

zone corresponding to each cyclitol was cut out and eluted into 100 ml. of water. The amount of cyclitol in each was estimated by periodate oxidation. The oxidation was carried out with periodic acid at room temperature for 24 hours; standard oxidations upon which the calculations are based showed an uptake for myo-inositol of 6.5 moles and for pinitol of 5.5 moles of periodate per mole of cyclitol.<sup>19</sup> Results of an assay on random samples of heartwood and sapwood are given in Table I.

(19) A. M. Stephens, *J. Chem. Soc.*, 738 (1952).

TABLE I  
QUANTITATIVE ESTIMATION OF CYCLITOLS IN WATER EXTRACT OF SUGAR PINE SAWDUST (FROM 500 G. SAWDUST)

	Pinitol, g.	Myo-inositol, g.	Sequooyitol, g.	<i>d</i> -Inositol
Heartwood	15.0	0.58	0.40	Trace
Sapwood	0.55	.01	..	...

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM VISTER RESEARCH LABORATORIES]

## An Improved Method of Preparing Testosterone, Dihydrotestosterone and Some of their Esters

BY ALBERTO ERCOLI AND PIETRO DE RUGGIERI

RECEIVED JULY 10, 1952

The 17-monocyanohydrin of  $\Delta^4$ -androstene-3,17-dione can be prepared in excellent yield by an interchange reaction between the dione and acetone cyanohydrin. This derivative can be converted in almost quantitative yield into the 3-enol ethyl ether, which on reduction with sodium and *n*-propanol is converted into the enol ethyl ether of testosterone. This substance can be converted into testosterone by acid hydrolysis; esters of testosterone are prepared conveniently by reaction with acid anhydrides in pyridine before hydrolysis. Two alternative routes through the 3-enol benzyl ether and the 3-ethylene glycol ketal of the 17-cyanohydrin derivative of  $\Delta^4$ -androstenedione are described. The method has also been applied to the preparation of androstane-17 $\beta$ -ol-3-one and the acetyl derivative.

The usual precursor for the preparation of testosterone (Ic) is  $\Delta^4$ -androstene-3,17-dione (Ia), readily available by oxidation of dehydroepiandrosterone. Direct reduction of the 17-keto group by the Meerwein-Ponndorf method without protection of the 3-keto group does furnish testosterone,<sup>1</sup> but in unsatisfactory yield. Serini and Köster<sup>2</sup> found that the 3-ketone group reacts preferentially with ethyl orthoformate to form an enol ethyl ether (IIb) and that this derivative can be converted into testosterone by sodium-propanol reduction of the 17-keto group followed by regeneration of the 3-keto group by acid hydrolysis. The same reaction sequence was also applied to androstane-3,17-dione (IIIa)<sup>2</sup>; in this case the intermediate is the 3-diethyl ketal (IIIe). The Serini-Köster method has been used extensively, but has certain disadvantages. If the theoretical amount of ethyl orthoformate is employed, the yield of the 3-enol ether is far from quantitative; if an excess is employed, the 17-keto group also reacts.

We have been able to eliminate these drawbacks by prior protection of the 17-carbonyl group in the form of the cyanohydrin, prepared by an improved method. The reaction of a 3,17-diketone with alcoholic hydrogen cyanide under the usual conditions is not satisfactory because some dicyanohydrin is always formed. We have found that cyanohydrins of steroid ketones are obtained readily by hydrogen cyanide interchange with acetone cyanohydrin<sup>3</sup> and that by this procedure the 17-monocyanohydrin of  $\Delta^4$ -androstene-3,17-dione (Ib) can be obtained in practically quantitative yield. This cyanohydrin has also been prepared, but in

only 43% yield, from the known cyanohydrin of dehydroepiandrosterone<sup>4</sup> by bromination of the double bond, chromic acid oxidation of the hydroxyl group and debromination with zinc and acetic acid. When this cyanohydrin derivative of androstenedione (Ib) is treated in hot benzene with an excess of ethyl orthoformate in the presence of alcoholic hydrogen chloride, the 3-enol ethyl ether (IIa) is formed in 90% yield. The structure is established by alkaline hydrolysis to the known 3-enol ether of  $\Delta^4$ -androstene-3,17-dione (IIb). Reduction with sodium and *n*-propanol of the enol ether cyanohydrin (IIa) leads to the known enol ether of testosterone (IIc), from which testosterone is obtained by mild acidic hydrolysis. The yield in these two steps, without isolation of the intermediate enol ether, is 92%.

