

to that of isodextropimaric acid, thus confirming its identity.

### Summary

The aldehyde of isodextropimaric acid, termed isodextropinarinal, has been isolated from the

neutral fraction of both wood and gum rosins. Its identity was proved by oxidation with chromic acid to obtain the pure resin acid in good yield.

WILMINGTON, DELAWARE

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE GLIDDEN COMPANY, SOYA PRODUCTS DIVISION]

## Sterols. VI. 16-Methyltestosterone

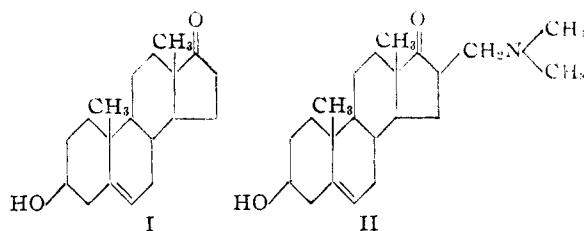
BY PERCY L. JULIAN, EDWIN W. MEYER AND HELEN C. PRINTY

The synthesis of homologs and analogs of the naturally-occurring steroidal hormones has elicited our interest not only with reference to the relation between physiological specificity of these regulators and chemical structure, but also with regard to the structural requirements necessary for the highest therapeutic efficiency. Thus while a naturally-occurring hormone might be much more active than a synthetic analog, when administered parenterally, the latter may conceivably show much greater therapeutic efficiency when administered orally, because of resistance to destruction or inactivation by metabolic processes. It therefore becomes important to define the structural limits within which physiological specificity might be correlated with therapeutic efficiency. As part of a broad program devoted to such considerations,<sup>1</sup> we have been particularly interested in the various possible methyl-testosterones. This paper reports the synthesis of a 16-methyltestosterone.

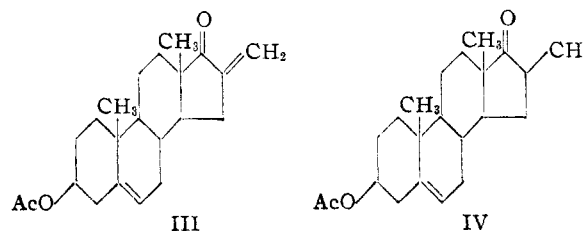
As one route to the various possible 16-methyltestosterones, we have chosen the Mannich reaction applied to dehydroisoandrosterone (I). Although the Mannich reaction has been employed extensively for the preparation of  $\alpha$ -substituted cyclic ketones,<sup>2</sup> its application to steroidal ketones has not been recorded in the literature. This application offers many intriguing possibilities for the synthesis of a variety of new steroid-hormone types, especially in view of the fact that the majority of the steroid hormones are either ketonic in nature or may be readily derived from parent ketonic substances.

The condensation of dehydroisoandrosterone (I), dimethylamine hydrochloride and paraformaldehyde proceeded smoothly in isoamyl alcohol to yield the Mannich base, 16-dimethylamino-methyldehydroisoandrosterone (II). Similar condensations with piperidine hydrochloride and diethylamine hydrochloride gave the corresponding 16-aminomethyl derivatives of dehydroisoandrosterone (I). These steroidal amines formed hydrochlorides which were soluble in water to the extent of about 10 mg./ml.

In order to achieve our immediate goal, the



preparation of 16-methyltestosterone, it was necessary to eliminate dimethylamine from the amino-ketone (II). This elimination took place readily in acetic acid-acetic anhydride to yield 16-methylenedehydroisoandrosterone acetate (III). This method affords an interesting alternative to the known methods<sup>2</sup> for elimination of amines from  $\beta$ -alkylaminoketones.



