(0.045 mole) of hydrazine hydrate dissolved in 25 ml. of ethyl alcohol. The solution was refluxed for 2 hours. After removal of the alcohol *in vacuo*, 20 ml. of water was added and the residue and the solution were acidified to *p*H 3 with concentrated hydrochloric acid. On standing, crystals were formed which were collected and recrystallized from ethyl ether–petroleum ether (1:1). The yield of product melting at 210–212° was 2.5 g.

*Anal.* Calcd. for $C_4H_3F_3N_2O$: C, 31.59; H, 1.99; N, 18.42. Found: C, 31.77; H, 2.10; N, 18.47.

**1-Methyl-3-trifluoromethylpyrazolin-5-one.**—Methylhydrazine sulfate (5.9 g., 0.041 mole) was dissolved in 10 ml. of water. This solution was neutralized with an equivalent amount of potassium hydroxide whereupon a clear solution was obtained. To this solution there was added 5.0 g. (0.027 mole) of ethyl trifluoroacetoacetate. After the addition of another 10 ml. of water the solution was refluxed for 2 hours. During the reflux period an oil separated out of solution which, on chilling, crystallized. The solid material was collected and, after drying, was recrystallized from ethyl ether–petroleum ether (1:1) to give 1.1 g. of product, m.p. 174–175.5°.

*Anal.* Calcd. for $C_5H_5F_3N_2O$: C, 36.15; H, 3.04; N, 16.87. Found: C, 36.38; H, 3.28; N, 16.88.

**1-Phenyl-3-trifluoromethylpyrazolin-5-one**, m.p. 185–187°, was prepared in 75% yield by condensing ethyl trifluoroacetoacetate with phenylhydrazine at 125° for 4 hours. The compound as recrystallized from ethyl alcohol–water mixture.

*Anal.* Calcd. for $C_{10}H_7F_3N_2O$: C, 52.64; H, 3.09; N, 12.28. Found: C, 52.53; H, 3.19; N, 12.45.

**6-Benzyloxy-2,3,4,4a,5,6,7,8-octahydro-3-cinnoline**, m.p. 137–138°, was prepared essentially according to the method outlined by Clarke and Lapworth.[14]

*Anal.* Calcd. for $C_{15}H_{18}N_2O_2$: C, 69.75; H, 7.03; N, 10.58. Found: C, 69.98; H, 7.08; N, 11.10.

(14) R. W. J. Clarke and A. Lapworth, *J. Chem. Soc.*, **89**, 1869 (1906).

SUMMIT, N. J.

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[CONTRIBUTION FROM THE BIOLOGICAL AND CHEMICAL RESEARCH DIVISIONS OF G. D. SEARLE AND CO.]

# Microbiological Transformations. IV. The Oxidation of Dehydroisoandrosterone at C-7

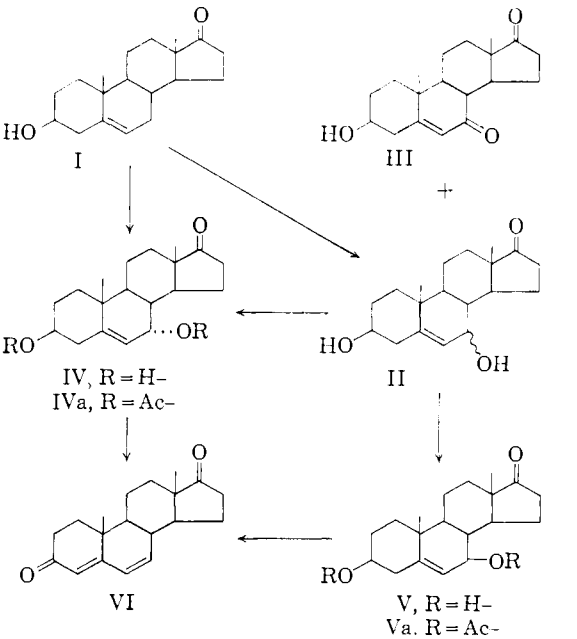
BY R. M. DODSON, R. T. NICHOLSON AND R. D. MUIR

RECEIVED JUNE 15, 1959

The structure and configurations of 7α-hydroxydehydroisoandrosterone and 7β-hydroxydehydroisoandrosterone, obtained by microbiological oxidation of dehydroisoandrosterone, were established. A mixture of these two materials behaves like a molecular compound.

