and 89% and the melting points 191–194° and 188–191°, respectively. Recrystallization as above gave in both instances thin needles, m.p. 200.4–201.0°.

Ethyl Isodesoxypodophyllate (XV, R′ = C_2H_5).—When IDPT (X) or IDPP (XI) was treated as in the preparation of the methyl ester, substituting ethanol for methanol, the crude products were amorphous solids. The yields were 78% and 88%, and the melting points 128–134° and 128–135°, respectively. Purification proved somewhat difficult because the compound tended to separate as a gel in the presence of impurities. It was dissolved in chloroform, adsorbed in alumina,[47] unchanged starting material eluted with chloroform, and the ester then eluted with chloroform–ethanol (9:1). Crystallization from 50% ethanol gave tiny colorless needles, m.p. 140–148°, and recrystallization from benzene–hexane (2:1) material melting at 148.8–149.6°, $[\alpha]^{21}D$ −22° (*c* 1.30, chloroform). The infrared spectra of the crude products were identical with that of the pure compound, except for the presence of a weak lactone band.

Anal. Calcd. for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35; 4 alkoxyl calcd. as OCH_3, 27.93. Found: C, 64.72; H, 6.42; OCH_3, 28.10.

Attempted Methylation of Desoxypodophyllic Acid.—When 400 mg. of desoxypodophyllic acid[24] (XIII) was treated with diazomethane as in the preparation of methyl isodesoxypodophyllate, 360 mg. (94%) of crystalline material, m.p. 164–171°, was isolated. Recrystallization from chloroform–hexane, then from methanol gave DPP (IX)[26] as strongly electrified thin needles, m.p. 171–172°. In another experiment, 450 mg. of XIII in 10 ml. of methanol was treated at 0° with 40 ml. of ethereal diazomethane (distilled from 2 g. of nitrosomethylurea) and kept in ice with occasional shaking for two hours. The long colorless needles of DPP[26] (330 mg.) were collected and washed with ether; m.p. 172–173°. Another 53 mg. of DPP, m.p. 171–172°, was obtained from the mother liquor, bringing the yield to 89%.

Attempted Epimerization of the Isodesoxylactones.—When 150 mg. of IDPT (X) or IDPP (XI) was refluxed with 150 mg. of anhydrous sodium acetate in 3 ml. of acetic anhydride for one hour, and the solutions gradually diluted with water, starting materials[26] (m.p. 250–251 and 199–202°) were recovered in 94% and 97% yields, respectively.

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[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Components of Podophyllin. XIII.[1] The Structure of the Peltatins[2]

BY ANTHONY W. SCHRECKER AND JONATHAN L. HARTWELL

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The location of the substituents in ring A and the point of attachment of ring C of α- and β-peltatin (I) have been established by permanganate oxidation of their methyl ether (I, R = R′ = CH_3) to the keto acid II and to cotarnic acid (VI). Further degradation of II yielded myristicinic acid (IV). The positions at which the lactone ring is attached are postulated by analogy with desoxypodophyllotoxin.

In preceding publications of this series[3,4] the general features of the carbon skeleton of α- and β-peltatin (I) have been established. The positions at which the substituents in rings A and B are attached were, however, yet unknown. The present paper provides evidence required for the complete structure determination of the peltatins. While this evidence does not include proof for the positions of attachment of the lactone ring, they are made quite probable by analogy with podophyllotoxin and some of its derivatives.

Permanganate oxidation at 100° of α-peltatin-B dimethyl ether (β-peltatin-B methyl ether[4]) (I, R = R′ = CH_3) and also of β-peltatin-B ethyl ether (I, R = C_2H_5, R′ = CH_3) had previously provided 3,4,5-trimethoxybenzoic acid (V), while α-peltatin-B diethyl ether (I, R = R′ = C_2H_5) yielded similarly syringic acid ethyl ether.[3] This established the presence of a 4-hydroxy-3,5-dimethoxyphenyl and of a 3,4,5-trimethoxyphenyl residue in α- and β-peltatin, respectively. Using milder conditions in the permanganate oxidation of I (R = R′ = CH_3), two additional degradation products have now been isolated. One of them was a substituted benzoylbenzoic acid, $C_{19}H_{18}O_9$, m.p. 183.5–185°. Decarboxylation of this acid by boiling with copper powder in quinoline[5] gave the corresponding benzophenone, $C_{18}H_{18}O_7$, m.p. 164–165°, which was subjected to cleavage with potassium *t*-butoxide.[6] From the resulting mixture of acids, myristicinic acid (IV)[7] was isolated, in addition to 3,4,5-trimethoxybenzoic acid (V), thus establishing structure III for the benzophenone. In view of the previous isolation of V from I (R = R′ = CH_3),[3] there remain two possible keto acids which could lead to III by decarboxylation. However, the other new degradation product obtained was cotarnic acid (VI), which was characterized as the anhydride and N-methylimide, both identical[7] with authentic samples.[8,9] This demonstrates that the