Several 16-alkylidene-dehydroisoandrosterones have been described previous to the present investigation. Butenandt, Schmidt-Thomé and Weiss<sup>3</sup> prepared 16-alkylidene derivatives in poor yield by the condensation of dehydroisoandrosterone (I) with acetone and methyl ethyl ketone in the presence of sodium or sodamide. Ross<sup>4</sup> recorded an improved preparation of 16-isopropylidenedehydroisoandrosterone.

16-Methylenedehydroisoandrosterone acetate (III) possesses a light absorption maximum at 228  $m\mu$  ( $\log \epsilon = 3.9$ ), a value in good agreement with that ( $225 \pm 5 m\mu$ ) postulated by Woodward<sup>5</sup> for an  $\alpha$ -substituted  $\alpha,\beta$ -unsaturated ketone. The hydrogenation of this ketone in the presence of Raney nickel catalyst proceeded in a stepwise fashion; the first mole of gas was absorbed rapidly, while the second was taken up more slowly. By stopping the reaction after the rapid addition, there was isolated from the reaction mixture a

(1) Cole and Julian, *THIS JOURNAL*, **67**, 1369 (1945).

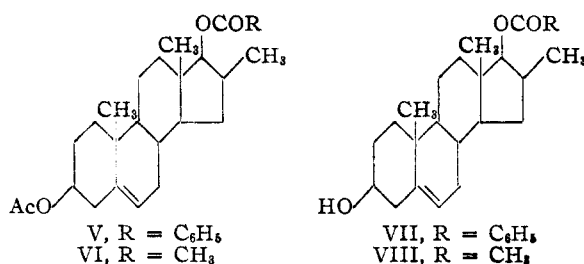
(2) Cf. Blicke, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, pp. 303-341.

(3) Butenandt, Schmidt-Thomé and Weiss, *Ber.*, **72**, 417 (1939).

(4) Ross, *J. Chem. Soc.*, 25 (1945).

(5) Woodward, *THIS JOURNAL*, **63**, 1123 (1941); **64**, 76 (1942).

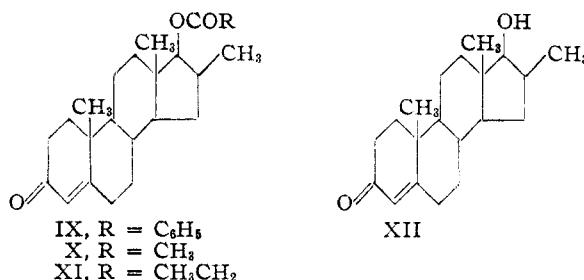
crystalline ketonic substance whose properties are in accord with the structure of 16-methyldehydroisoandrosterone acetate (IV).<sup>6</sup> Complete reduction in the presence of Raney nickel gave what appeared to be a mixture of isomeric 17-ols. Since the complete hydrogenation of the  $\alpha,\beta$ -unsaturated ketonic system in III creates two new asymmetric centers at C 16 and C 17, this reduction may give rise to two ketones of the structure IV and four 17-ols of the structure corresponding to V. The study of the stereo-configuration of the particular isomers described here and those present in the reduction mixtures is reserved for later publication. After benzooylation of the 17-ol mixture, there was isolated an acetate-benzoate (V) which melted at 148–151°. Acetylation of the



same type of reduction mixture gave a crystalline diacetate (VI) which melted at 175–177.5°.

It was found that both the acetate-benzoate (V) and the diacetate (VI) could be preferentially hydrolyzed at the C 3 position with potassium hydroxide in methanol. The latter instance is in contrast to the mild conditions necessary for the preferential hydrolysis of 3( $\beta$ ),17( $\alpha$ )-diacetoxy-5-androstene.<sup>7</sup> This result is not wholly unpredictable for the introduction of a methyl group at C 16 would increase the hindrance at C 17.

Oxidation of the monobenzoate (VII) with chromic acid in acetic acid after protection of the 5–6 double bond with bromine gave the 3-keto derivative, 16-methyltestosterone benzoate (IX).