The selective hydroxylation of steroids by microörganisms was first demonstrated by Kramli and Horvath[1] by their conversion of cholesterol to 7-hydroxycholesterol with *Proactinomyces roseus*. More recently Murray and Peterson[2] have demonstrated the 7β-hydroxylation of 3β-hydroxyallopregnan-20-one and 3β-hydroxy-5-pregnen-20-one with *Rh. arrhizus*. The latter compound also was hydroxylated 7β by *Rh. nigricans*.[2] Kahnt and coworkers[3] also have shown the 7β-hydroxylation of a 3β-hydroxyallopregnane with a species of Rhizopus. During our studies on the C-1 hydroxylation of dehydroisoandrosterone,[4] we uncovered a number of organisms which oxidized dehydroisoandrosterone at C-7.

Fermentation of dehydroisoandrosterone (I) by the methods previously described[5] with a species of Rhizopus (M 2045) isolated in our laboratories gave a monohydroxydehydroisoandrosterone (II), m.p. 193–196°, which crystallized readily from acetone and which, in most respects, behaved like a pure compound.[6] Chromatography of the residues from the isolation of II yielded 7-oxodehydroisoandrosterone (III) and 7β-hydroxydehydroisoandrosterone (V), m.p. 215–216°, but no II. Chromatography of II on silica gel yielded a small quantity of V, in the initial fractions eluted with 50% ethyl acetate in benzene, but most of the material was recovered unchanged, m.p. 195–197.5°. A paper chromatography study of II failed to show the presence of more than one compound.




(1) A. Kramli and J. Horvath, *Nature*, **162**, 619 (1948); **163**, 219 (1949).

(2) H. C. Murray and D. H. Peterson, U. S. Patent 2,702,809, Feb. 22, 1955; U. S. Patent 2,703,326, March 1, 1955; U. S. Patent 2,602,769, July 8, 1952; S. H. Epstein, P. D. Meister, H. C. Murray and D. H. Peterson, "Vitamins and Hormones," Vol. XIV, Academic Press, Inc., New York, N. Y., 1956, p. 359.

(3) F. W. Kahnt, Ch. Meystre, R. Neher, E. Vischer and A. Wettstein, *Experientia*, **8**, 422 (1952).

(4) R. M. Dodson, A. H. Goldkamp and R. D. Muir, THIS JOURNAL, **79**, 3921 (1957).

(5) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, *ibid.*, **74**, 5933 (1952).

(6) This same material was also separated directly by crystallization from fermentations of dehydroisoandrosterone with two other strains of Rhizopus (M 2052 and M 2051). The identity of these materials was established by comparison of their infrared spectra and by the lack of depression in the m.p.'s of their mixtures.

Acetylation of V produced the expected diacetate Va, m.p. 161.5–162.5°. Acetylation of II gave an oil which could not be purified by chromatography on silica gel or separated into distinct spots on paper chromatography. Repeated crystallization of this material from petroleum ether gave the diacetate IVa, m.p. 168.5–170°.

The nature of II was resolved by the discovery that fermentation of dehydroisoandrosterone with a species of Fusidium (M 61-1) yielded 5-androstene-3β,17β-diol and 7α-hydroxydehydroisoandrosterone (IV), m.p. 180–182.5°. The acetate of IV was identical in all respects with the acetate IVa, m.p. 168.5–170°, from II. Thus II appeared to be a molecular compound consisting of approximately one-third 7β-hydroxydehydroisoandrosterone and two-thirds 7α-hydroxydehydroisoandrosterone. This molecular compound could be prepared by crystallization of a mixture of 7α-hydroxydehydroisoandrosterone and 7β-hydroxydehydroisoandrosterone from dilute acetone.

The structures of IV and V were established by their conversion on Oppenauer oxidation to 4,6-androstadiene-3,17-dione (VI).[7] The configurations of the 7-hydroxyl groups were assigned from the comparison of their molecular rotatory contributions ($\Delta M_D^{7OH\text{-}7H}$) with those of the corresponding, and known, 7-hydroxycholesterols (Table I). It should be noted that *Rhizopus sp.* (M 2045) converted 4-androstene-3,17-dione to 11α-hydroxy-4-androstene-3,17-dione and 6β-hydroxy-4-androstene-3,17-dione.