We have also found that the enol ethyl ether of testosterone (IIc) can be used directly for the preparation of esters of testosterone. Thus this derivative IIc can be acylated in pyridine solution by acid anhydrides<sup>5</sup> such as propionic or  $\beta$ -cyclopentylpropionic anhydride to give the corresponding esters (IIId and IIe, respectively). Hydrolysis with hydrochloric acid in acetone furnishes the corresponding esters of testosterone.

Our procedure for the preparation of testosterone and its esters can be modified in various respects. The 3-keto function can be protected in other ways; in the Experimental part routes through the 3-enol benzyl ether (IIIf) and the 3-glycol ketal (Ie) of androstenedione cyanohydrin are described.

This procedure is also applicable to the preparation of dihydrotestosterone (androstane-17 $\beta$ -ol-3-one, IIIg) and its esters from androstane-3 $\beta$ -ol-17-

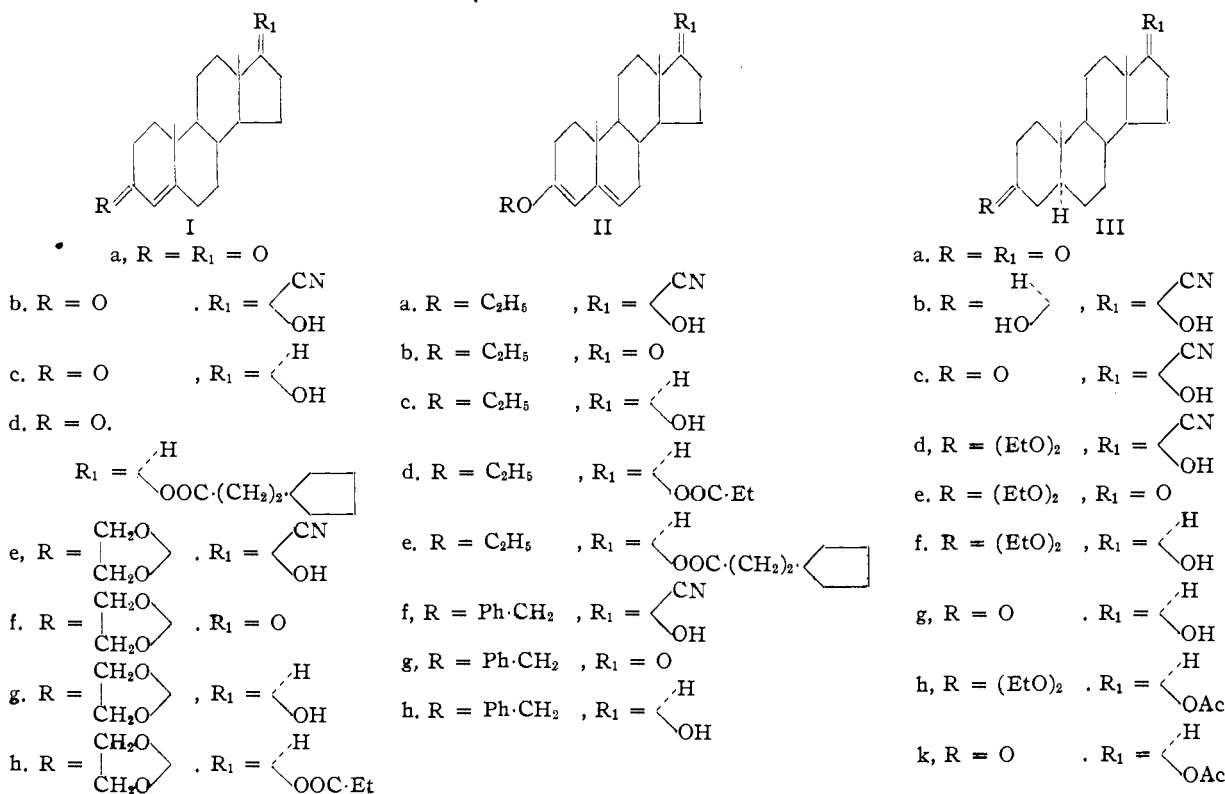
(1) K. Miescher and W. H. Fischer, *Helv. Chim. Acta*, **22**, 158 (1939).