16-Methyltestosterone acetate (X) was prepared by the Oppenauer oxidation of the monoacetate (VIII). Hydrolysis of these compounds with potassium hydroxide in methanol afforded the same 16-methyltestosterone (XII), m. p. 182–184°,  $[\alpha]_D + 106^\circ$ . It is interesting to note that hydrolysis of IX under conditions which brought

(6) In the structural formulas, the solid lines at C16 and C17 are not intended to indicate a particular stereo-configuration.

(7) Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935).

about complete hydrolysis of testosterone benzoate gave considerable unchanged starting material. This again gives some evidence of the spatial condition at C 17 arising from the introduction of an alkyl group at C 16. Butenandt, *et al.*,<sup>3</sup> and Ross<sup>4</sup> found that their branched chain alkylidene ketones were unreactive toward ketonic reagents; however, in 16-methyldehydroisoandrosterone acetate (IV) the hindrance is insufficient to prevent semicarbazone formation.

16-Methyltestosterone has been assayed for androgenic activity. An examination by the capon-comb growth method<sup>8</sup> indicates that this material has approximately one-tenth the activity of testosterone when administered intramuscularly. Furthermore, when examined for its effect upon the seminal vesicle weight of immature rats, 16-methyltestosterone appeared to be one-eighth to one-tenth as effective as testosterone.<sup>9</sup> It will be interesting to compare the biological activity of the 16-methyltestosterone discussed in this report with its C 16 and C 17 isomers.

Work in progress indicates that the Mannich reaction may be applied to other steroid ketones such as 3( $\beta$ )-hydroxy-5-pregnene-20-one and 3( $\beta$ )-hydroxyandrostane-17-one. Moreover, these Mannich bases and those described herein may be condensed with various active methylene compounds. These studies will be reported in a later communication.

### Experimental<sup>10</sup>

**16-Dimethylaminomethyldehydroisoandrosterone (II).**—A mixture of 14.4 g. (0.05 mole) of dehydroisoandrosterone, 7.5 g. (0.25 mole) of paraformaldehyde and 25 g. (0.375 mole) of dry dimethylamine hydrochloride in 125 ml. of isoamyl alcohol was refluxed for two hours. The solid material dissolved rapidly and after fifteen minutes, crystals began to separate. The reaction mixture was allowed to stand in the refrigerator at 5° for sixteen hours. The solid mass was then shaken with 200 ml. of hydrochloric acid (1:9), diluted with water and extracted with ether. The aqueous suspension was separated, made alkaline with saturated sodium carbonate solution and again extracted with ether. A considerable quantity of ether was necessary for this extraction.<sup>11</sup> The ether extract was washed with water, dried and concentrated until a goodly amount of crystalline material had separated. Petroleum ether (b. p. 35–60°) was added and concentration was continued until most of the ether was removed and crystallization made further concentration difficult. The mixture was chilled, filtered and washed with cold petroleum ether. There was obtained 13.3 g. (77%) of a white solid which melted at 158–163°, dec. Several recrystallizations from ether-petroleum ether (b. p. 35–60°) containing a small quantity of methanol gave silky, white needles melting at 173–174.5°, dec.;  $[\alpha]_D^{20} - 62.3 \pm 2^\circ$  (31.3 mg. made up to 5 ml. with chloroform,  $\alpha_D - 0.39^\circ$ ,  $l$ , 1 dm.).

(8) We are greatly indebted to Dr. T. F. Gallagher of the Sloan-Kettering Institute for Cancer Research and Dr. Lincoln V. Domm of the University of Chicago, Department of Zoology, for these bioassays. Dr. Gallagher informs us that he has initiated a study of the oral activity of this compound.

(9) We are greatly indebted to Dr. M. H. Kuizenga of The Upjohn Company for these results.

(10) Carbon-hydrogen analyses by Mr. C. W. Beazley of Micro-Tech Laboratories, Skokie, Illinois. We are indebted to Miss Isabelle Ryden of this Laboratory for certain technical assistance.