TABLE I[a]

| Compound | $M_D$ | $\Delta M_D$ substitution |
|---|---|---|
| 7α-Hydroxycholesterol | −358 | −207 |
| 7α-Hydroxydehydroisoandrosterone (IV) | −215 | −209 |
| 7α-Acetoxycholesterol acetate | −851 | −667 |
| 3β,7α-Diacetoxy-5-androsten-17-one (IVa) | −694 | −669 |
| 7β-Hydroxycholesterol | + 16 | +167 |
| 7β-Hydroxydehydroisoandrosterone (V) | +206 | +200 |
| 7β-Acetoxycholesterol acetate | +263 | +447 |
| 3β,7β-Diacetoxy-5-androsten-17-one (Va) | +408 | +433 |

[a] All rotations, other than those first reported in this paper, were taken from "Constantes Sélectionnées Pouvoir Rotatoire Naturel, I. Steroides" by J. P. Mathieu and A. Petit, Masson and Co., Editors, 120, Boulevard Saint-Germain, Paris, 1956. They were determined in chloroform.

## Experimental[b]

**Fermentation of Dehydroisoandrosterone (I) with *Rhizopus sp.* (M 2045).**—A 40-l. stainless steel fermenter was charged with medium consisting of 150 g. of cotton seed flour, 1000 g. of glucose, 90 ml. of corn steep liquor, 5.0 g. of silicone antifoam[9] and 30 l. of water. The *p*H of the solution was adjusted to 4.3 with hydrochloric acid, and the vessel and medium were sterilized at 120°. After having been cooled to 25°, the fermenter was inoculated with a suspension of spores of *Rhizopus sp.* (M 2045). The culture was stirred and was aerated with sterile air at the rate of 10 l./min. for a period of 25 hours. A solution of 10.0 g. of dehydroisoandrosterone in 250 ml. of acetone then was added and the fermentation was continued for 17 hours.

The culture then was extracted with one volume of methylene chloride in two portions. The methylene chloride extract was evaporated to dryness. The residue was triturated with 20 ml. of acetone until crystalline; the resulting suspension was diluted with 20 ml. of ether, and the solid material was separated by filtration and was washed on the filter with 40 ml. of ether. Crystallization of this material from acetone yielded 1.98 g. of II,[10] m.p. 193–196°.

The mother liquors from the isolation of II were combined, evaporated to dryness, and the residue was chromatographed on 1.3 kg. of silica gel. Crystallization of the material, eluted with 50% ethyl acetate in benzene, from acetone–cyclohexane yielded 0.46 g. of 7-ketodehydroisoandrosterone (III),[11] m.p. 243–244°; an additional 0.10 g. of product, m.p. 238–240°, was obtained from the mother liquors. The analytical sample, prepared by crystallizing III from dilute acetone, had the properties: m.p. 243–244.5°, $[\alpha]_D$ −82.8°; $\lambda_{max}^{methanol}$ 238 mμ, ε13,500. The infrared spectrum was identical with that of an authentic sample.

*Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 75.45; H, 8.67. Found: C, 75.18; H, 8.63.

The solid material eluted with 65% ethyl acetate in benzene, after being crystallized from acetone, then from acetone–cyclohexane, yielded 1.28 g. of 7β-hydroxydehydroisoandrosterone (V), m.p. 214–216°, $[\alpha]_D$ +67.5°.

*Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.28; H, 9.67.

Careful chromatography of 1.95 g. of II, isolated above, on silica gel yielded 98 mg. of 7β-hydroxydehydroisoandrosterone, m.p. 215–216°, after crystallization from acetone and acetone–cyclohexane, and 0.52 g. of II, m.p. 195–197.5°, after crystallization from acetone and dilute acetone.

**Fermentation of Dehydroisoandrosterone (I) with *Fusidium sp.* (M 61-1).**—A 40-l. stainless steel fermenter was charged with a medium consisting of 10 g. of cotton seed flour, 120 ml. of corn steep liquor, 40 g. of potassium dihydrogen phosphate, 5.0 g. of silicone antifoam, 10.0 g. of dehydroisoandrosterone and 30 l. of water. The vessel and medium were sterilized at 120°. After having cooled to 25° the fermenter was inoculated with a suspension of spores of *Fusidium sp.* (M 61-1). The culture was stirred and was aerated with 10 l. of sterile air per minute for 65 hours. It then was extracted with one volume of methylene chloride in two portions. The methylene chloride solution was evaporated to dryness. The residue (7.14 g.) was dissolved in benzene and chromatographed on 700 g. of silica gel. After the elution of a small quantity of dehydroisoandrosterone with 20% ethyl acetate in benzene, 5-androstene-3β,17β-diol (419 mg.) was eluted with 35% ethyl acetate in benzene. The 5-androstene-3β,17β,diol[12] was purified by crystallization from acetone, m.p. 180.5–183°. Comparison of the infrared spectrum of this material with that of an authentic sample of 5-androstene-3β,17β-diol confirmed its identity.