(1) Paper XII, A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **75**, 5916 (1953).

(2) Presented in part before the Medicinal Chemistry Division of the American Chemical Society at Chicago, Ill., Sept. 7, 1953; *cf.* Abstracts of Papers, Am. Chem. Soc., **124**, 8N (1953).

(3) J. L. Hartwell and W. E. Detty, THIS JOURNAL, **72**, 246 (1950).

(4) J. L. Hartwell, A. W. Schrecker and G. Y. Greenberg, *ibid.*, **74**, 6285 (1952).

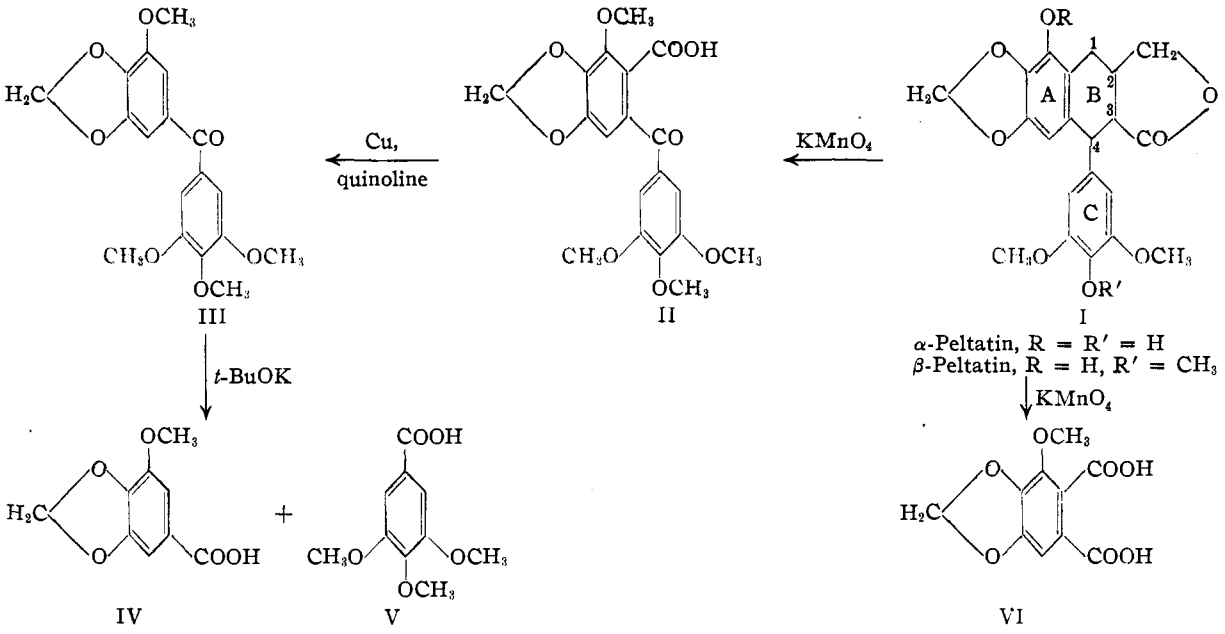
(5) E. Späth, F. Wessely and E. Nadler, *Ber.*, **66**, 125 (1933).

(6) G. A. Swan, *J. Chem. Soc.*, 1408 (1948).

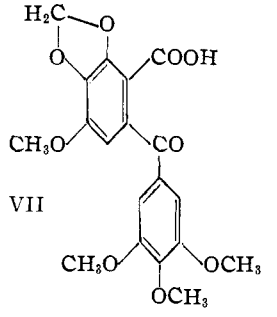
(7) Identity proven by mixed melting point determination and comparison of infrared spectra.