(11) An attempt to substitute chloroform for ether in this extraction gave material of poorer quality.

*Anal.* Calcd. for  $C_{22}H_{35}NO_2$ : C, 76.47; H, 10.22. Found: C, 76.27; H, 10.26.

A study of variations in quantities of reactants and time of reaction indicated that the described conditions were about optimum. The addition of hydrochloric<sup>12</sup> acid or the removal of water formed during the reaction did not improve the yield.

The hydrochloride was prepared from 2.0 g. of the base in ether-methanol with dry hydrogen chloride. The product, 2.1 g. of silky crystals, melted at 231–235°, dec. One recrystallization from methanol gave thin, colorless prisms melting at 242–243.5° dec. At 20°, the hydrochloride dissolved in water to the extent of 12.3 mg./ml.;  $[\alpha]_D^{20} + 15.1 \pm 1^\circ$  (39.8 mg. made up to 5 ml. with water,  $\alpha_D + 0.12^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{22}H_{35}ClNO_2$ : C, 69.16; H, 9.50. Found: C, 69.03; H, 9.47.

Treatment of the Mannich base (II) in benzene-ether with methyl iodide gave the unstable, insoluble methiodide. Recrystallization of this material from methanol gave slightly yellow prisms which decomposed at 250–252°, but could not be purified to give an accurate analysis.

In much the same fashion as described above, a Mannich base was prepared from dehydroisoandrosterone, piperidine hydrochloride and paraformaldehyde. This compound, white needles, melted at 175.5–177.5°.

*Anal.* Calcd. for  $C_{25}H_{39}O_2N$ : C, 77.87; H, 10.19. Found: C, 77.91; H, 10.02.

The reaction of dehydroisoandrosterone, diethylamine hydrochloride and paraformaldehyde gave the crystalline Mannich base, 16-diethylaminomethyldehydroisoandrosterone, m. p. 142°.

*Anal.* Calcd. for  $C_{24}H_{39}O_2N$ : C, 77.16; H, 10.52. Found: C, 77.14; H, 10.28.

**16-Methylenedehydroisoandrosterone Acetate (III).**—A solution of 10.0 g. of the crude Mannich base (II) in 25 ml. of acetic acid and 25 ml. of acetic anhydride was heated on the steam-bath for two hours. The majority of the solvent was then removed in partial vacuum at steam-bath temperature and finally water was added to the residual slurry. The mixture was extracted with ether. The ethereal solution was washed with 10% sodium hydroxide solution followed by water. The residue remaining after concentration of the dried ether solution crystallized upon addition of petroleum ether (b. p. 35–60°). There resulted 8.0 g. (84%) of a white solid melting at 160–165°. Recrystallization from ether-petroleum ether gave needles which possessed the same melting point;  $[\alpha]_D^{20} - 57.5^\circ$  (40.0 mg. made up to 5 ml. with chloroform,  $\alpha_D - 0.46^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{22}H_{35}O_3$ : C, 77.16; H, 8.82. Found: C, 77.22; H, 8.80.

The absorption spectrum was obtained with a Beckmann spectrophotometer, Model DU, using absolute ethanol as the solvent.

**16-Methyldehydroisoandrosterone Acetate (IV).**—A 2.5-g. sample of 16-methylenedehydroisoandrosterone acetate was hydrogenated in 75 ml. of ethanol over Raney nickel catalyst<sup>13</sup> at atmospheric pressure and 30° until 160 ml. of hydrogen was absorbed. At this point the speed of absorption decreased noticeably. The catalyst was separated by centrifugation and the ethanol removed under diminished pressure. The remaining white solid was crystallized from ether-petroleum ether (b. p. 35–60°) and weighed 1.3 g. (53%), m. p. 137–143°. Several recrystallizations from methanol gave long, heavy needles melting at 144–146°;  $[\alpha]_D^{20} 0 \pm 1^\circ$  (47.5 mg. made up to 5 ml. with chloroform,  $\alpha_D 0^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{22}H_{35}O_3$ : C, 76.71; H, 9.35. Found: C, 76.82; H, 9.59.