Further elution of the silica gel column with 75% ethyl acetate gave 2.55 g. of 7α-hydroxydehydroisoandrosterone, which after crystallization from acetone melted at 181.5–183.5° (1.90 g.), $[\alpha]_D$ −70.7°.

*Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.69; H, 9.38.

**3β,7β-Diacetoxy-5-androsten-17-one (Va).**—Acetylation of 0.20 g. of 7β-hydroxydehydroisoandrosterone (V) with 3 ml. of acetic anhydride in 3 ml. of pyridine at room temperature overnight yielded, after crystallization from dilute ace-

(7) See, *e.g.*, C. W. Greenhalgh, H. B. Henbest and E. R. H. Jones, *J. Chem. Soc.*, 2375 (1952).

(8) All melting points were taken on a Fisher–Johns melting point apparatus. The rotations were taken in chloroform at 24 ± 2° and are accurate to ±2°. We are indebted to Dr. R. T. Dillon and the Analytical Division of G. D. Searle and Co. for the analytical and optical data reported.

(9) Antifoam AF Emulsion, Dow Corning Corp., Midland, Mich.

(10) The analytical data on this molecular compound were obtained on a sample of II prepared by the fermentation of dehydroisoandrosterone (I) with *Rhizopus sp.* (M 2052). The identity of this material with the above sample was confirmed by their identical melting points, lack of depressions in the melting point of their mixture, and the identity of their infrared spectra. The material obtained with *Rhizopus sp.* (M 2052) had the properties; m.p. 195–197°, $[\alpha]_D$ −26.9°, −30.5°. *Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.96; H, 9.47.

(11) W. Logemann and P. Giraldi, *Gazz. Chim. Ital.*, **81**, 548 (1951).

(12) P. Wieland and K. Miescher, *Helv. Chim. Acta*, **32**, 1768 (1949).

tone, 0.23 g. of 3β,7β-diacetoxy-5-androsten-17-one, m.p. 161.5–162.5°, $[\alpha]_D$ +105.0°.

*Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.32; H, 8.33.

**3β,7α-Diacetoxy-5-androsten-17-one (IVa).**—Acetylation of 0.20 g. of 7α-hydroxydehydroisoandrosterone (IV) with 3 ml. of acetic anhydride in 3 ml. of pyridine at room temperature overnight yielded, after crystallization from dilute methanol, 0.23 g. of 3β,7α-diacetoxy-5-androsten-17-one, m.p. 168–170°, $[\alpha]_D$ −178.5°.

*Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.95; H, 8.53.

**3β,7α-Diacetoxy-5-androsten-17-one (IVa) from II.**—Acetylation of 0.29 g. of II, m.p. 193–196°, by the method described above yielded, after repeated crystallizations from petroleum ether (b.p. 60–71°), acetone–petroleum ether, and dilute acetone, 103 mg. of 3β,7α-diacetoxy-5-androsten-17-one, m.p. 168.5–170°. This material was identical in all respects, m.p., mixed m.p. and infrared spectrum, with that described above.

**Molecular Compound II from IV and V.**—A mixture of 100 mg. of 7α-hydroxydehydroisoandrosterone, m.p. 183–184.5°, and 50 mg. of 7β-hydroxydehydroisoandrosterone, m.p. 215–216°, was crystallized twice from dilute acetone. From these crystallizations there was obtained 79 mg. of II, m.p. 196–197.5°, $[\alpha]_D$ −32.0°. The infrared spectrum of this material was identical with that obtained above and was different from that of either of the starting materials.

*Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.78; H, 9.16.

**4,6-Androstadiene-3,17-dione (VI) from IV.**—A solution of 0.50 g. of 7α-hydroxydehydroisoandrosterone, m.p. 181.5–183.5°, in 20 ml. of toluene and 7.0 ml. of cyclohexanone was evaporated to approximately one-half its volume to free the solution of water. To this was added 4.00 ml. of a toluene solution containing 1.00 g. of aluminum isopropoxide. The resulting solution was heated under reflux for 20 minutes during which time the solution became yellow. The reaction mixture then was poured into 50 ml. of a saturated Rochelle salt solution; the flask was rinsed with benzene and an additional 50 ml. of Rochelle salt solution; then the mixture was distilled with steam. The suspension became brick-red during this steam distillation. The organic material was separated from the suspension by extraction with ether plus a little ethyl acetate. The ether was removed by evaporation and the residue chromatographed on silica gel. The crystalline material eluted with 10% ethyl acetate in benzene weighed 230 mg. and, after being crystallized from acetone–petroleum ether (b.p. 60–71°), yielded 112 mg. of 4,6-androstadiene-3,17-dione,[13] m.p. 170.5–172.5°, $\lambda_{max}^{methanol}$ 283 (ε26,600). This material was identical in all respects (m.p., mixed m.p. and infrared spectrum) with an authentic sample of 4,6-androstadiene-3,17-dione.