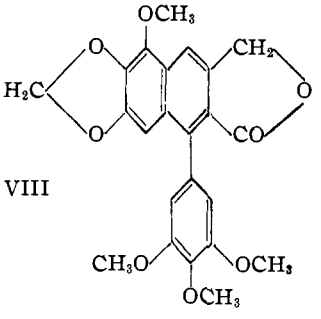
(8) (a) W. Roser, *Ann.*, **249**, 156 (1888); **254**, 334 (1889); (b) M. Freund and G. Wulff, *Ber.*, **35**, 1737 (1902); (c) E. Späth, L. Schmid and H. Sternberg, *ibid.*, **67**, 2095 (1934).

(9) Direct oxidation of cotarnine[8b] proved unsatisfactory. Oxidation of cotarnolactone[8a] yielded cotarnic acid, which was converted to the anhydride by vacuum sublimation[8c] and to the methylimide by heating with methylamine, as described in the Experimental section



keto acid possesses structure II and eliminates the alternative structure VII, which by further oxidation would have yielded isocotarnic acid.[8a] Formation of the keto acid II from I (R = R′ = CH_3) proves that the substituents in ring A and the trimethoxyphenyl group are located in the positions shown in that formula. Dehydrogenation of I





(R = R′ = CH_3) by means of palladium yielded dehydro-β-peltatin methyl ether (VIII), $C_{23}H_{20}O_8$, m.p. 271–272°, with an ultraviolet spectrum (Fig. 1) characteristic of a substituted naphthalene. This compound is analogous to dehydroanhydropicropodophyllin.[10,11]

From the infrared spectrum of I (R = R′ = CH_3) it was concluded[4] that a γ-lactone ring is present in the peltatins. The location of this ring has not yet been determined with certainty. However, the epimerization of the peltatins-A to the peltatins-B by means of sodium acetate[3] closely parallels the analogous conversion of desoxypodophyllotoxin to desoxypicropodophyllin.[1,12] Similarly, saponification of the peltatins yielded hydroxy acids which were lactonized to the "B"-lactones,[4] again duplicating the formation of desoxypicropodophyllin from desoxypodophyllotoxin *via* desoxypodophyllic acid.[12] Therefore, it is reasonable to assume that the lactone ring in the pelta-

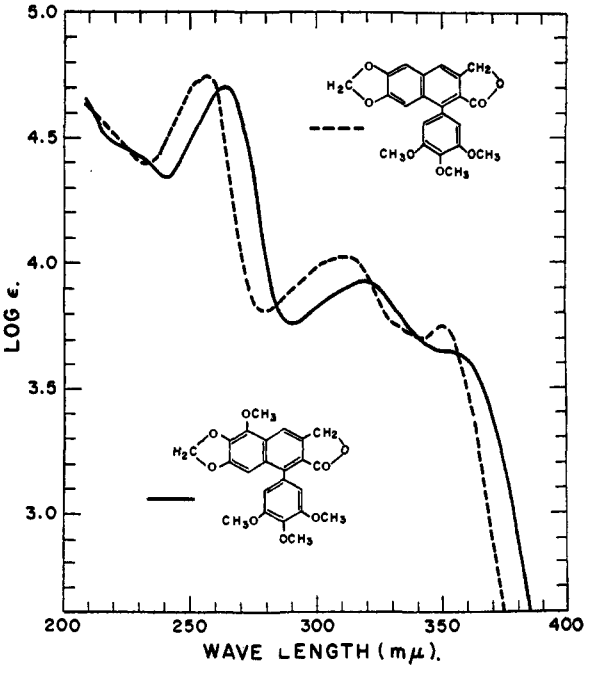


Fig. 1.—Ultraviolet absorption spectra in 95% ethanol of: ——, dehydro-β-peltatin methyl ether (VIII); ---, dehydroanhydropicropodophyllin.

(10) E. Späth, F. Wessely and L. Kornfeld, *Ber.*, **65**, 1536 (1932).

(11) R. D. Haworth and T. Richardson, *J. Chem. Soc.*, 348 (1936); A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **74**, 5676 (1952).