This ketone formed a semicarbazone which melted at 243–245°, dec., after recrystallization from chloroform-methanol.

*Anal.* Calcd. for  $C_{23}H_{35}N_3O_3$ : C, 68.79; H, 8.79. Found: C, 68.67; H, 8.68.

**3( $\beta$ )-Acetoxy-17-benzoyloxy-16-methyl-5-androstene (V).**—A solution of 3.4 g. of the methylene compound (III) in 60 ml. of purified ethanol was reduced with fresh Raney nickel catalyst at four atmospheres pressure and 25–30°. The catalyst was separated and the solution combined with that of a similar hydrogenation of 3.4 g. of methylene compound. The ethanol was removed in partial vacuum at steam-bath temperature. The last traces of ethanol were removed by addition of benzene and subsequent evacuation and warming. The remaining solid was dissolved in 60 ml. of pure, dry benzene to which 30 ml. of pyridine had been added. After the addition of 12 ml. of benzoyl chloride in 20 ml. of benzene, the mixture was allowed to stand overnight. It was then diluted with water and extracted with ether. The ethereal solution was washed with water, dilute hydrochloric acid followed by water. After removal of ether, the residue was steam distilled. The remaining material was taken up in ether and washed with dilute sodium hydroxide solution and water. Crystallization of the residue from the dried ethereal solution from methanol gave 4.0 g. (42.5%) of an acetate-benzoate melting at 125–147°. Recrystallization of the solid from methanol gave glistening white plates melting at 148–151°;  $[\alpha]_D^{20} + 11.1 \pm 1^\circ$  (94.5 mg. made up to 5 ml. with chloroform,  $\alpha_D + 0.21^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{28}H_{38}O_4$ : C, 77.29; H, 8.50. Found: C, 77.14; H, 8.57.

The mother liquors of crystallization were reserved for isolation of the isomeric materials.

**3( $\beta$ )-Hydroxy-17-benzoyloxy-16-methyl-5-androstene (VII).**—To a solution of 4.0 g. of the acetate-benzoate (V) in 200 ml. of methanol, there was added a solution of 0.8 g. of potassium hydroxide in 7.0 ml. of water and 33 ml. of methanol. The resulting solution was refluxed for twenty-five minutes and then concentrated *in vacuo* on a steam-bath. The last of the concentration was accomplished with the steam turned off. The concentration was continued until a goodly amount of crystalline solid had separated. This was filtered and washed with cold methanol; 3.1 g. (85.6%) of glistening white plates melting at 152–154°;  $[\alpha]_D^{20} + 13.4 \pm 1^\circ$  (67.3 mg. made up to 5 ml. with chloroform,  $\alpha_D + 0.18^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{27}H_{38}O_3$ : C, 79.37; H, 8.88. Found: C, 79.27; H, 8.93.

**16-Methyltestosterone Benzoate (IX).**—A solution of 3.0 g. of the hydroxy-benzoate (VII) in 50 ml. of chloroform was brominated with 1.14 g. of bromine in 10 ml. of chloroform. The solvent was removed *in vacuo* and the residue dissolved in 275 ml. of glacial acetic acid. An oxidizing mixture composed of 1.2 g. of chromic acid, 14 ml. of water and 35 ml. of acetic acid was added. The brown solution was allowed to stand at room temperature for two hours. After addition of 4 ml. of 3 *N* sulfuric acid in 17 ml. of acetic acid and then 14 ml. of methanol (to decompose excess chromic acid), the solution was debrominated under carbon dioxide with 95 ml. of 1 molar chromous chloride solution<sup>14</sup> in 90 ml. of acetic acid. The solution was allowed to stand overnight, concentrated *in vacuo*, diluted with water and extracted with ether. The extract was washed with water, dilute sodium hydroxide solution and water. It was dried and concentrated and the product crystallized from ether-petroleum ether (b. p. 35–60°). There resulted 2.0 g. (67%) of white prisms, m. p. 223–225.5°;  $[\alpha]_D^{20} + 150.6 \pm 1^\circ$  (54.1 mg. made up to 5 ml. with chloroform,  $\alpha_D + 1.63^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{27}H_{38}O_3$ : C, 79.76; H, 8.44. Found: C, 79.72; H, 8.72.