(2) A. Serini and H. Köster, *Ber.*, **71**, 1766 (1938).

(3) ADDED IN PROOF.—F. E. Küng (U. S. Patent 2,259,167, *C. A.*, **36**, 494 (1942)) had prepared the cyanohydrins of some non-steroid carbonyl compounds, such as  $\text{CH}_2\text{O}$ , by *trans*-cyanohydrination with a cyanohydrin, such as methyl ethyl ketone cyanohydrin.

(4) S. Kuwada and M. Miyasaka, *Chem. Zentr.*, **108**, II, 1825 (1937). This cyanohydrin can also be prepared in excellent yield by our method.

(5) G. Rosenkranz, St. Kaufmann and J. Romo, *THIS JOURNAL*, **71**, 3689 (1949), have acylated thioenol ethers of testosterone with acyl chlorides in pyridine solution.



one. The 3 $\beta$ -ol-17-one is converted into the known 17-cyanohydrin<sup>6</sup> IIIb by our interchange method (96% yield), and the 3-hydroxyl group is oxidized by chromic acid to the corresponding ketone IIIc. This substance is transformed into the diethyl ketal IIIId and then reduced in the usual manner to give the diethyl ketal of androstane-17 $\beta$ -ol-3-one (IIIIf). Free dihydrotestosterone IIIg is obtained by hydrolysis.

The 17-acetate (IIIk) is obtainable by acetylation of IIIf and elimination of the diethyl ketal group with hydrochloric acid in acetone.

We have noted variations in the melting or decomposition points of our crude cyanohydrins, indicative of the presence of epimer mixtures. The heating rate also exerts an effect on these constants.

### Experimental<sup>7</sup>

**Cyanohydrin of Dehydroepiandrosterone.**<sup>4</sup>—To a solution of 3 g. of dehydroepiandrosterone in 30 cc. of ethanol 6 cc. of freshly prepared acetone cyanohydrin<sup>8</sup> was added. Crystallization occurred rapidly and was practically complete after 3–4 hr. The product, washed with ethanol and then with water, was dried *in vacuo* (3.06 g.); m.p. 212° dec.<sup>9</sup>

*Anal.* Calcd. for C<sub>20</sub>H<sub>28</sub>O<sub>2</sub>N: N, 4.44. Found: N, 4.35.

(6) S. Kuwada and M. Miyasaka, *Chem. Zentr.*, **110**, I, 1372 (1939).

(7) All melting points are uncorrected. Microanalyses for carbon, hydrogen and some for nitrogen were performed by Mr. A. Peisker-Ritter, Brugg (Switzerland). For several nitrogen determinations we are indebted to Dr. Dina Della Morte.

(8) Prepared according to *Org. Syntheses*, **20**, 43 (1940). When pure, distilled acetone cyanohydrin is employed, the addition of 1–2 drops of diluted alkaline solution (*i.e.*, NH<sub>4</sub>OH, KCN or K<sub>2</sub>CO<sub>3</sub>) is advisable.

(9) Kuwada<sup>4</sup> gives m.p. 236°. A. Butenandt and J. Schmidt-Thomé, *Ber.*, **71**, 1487 (1938), give m.p. 221°. According to the latter authors the dec. pt. of this product ranges from 210 to 250° depending on the rate of heating. We have also observed widely different values ranging from 160 to 240°.