**4,6-Androstadiene-3,17-dione (VI) from V.**—The treatment of 0.40 g. of 7β-hydroxydehydroisoandrosterone (V) by the methods given above yielded, after chromatography and crystallization from acetone–petroleum ether (b.p. 60–71°) and dilute acetone, 37 mg. of 4,6-androstadiene-3,17-dione, m.p. 169.5–171°, $\lambda_{max}^{methanol}$ 282 (ε 25,400). The structure was confirmed by mixed m.p. and comparison of infrared spectra.

(13) L. Ruzicka and W. Bosshard, *Helv. Chim Acts*,, **20**, 328 (1937); C. Djerassi, G. Rosenkranz, J. Romo, St. Kaufmann and J. Pataki, THIS JOURNAL, **72**, 4534 (1950).

CHICAGO 80, ILL.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF TEMPLE UNIVERSITY]

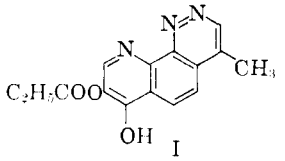
# Substituted 1,10-Phenanthrolines. XI. Aza Derivatives[1]

BY FRANCIS H. CASE AND JAMES A. BRENNAN

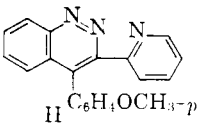
RECEIVED MAY 4, 1959

The syntheses of 2-, 3-, 4- and 5-aza-1,10-phenanthrolines and of 4,7-diaza-1,10-phenanthroline have been described. These compounds are expected to form chelates with Fe(II) and possibly Cu(I).

The compounds 2-aza-8-carbethoxy-7-hydroxy-4-methyl-1,10-phenanthroline[2] (I) and 3-(2-pyridyl)-4-*p*-methoxyphenylcinnoline[3] (II) have been shown by Irving and Williams[4] to have certain advantages over 1,10-phenanthroline as chelating agents.

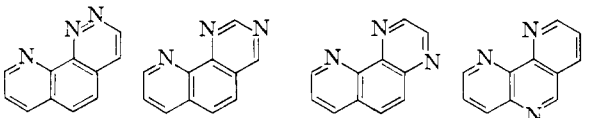



I




II

The difference in absorptive power of the chelates of these substances is ascribed to the presence of the third nuclear nitrogen atom in the 2-position. It seemed desirable to us to prepare the parent substance, 2-aza-1,10-phenanthroline (III) and in addition the other three isomeric monaza derivatives of 1,10-phenanthroline, *i.e.*, the 3- (IV), 4- (V) and 5- (VI). These can be considered as pyrido derivatives of cinnoline, quinazoline, quinoxaline and 1,5-naphthyridine, respectively.



III IV V VI

The synthesis of III was accomplished in the following manner: 8-amino-4-methylcinnoline[2,5] was converted to 4-methyl-2-aza-1,10-phenanthroline by a modified Skraup reaction[6] involving acrolein. The methyl group then was removed by conversion to the styryl derivative, oxidation to the acid and decarboxylation.

The 4-hydroxy derivative of 2-aza-1,10-phenanthroline (X) also was prepared, but by an entirely different procedure. 3-Acetamido-2-nitroacetophenone[7] was converted in a modified Skraup reaction to 7-acetyl-8-nitroquinoline (VII). Reduction by iron and acetic acid yielded the corresponding amine VIII, but stannous chloride and hydrochloric acid produced 3-methyl-(pyrido-(3,2-g)-anthranil) (IX). It was found that the anthranil then could be reduced further to VIII by iron and acetic acid. Reduction of VII using

(1) This work was supported by a grant from the Committee on Research and Publications of Temple University.

(2) L. McKenzie and C. S. Hamilton, *J. Org. Chem.*, **16**, 1414 (1951).

(3) K. Schofield, *J. Chem. Soc.*, 2408 (1949).

(4) H. Irving and R. Williams, *Analyst*, **77**, 813 (1952).

(5) K. Schofield and T. Swain, *J. Chem. Soc.*, 1367 (1949).

(6) H. L. Yale and J. Bernstein, THIS JOURNAL, **70**, 254 (1948).

(7) N. Leonard and S. Boyd, *J. Org. Chem.*, **11**, 405 (1946).