**16-Methyltestosterone (XII).**—16-Methyltestosterone benzoate, 1.5 g., was hydrolyzed with 0.8 g. of potassium hydroxide in 5 ml. of water and 15 ml. of methanol by refluxing for eleven hours. After dilution with water, the

(12) Spaeth, Geissman and Jacobs, *J. Org. Chem.*, **11**, 399 (1946).

(13) Covert and Adkins, *This Journal*, **54**, 4116 (1932).

(14) Julian, Cole, Magnani and Meyer, *ibid.*, **67**, 1728 (1945).

product was extracted with ether. From the washed and dried ethereal solution, the crude 16-methyltestosterone was separated by concentration and chilling. The product 1.0 g. (89.5%) melted at 175–182°. Several recrystallizations from acetone gave prisms melting at 182–184°;  $[\alpha]_D^{25} + 106 \pm 2^\circ$  (56.6 mg. made up to 5 ml. with chloroform,  $\alpha_D + 1.20^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{20}H_{30}O_2$ : C, 79.43; H, 9.99. Found: C, 79.50; H, 9.93.

Hydrolysis of 16-methyltestosterone acetate in this manner gave the same product as described above. In one experiment, after one hour of hydrolysis, a considerable portion of the benzoate was recovered unchanged. A comparable hydrolysis of testosterone benzoate under these conditions went to completion.

A sample of the 16-methyltestosterone was converted to the propionate (XI) by treatment with propionic anhydride at steam-bath temperature for two hours. After several recrystallizations from methanol, the propionate melted at 138–140.5°.

*Anal.* Calcd. for  $C_{23}H_{34}O_3$ : C, 77.04; H, 9.57. Found: C, 77.26; H, 9.71.

**3( $\beta$ ),17-Diacetoxy-16-methyl-5-androstene (VI).**—In a manner similar to that described for the preparation of the acetate-benzoate (V), 16-methylenedehydroisoandrosterone acetate was converted to the diacetate (VI). In this instance, after removal of solvent from the reduction solution, the residue gave upon acetylation with acetic anhydride–acetic acid 45.5% of crude crystalline material, from ether–petroleum ether (b. p. 35–60°), melting at 165–175°. Several recrystallizations from the same solvent mixture yielded glistening white plates which melted at 175–177.5°;  $[\alpha]_D^{25} - 39.9 \pm 1^\circ$  (36.3 mg. made up to 5 ml. with chloroform,  $\alpha_D - 0.29^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{24}H_{36}O_4$ : C, 74.18; H, 9.34. Found: C, 74.00; H, 9.24.

**3( $\beta$ )-Hydroxy-17-acetoxy-16-methyl-5-androstene (VIII).**—To a hot solution of 9.7 g. of the diacetate (VI) in 450 ml. of methanol, there was added a solution of 1.8 g. of potassium hydroxide in 18 ml. of water and 50 ml. of methanol. After being refluxed for twenty-five minutes, the mixture was rapidly concentrated *in vacuo* with little heat to about 100 ml. The cold mixture was filtered and the solid washed sparingly with cold methanol. There resulted 7.0 g. (80.9%) of white crystalline material melting

at 156–164°. Several recrystallizations from methanol gave glistening plates, m. p. 164.5–168.5°, which lose solvent of crystallization upon careful drying *in vacuo*.  $[\alpha]_D^{25} - 36.7 \pm 1^\circ$  (38.1 mg. made up to 5 ml. with chloroform,  $\alpha_D - 0.28^\circ$ ,  $l$ , 1 dm.).