**17-Cyanohydrin of  $\Delta^4$ -Androstene-3,17-dione (Ib).** (a) **From the Cyanohydrin of Dehydroepiandrosterone.**—A suspension of 2 g. of the above product in 80 cc. of acetic acid was slowly treated with 1.13 g. (10% excess) of bromine in 14 cc. of acetic acid. During the bromination the product went into solution. Then chromic anhydride (1.43 g.) in 14 cc. of 50% acetic acid was gradually added. After standing at room temperature overnight the mixture was stirred for 45 min. with 10 g. of zinc dust with control of the temperature below 40°; the zinc was then filtered off, and the solution was warmed for 10 min. on the steam-bath. The water-diluted solution was extracted three times with ether, the ether layers were washed with water, dried over sodium sulfate, filtered and concentrated to a small volume. The crystalline product was recrystallized from ether; 940 mg. (43%); m.p. 192°, dec.;  $\lambda_{\text{max}}^{\text{EtOH}}$  240 m $\mu$ , log *E* 4.20.

*Anal.* Calcd. for C<sub>20</sub>H<sub>27</sub>O<sub>2</sub>N: C, 76.64; H, 8.68; N, 4.46. Found: C, 76.59; H, 8.72; N, 4.48.

The product (50 mg.) when warmed for 10 minutes on the steam-bath with 2 cc. of ethanol and 2 drops of pyridine afforded, after dilution with water, 35 mg. of androstenedione (Ia), m.p. 169–171°.

(b) **From  $\Delta^4$ -Androstene-3,17-dione (Ia).**—Androstenedione (20 g.) was dissolved by gentle warming and swirling in 30 cc. of freshly prepared crude acetone cyanohydrin.<sup>8</sup> Crystallization began in a few minutes and was complete within 2 hr. at room temperature. The petroleum ether-washed crystals (19.90 g.) melted at 178° dec. The water-diluted mother liquor furnished another 1.20 g., m.p. 176–178° dec. The total yield was 21.1 g. (96%). The ultraviolet absorption spectrum was identical with that of the product obtained in (a). Also the analytical figures (Found: C, 76.57; H, 8.67; N, 4.42) agreed with the theoretical values.

**3-Enol Ethyl Ether of  $\Delta^4$ -Androstene-3,17-dione-17-cyanohydrin (IIa).**—From a suspension of 1.56 g. of the above androstenedione cyanohydrin (Ib) in 20 cc. of benzene, 10 cc. of the solvent was distilled. After the temperature was lowered to 65°, 1.76 g. of ethyl orthoformate, 0.9 cc. of absolute ethanol and 0.08 cc. of an ethanolic solution of hydrochloric acid (5.6 mg. of HCl) were added. After 15 min. all the product went into solution and then crystals of the enol ether began to separate. After a further 45 min. the mixture was cooled, and the crystals were filtered and then dried *in vacuo*; 880 mg., m.p. 207° dec.

After addition of 0.014 cc. of pyridine the mother liquor was evaporated *in vacuo*. The crystalline solid, rapidly washed with a small quantity of ethanol containing a trace of pyridine, weighed 650 mg., m.p. 203–205° dec., total yield 1.53 g. (90%).

*Anal.* Calcd. for  $C_{22}H_{31}O_2N$ : C, 77.37; H, 9.15; N, 4.10. Found: C, 77.42; H, 9.11; N, 4.13.

The above product (100 mg.), dissolved in 2 cc. of ethanol containing two drops of pyridine, was warmed on the steam-bath for 20 minutes. Dilution with hot water afforded 80 mg. of the 3-enol ethyl ether of androstenedione (IIb), m.p. 152°, undepressed in mixture with an authentic specimen.<sup>2</sup>

**3-Enol Ethyl Ether of Testosterone (IIc).**—To a boiling solution of 500 mg. of the above 3-enol ethyl ether of  $\Delta^4$ -androstene-3,17-dione-17-cyanohydrin (IIa) in 13 cc. of absolute *n*-propanol in which a small quantity of sodium had been previously dissolved, 500 mg. of sodium was added portionwise during half an hour. Dilution with hot water induced crystallization. The product obtained after cooling, filtering and washing with water weighed 440 mg., m.p. 119–121°, and did not depress the melting point of the 3-enol ethyl ether of testosterone.<sup>2</sup>

A hot solution of the product (400 mg.) in 5 cc. of ethanol was diluted with warm 1 *N* hydrochloric acid solution. After cooling, 370 mg. of testosterone was obtained, m.p. (mixed) 151–153°.

