 24 hours.

At the end of this period of growth, 500 mg. of Reichstein's Compound S was added as a sterile ethanolic solution. The resulting concentration of ethanol in the fermentation mixture was 6%. The incubation was allowed to proceed for 16 hours.

Extraction.—After the mycelium was removed by filtration through a Celite[7] pad, the filtrate was extracted three times with 2-l. portions of ethylene chloride. Small amounts of steroids remaining in the mycelium were removed by further extractions in which the filter cake was stirred twice with 1-l. portions of ethylene chloride. All extracts were combined, and the solvent was removed[8] to give a crude steroid residue.

The presence of 17α-hydroxycorticosterone, 11-dehydro-17α-hydroxycorticosterone (cortisone) and unreacted Compound S in the crude steroid residue was indicated by the paper chromatographic techniques of Zaffaroni, *et al.*[9] The positions of the steroids on the papergram were determined by means of their absorption of ultraviolet light.[10] A quantitative determination of the 17α-hydroxycorticosterone present was made by eluting the steroid from the paper and measuring the absorption at 242 mμ. It was found that 35.2% of the Compound S had been converted to 17α-hydroxycorticosterone.

Chromatography.—The crude steroid residue from the fermentation was chromatographed first on a Florisil[11] adsorption column to effect the removal of extraneous lipids and secondly on a silica partition column to fractionate the steroidal components.

After the Florisil column (50 g. of adsorbent), bearing the crude steroid residue (about 3.0 g.), was washed with ethylene chloride (500 ml.) for removal of the extraneous lipids, the steroids of medium polarity were recovered by elution with 900 ml. of a mixture of ethylene chloride and acetone (2:1). This latter fraction (428.6 mg.), containing the 17α-hydroxycorticosterone, was separated into its constituent steroids by use of a partition column and an automatic chromatographic fraction cutter.[12,13] This column

(1) (a) Paper VII in this series: S. H. Eppstein, D. H. Peterson, H. Marian Leigh, H. C. Murray, A. Weintraub, L. M. Reineke and P. D. Meister, THIS JOURNAL, **75**, 421 (1953); (b) D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952); (c) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. Marian Leigh, *ibid.*, **74**, 5933 (1952); (d) H. C. Murray and D. H. Peterson, U. S. Patent 2,602,769 (July 8, 1952), based on an original application filed August 19, 1950.

(2) D. H. Peterson, S. H. Eppstein, P. D. Meister, B. J. Magerlein, H. C. Murray, H. Marian Leigh, A. Weintraub and L. M. Reineke, THIS JOURNAL, **75**, 412 (1953).

(3) (a) D. R. Colingsworth, M. P. Brunner and W. J. Haines, *ibid.*, **74**, 2381 (1952); (b) D. R. Colingsworth, J. N. Karnemaat, F. R. Hanson, M. P. Brunner, K. M. Mann and W. J. Haines, *J. Biol. Chem.*, **203**, 807 (1953).

(4) Melting points were taken on a Kofler micro hot stage.

(5) Soybean meal, 5 g.; dextrose, 20 g.; NaCl, 5 g.; K_2HPO_4, 5 g.; Brewers' yeast, 5 g.; tap H_2O, 1 l.; *p*H adjusted to 6.4.

(6) *C. blakesleeana* grown in soybean–dextrose medium on a reciprocating shaker at 26° for 48 hours.

(7) Diatomaceous earth produced by Johns-Manville, 22 East 40th Street, New York 16, New York.

(8) Solvents were always removed at 10 mm. pressure, under nitrogen, at temperatures less than 50°.

(9) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

(10) W. J. Haines and N. A. Drake, *Federation Proc.*, **9**, 180 (1950).

(11) Florisil is an activated magnesium silicate produced by the Floridin Company, 220 Liberty Street, Warren, Pennsylvania.

(12) W. J. Haines, N. A. Drake, C. D. Alway and M. P. Brunner, Abstracts of Papers, 118th Meeting Am. Chem. Soc., Chicago, Illinois, Sept. 1950, p. 11-M.

(13) W. J. Haines, "Recent Progress in Hormone Research," Vol. VII, Academic Press, Inc., New York, N. Y., 1952, p. 255.