*Anal.* Calcd. for  $C_{22}H_{34}O_3$ : C, 76.25; H, 9.90. Found: C, 75.79; H, 9.77.

**16-Methyltestosterone Acetate (X).**—A solution of 4.5 g. of the monoacetate in 40 ml. of toluene containing 4.0 g. of aluminum isopropoxide and 15 ml. of cyclohexanone was refluxed for two hours. After cooling, the mixture was diluted with 10% hydrochloric acid and extracted with ether. The ethereal solution was washed with water, 10% sodium hydroxide solution, water and steam distilled. The chilled residue was taken up in ether and washed with 2% sodium hydroxide and water. The product was crystallized from the dried, concentrated ethereal solution. After chilling, it was filtered and washed with cold ether; 3.9 g. (87%), m. p. 150–160°. After a number of crystallizations from ether–petroleum ether (b. p. 35–60°), the melting point was still not sharp; however, this material gave a good yield of 16-methyltestosterone (80%). This range may be due to minor contamination with isomeric material which is difficult to separate.

*Anal.* Calcd. for  $C_{22}H_{32}O_2$ : C, 76.71; H, 9.35. Found: C, 76.77; H, 9.55.

### Summary

1. Dehydroisoandrosterone has been subjected to the Mannich reaction to yield the expected 16-aminomethyl derivatives.

2. 16-Dimethylaminomethyldehydroisoandrosterone was converted into 16-methylenedehydroisoandrosterone acetate on treatment with acetic acid–acetic anhydride.

3. By reduction of 16-methylenedehydroisoandrosterone acetate followed by esterification, partial saponification, oxidation, and hydrolysis, a 16-methyltestosterone was prepared. This testosterone showed androgenic activity.

CHICAGO, ILLINOIS

RECEIVED MAY 13, 1948

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

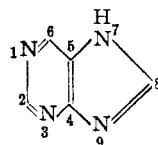
## Ultraviolet Absorption Spectra of Purines, Pyrimidines and Triazolopyrimidines<sup>1</sup>

BY LIEBE F. CAVALIERI, AARON BENDICH, JOHN F. TINKER AND GEORGE BOSWORTH BROWN<sup>2</sup>

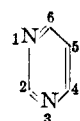
### Introduction

The ultraviolet absorption spectra of purines and pyrimidines have received considerable attention in the past<sup>3,4</sup> but the specific chromophore or chromophores responsible for the absorption have not been definitely assigned. The purpose of the present investigation was to correlate the spectra of various substituted purines, pyrimidines and triazolopyrimidines and ascertain

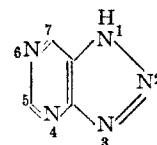
which functional groups are the chromophores.



Purine



Pyrimidine



Triazolopyrimidine

The purity of the compounds studied here is not readily determined by ordinary means and it was necessary to resort to another technique to determine their homogeneity. The counter-current distribution procedure has been applied successfully to this class of compounds<sup>5</sup> and the detection and separation of small amounts of related sub-

(1) The material in this paper was presented before the 113th Meeting of the American Chemical Society, Chicago, Ill., April, 1948.

(2) The authors gratefully acknowledge the assistance of the Office of Naval Research, the James Foundation of New York, Inc., and the Lord and Taylor Fund, New York.

(3) (a) Heyroth and Loofbrouow, *THIS JOURNAL*, **66**, 1728 (1934);

(b) Stimson and Reuter, *ibid.*, **65**, 154 (1943).

(4) Loofbrouow and Stimson, *J. Chem. Soc.*, **844** (1940).

(5) Tinker and Brown, *J. Biol. Chem.*, **178**, 585 (1948).