**Testosterone from  $\Delta^4$ -Androstene-3,17-dione-17-cyanohydrin (Ib) without Isolation of Intermediates.**—The reaction of 1.56 g. of androstenedione cyanohydrin with ethyl orthoformate was carried out exactly as described above for the preparation of IIa. Instead of filtering the crystallized product, the whole mixture, after addition of 0.014 cc. of pyridine, was evaporated *in vacuo*. The reduction of the crystalline solid was performed as described above, with 40 cc. of *n*-propanol and 1.5 g. of sodium added during 40 minutes. After diluting with hot water, acidifying the mixture to congo red with 2 *N* hydrochloric acid, removing the main part of the propanol *in vacuo* and cooling, the crystalline product which separated was filtered, washed with water and dried; yield 1.31 g. (92%), m.p. 149–151°, undepressed on admixture with an authentic specimen of testosterone.

**3-Enol Ethyl Ether of Testosterone Propionate (IId).**—A solution of 1 g. of the enol ethyl ether of testosterone (IIc) in 10 cc. of pyridine was treated with 7 cc. of propionic anhydride and kept at room temperature overnight. Dilution with water yielded 1.03 g. of crystals, m.p. 145–148°. An analytical sample, crystallized twice from acetone, melted at 156°.

*Anal.* Calcd. for  $C_{24}H_{35}O_3$ : C, 77.37; H, 9.74. Found: C, 77.40; H, 9.80.

**Testosterone Propionate.**—A drop of 36% hydrochloric acid was added to a solution of 200 mg. of the above product (IId), m.p. 145–148°, in 2 cc. of acetone and then the mixture was heated 5 minutes on the steam-bath. Dilution with water yielded 170 mg. of crystals, m.p. 115–118°. After recrystallization from ether-petroleum ether the m.p. was 121°, undepressed on admixture with an authentic specimen.

Hydrolysis with alcoholic potassium hydroxide gave pure testosterone, m.p. and mixed m.p. 151–152°.

**3-Enol Ethyl Ether of Testosterone  $\beta$ -Cyclopentylpropionate (IIe).**—One gram of the enol ethyl ether of testosterone (IIc) was treated overnight with 2 cc. of  $\beta$ -cyclopentylpropionic anhydride (obtained from  $\beta$ -cyclopentylpropionic acid and acetic anhydride, b.p. 180° (2 mm.)) in 3 cc. of pyridine. After dilution with water and extraction with ether, the extract was neutralized, dried and evaporated. The oily residue was dissolved in petroleum ether and filtered through an alumina column to retain some contaminating cyclopentylpropionic anhydride. Elution of the column with petroleum ether yielded 1 g. of crystals, m.p. 86–88°. The analytical sample, recrystallized from alcohol, melted at 90–91°.

*Anal.* Calcd. for  $C_{25}H_{40}O_3$ : C, 79.04; H, 10.07. Found: C, 79.01; H, 10.12.

Hydrolysis either of the oily or of the crystalline product, carried out with hydrochloric acid in acetone as described above for the hydrolysis of Id, afforded testosterone  $\beta$ -cyclopentylpropionate (Id), m.p. 99–101°,  $[\alpha]_D^{25} +90^\circ$  (c

1.7, EtOH).<sup>10</sup> The melting point was undepressed on admixture with a specimen prepared from testosterone and  $\beta$ -cyclopentylpropionic anhydride in pyridine.

**3-Enol Benzyl Ether of  $\Delta^4$ -Androstene-3,17-dione-17-cyanohydrin (IIf).**—In a flask fitted with a Marcussen-like apparatus surrounded by a reflux condenser, 1.56 g. of Ib, m.p. 178°, together with a few crystals of *p*-toluenesulfonic acid was suspended in a mixture of 50 cc. of benzene and 1.56 g. of benzyl alcohol. Some phosphorus pentoxide was placed in the hanging arm of the Marcussen apparatus in order to dry the refluxing benzene. After being heated for 16 hr., the mixture was filtered and 420 mg. of crystals, m.p. 237° dec., was collected. Examination of the product (analytical data, ultraviolet absorption spectrum, alkaline hydrolysis to androstenedione) showed that it was starting material in spite of the change in melting point. The filtered benzene solution, after addition of a drop of pyridine to neutralize the *p*-toluenesulfonic acid, was concentrated *in vacuo* to dryness. The residue, recrystallized from methanol-petroleum ether, gave 820 mg. of product, m.p. 199–200° dec.

*Anal.* Calcd. for  $C_{27}H_{35}O_2N$ : C, 80.36; H, 8.24; N, 3.47. Found: C, 80.37; H, 8.30; N, 3.49.

Heating of IIf with pyridine-containing ethanol yielded, after water dilution and filtration, the 3-enol benzyl ether of  $\Delta^4$ -androstene-3,17-dione (IIg), m.p. 184°, undepressed on admixture with an authentic specimen.<sup>11</sup>

**3-Enol Benzyl Ether of Testosterone (IIh).**—The reduction of IIf was carried out with 1-propanol and sodium as described above for similar cases. From 300 mg. of starting substance 240 mg. of crystals melting at 148–150° was obtained. After recrystallization from pyridine-containing ethanol, the m.p. was 153°, unchanged on admixture with an authentic specimen of the 3-enol benzyl ether of testosterone.<sup>11</sup>

Mild acidic hydrolysis, as above described for similar cases, furnished pure testosterone, m.p. 151–152°.

**3-Ethylene Glycol Ketal of  $\Delta^4$ -Androstene-3,17-dione-17-cyanohydrin (Ie).**—A suspension of 1.56 g. of Ib in 50 cc. of benzene and 2 cc. of ethylene glycol with some crystals of *p*-toluenesulfonic acid was refluxed for 16 hours in the Marcussen-like apparatus as described for the preparation of IIf. After cooling, the *p*-toluenesulfonic acid was neutralized with a little pyridine and the benzene was distilled *in vacuo* from the mixture. The residue, after crystallization from methanol, furnished 580 mg. of a product, m.p. 187° dec.

From the mother liquor, after dilution with water, another 1.150 g. of the same substance, m.p. 185° dec., was obtained: total yield 1.630 g. (92%).

*Anal.* Calcd. for  $C_{22}H_{33}O_2N$ : C, 73.50; H, 9.25; N, 3.90. Found: C, 73.70; H, 9.18; N, 3.86.

Hydrolysis of the product with pyridine-containing ethanol yielded the 3-ethylene glycol ketal of androstenedione (If), m.p. 198–199° dec., unchanged on admixture with an authentic specimen.<sup>12,13</sup>

**3-Ethylene Glycol Ketal of Testosterone (Ig).**—Reduction of Ie was carried out as described above for similar cases. From 500 mg. of starting material 460 mg. of a product, m.p. 172–177°, was obtained. Recrystallization from pyridine-containing ethanol gave the pure glycol ketal Ig, m.p. 180–181°, undepressed on admixture with an authentic specimen.<sup>13</sup>

A solution of 100 mg. of Ig in 3 cc. of acetone containing a drop of 2 *N* hydrochloric acid was heated for 10 min. on the steam-bath and then diluted with water; 75 mg. of testosterone, m.p. 151°, separated from the cooled solution.

**3-Ethylene Glycol Ketal of Testosterone Propionate (IIh).**—Overnight treatment of 200 mg. of Ig with 2 cc. of propionic anhydride in 2 cc. of pyridine furnished 210 mg. of a product, m.p. 176°. By recrystallization either from ether or from other solvents (acetone, ethanol) crystals melting at 208–209° were obtained.

*Anal.* Calcd. for  $C_{24}H_{35}O_4$ : C, 74.19; H, 9.34. Found: C, 74.47; H, 9.29.

A sample of Ih, prepared from testosterone propionate by

(10) A. C. Ott, M. H. Kuizenga, S. C. Lyster and B. A. Johnson, *J. Clin. Endocrin. Med.*, **12**, 15 (1952).

(11) Swiss Patent 220,206 (*Chem. Zentr.*, **124**, I, 1695 (1943)).

(12) Holland Patent 52656 (*ibid.*, **123**, II, 2615 (1942)).

(13) Holland Patent 64579 (*ibid.*, **123**, II, 1822 (1942)).

reaction with ethylene glycol as described above for the preparation of Ie, also was first obtained as crystals melting at 176°. After recrystallization the form melting at 208–209° was again obtained.

**Alkaline Hydrolysis.**—By refluxing Ib in 5% methanolic KOH under nitrogen for an hour, the 3-glycol ketal of testosterone (Ig), m.p. 180–181°, was restored.

**Acidic Hydrolysis.**—When 50 mg. of Ih was heated for 5 minutes in 5 cc. of acetone containing a drop of dilute hydrochloric acid, 38 mg. of testosterone propionate, m.p. 121–122°, was recovered.

**Cyanohydrin of Androstane-3 $\beta$ -ol-17-one (IIIb).**—Androstane-3 $\beta$ -ol-17-one (2.25 g.) was dissolved by gentle warming in 3 cc. of freshly prepared undistilled<sup>8</sup> acetone cyanohydrin. The cyanohydrin of the steroid ketone precipitated immediately. The product was collected after 2 hours and washed with petroleum ether, 2.370 g. (96% yield), m.p. 170° dec.<sup>14</sup> The analytical sample was recrystallized from ethanol (m.p. unchanged).

*Anal.* Calcd. for C<sub>26</sub>H<sub>31</sub>O<sub>2</sub>N: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.21; H, 9.59; N, 4.65.

**17-Cyanohydrin of Androstane-3,17-dione (IIIc).**—To a suspension of 2.37 g. of the above cyanohydrin (IIIb) in a mixture of 20 cc. of ethylene dichloride and 50 cc. of acetic acid, a solution of 725 mg. of chromic anhydride in 15 cc. of 90% acetic acid was added at room temperature. After 24 hr. all the product went into solution. Ether and water were added, the ether layer was separated, washed with water, dried and evaporated. The crystalline solid (2.1 g.) when recrystallized from ether afforded 1.980 g. (88%) of a product melting at 207° dec.

*Anal.* Calcd. for C<sub>26</sub>H<sub>29</sub>O<sub>2</sub>N: C, 76.15; H, 9.27; N, 4.44. Found: C, 76.45; H, 9.40; N, 4.58.

Hydrolysis of 200 mg. of the product with pyridine-containing ethanol gave 180 mg. of androstane-3,17-dione (IIIa), m.p. and mixed m.p. 130–131°.

**Androstane-17 $\beta$ -ol-3-one (IIIg) through the 3-Diethyl Ketal of Androstane-3,17-dione-17-cyanohydrin (IIIId).**—The reaction of the cyanohydrin IIIc with ethyl orthoformate and the purification was carried out as described

for the preparation of IIa. The 3-diethyl ketal of androstane-3,17-dione-17-cyanohydrin (IIIId) was obtained as an oily residue, which when heated with ethanol and a little pyridine yielded the known 3-diethyl ketal of androstane-3,17-dione (IIIe), m.p. 121°, undepressed on admixture with an authentic specimen.<sup>2</sup>

By repetition of the above procedure starting with 1.6 g. of IIIc, 1.7 g. of oily IIIId was obtained which, after reduction with 1.7 g. of sodium in 45 cc. of 1-propanol and the usual processing, yielded the 3-diethyl ketal of androstane-17 $\beta$ -ol-3-one (IIIIf) as a crude product melting at 120–130°. Attempts to recrystallize gave oily products.<sup>15</sup> All the fractions were recombined and heated for 15 min. on the steam-bath with 50 cc. of ethanol containing 1 cc. of 4 N hydrochloric acid. Dilution with water yielded 1.326 g. (91%) of androstane-17 $\beta$ -ol-3-one (IIIIf), m.p. 176–178°, undepressed on admixture with an authentic specimen.

**17 $\beta$ -Acetoxyandrostane-3-one (IIIk) through the 3-Diethyl Ketal of 17 $\beta$ -Acetoxyandrostane-3-one (IIIh).**—By repetition of the above procedure starting with 2.2 g. of androstane-3,17-dione-17-cyanohydrin (IIIc), 2.1 g. of crude 3-diethyl ketal of androstane-17 $\beta$ -ol-3-one (IIIIf), m.p. 120–130°, was obtained. This substance was dissolved in 20 cc. of pyridine and acetylated at room temperature overnight with 10 cc. of acetic anhydride. The water-diluted reaction mixture was extracted with ether. The alkali-washed and dried ether layers yielded after evaporation 2.2 g. of an oily residue of 3-diethyl ketal of 17 $\beta$ -acetoxyandrostane-3-one (IIIh), which was dissolved in 10 cc. of acetone and refluxed for 10 min. with 0.2 cc. of 36% hydrochloric acid. The crystalline product furnished after recrystallization from petroleum ether 1.850 g. (80% from IIIc) of dihydrotestosterone acetate (IIIk), m.p. 153–156°, undepressed on admixture with an authentic specimen.

**Acknowledgment.**—The authors wish to express their gratitude to Louis F. Fieser and Mary Fieser for revising the manuscript.

(15) A. Serini and H. Köster<sup>2</sup> give no description of IIIIf.

CASATENOVO (COMO), ITALY

(14) Literature<sup>8</sup> 210°.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

## Chemistry of Vitamin B<sub>6</sub>. IX. Derivatives of 5-Desoxypyridoxine

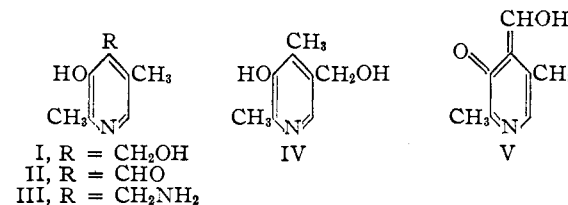
BY DOROTHEA HEYL, STANTON A. HARRIS AND KARL FOLKERS

RECEIVED AUGUST 29, 1952

5-Desoxy derivatives of pyridoxine, pyridoxal and pyridoxamine have been prepared and characterized. These compounds are of biological interest because they can participate normally in biochemical reactions involving the substituent at the 4-position, but they cannot be phosphorylated like pyridoxine, pyridoxal and pyridoxamine. As might be expected, the 5-desoxy compounds not only have no vitamin B<sub>6</sub> activity, but are effective antimetabolites. Codexcarboxylase has been catalytically hydrogenated to produce 5-desoxypyridoxine; under the same conditions, pyridoxal and pyridoxine each produce a mixture of 4-desoxypyridoxine and 5-desoxypyridoxine.

5-Desoxy derivatives of pyridoxine, pyridoxal and pyridoxamine have been prepared. These compounds are of interest, both biologically and chemically, because of their relationship to phosphates of the vitamin B<sub>6</sub> group. With the 5-hydroxymethyl group missing, these 5-desoxy derivatives cannot be converted to 5-phosphates, although the functional group in the 4-position is presumably still capable of normal participation in biochemical reactions. For these reasons, the 5-desoxy compounds would be expected to have no vitamin B<sub>6</sub> activity and might be antimetabolites. Actually, 5-desoxypyridoxal and 5-desoxypyridoxamine have been found to be potent vitamin B<sub>6</sub> inhibitors.<sup>1</sup> The inhibition is of the same high order of activity

as that caused by 4-desoxypyridoxine (IV),<sup>2</sup> in which the functional group in position 4 is blocked.



Because of the absence of the 5-hydroxymethyl group, the hemiacetal form of pyridoxal<sup>3</sup> is impossible in 5-desoxypyridoxal (II). This same restric-

(2) W. H. Ott, *Proc. Soc. Exptl. Biol. Med.*, **61**, 125 (1946); W. W. Cravens and E. B. Snell, *ibid.*, **71**, 73 (1949).

(3) D. Heyl, E. Luz, S. A. Harris and K. Folkers, *THIS JOURNAL*, **73**, 3431 (1951).

(1) J. C. Rabinowitz and E. E. Snell, *Arch. Biochem. Biophys.*, in press.