(12) J. L. Hartwell, A. W. Schrecker and J. M. Johnson, *ibid.*, **75**, 2138 (1953).

tins is attached as in desoxypodophyllotoxin. It may be pointed out that, were the carboxyl group attached at C_2, the epimerization of the peltatins by sodium acetate would not be expected to take place. Thus more strongly basic reagents (sodium methoxide or ammonia under pressure) are required to convert α- to β-conidendrin.[13] It has been postulated[14] that the easy conversion of podophyllotoxin to picropodophyllin is promoted by repulsion of the substituents at C_3 and C_4. This has been confirmed by the determination of the relative configuration of the asymmetric carbon atoms in podophyllotoxin and the finding that the reaction of isodesoxypodophyllotoxin (*trans*-(3:4)) with sodium acetate produced lactone ring opening rather than epimerization.[1] Thus it appears safe to assume not only that the peltatins have the lactone ring located as shown in I, but also that their relative configuration is *trans*-(2:3)-*cis*-(3:4) for the "A"-series (as in desoxypodophyllotoxin[1]) and *cis*-(2:3)-*trans*-(3:4) for the "B"-series (as in desoxypicropodophyllin).

Experimental[15]

2-Methoxy-3,4-methylenedioxy-6-(3',4',5'-trimethoxybenzoyl)-benzoic Acid (II).—One hundred grams of potassium permanganate, which was placed over a plug of glass wool in the extraction chamber of a Soxhlet apparatus, was extracted during 24 hours into a stirred and refluxed solution of 10.7 g. of β-peltatin-B methyl ether[4] in 250 ml. of acetone and 125 ml. of water. After removal of acetone by boiling, the mixture was cooled in ice-water and treated with sulfur dioxide until the disappearance of manganese dioxide, then acidified (congo red) with dilute sulfuric acid. It was then extracted thrice with chloroform (residual aqueous solution A). The chloroform solutions were washed once with water, dried over sodium sulfate, concentrated to about 25 ml. and, after addition of 100 ml. of ether, extracted with aqueous sodium carbonate. The extract was washed with chloroform–ether (1:4) and boiled with Norit. The cooled filtrate was acidified with dilute hydrochloric acid and the partly plastic solid collected after two hours in the refrigerator (filtrate B saved) and washed with water. It was then dissolved in 70 ml. of hot methanol, the solution treated with 35 ml. of hot water, then allowed to come slowly to room temperature. The pale tan leaflets, m.p. 183–185°, yield 2.33 g. (24%), were collected the next day (filtrate C saved). Recrystallization from methanol provided colorless leaflets, m.p. 183.5–185°.

Anal. Calcd. for $C_{19}H_{18}O_9$: C, 58.46; H, 4.65; OCH_3, 31.80. Found: C, 58.68; H, 4.68; OCH_3, 31.72.

Cotarnic Acid (VI). **(a) Anhydride.**—"Filtrate C" from the preparation of II was concentrated to remove methanol, then combined with the "residual solution A" and "filtrate B" and extracted continuously with ether for 48 hours. The extract was evaporated, and the residue dissolved in hot aqueous ammonia and treated with calcium chloride to remove oxalic acid. The filtrate was acidified with hydrochloric acid and extracted again with ether for 48 hours. The ether solution was dried with sodium sulfate, evaporated and the residue sublimed twice *in vacuo*. The sublimate was dissolved in ether, the solution extracted with cold dilute sodium bicarbonate to remove monocarboxylic acids (such as V), then dried with magnesium sulfate. Another vacuum sublimation, followed by recrystallization from acetone–ether–hexane, provided 45 mg. (0.81%) of cotarnic anhydride as pale yellow needles, m.p. 160–161° (lit.[8e] 161–163°), identical[7] with an authentic sample prepared from cotarnine.[8,9]

(b) Methylimide.—In a similar experiment, the residue obtained after removal of oxalic acid, extraction with ether and evaporation was treated with excess 25% aqueous methylamine. The mixture was gradually heated to 200° and kept at that temperature for five minutes. The melt was dissolved in chloroform, the solution extracted with aqueous sodium carbonate, dried over potassium carbonate and evaporated. The material was sublimed *in vacuo* three times, then recrystallized from ethanol to yield 80 mg. (1.4%) of pale yellowish needles, m.p. 197–203° (sintering 186°). Another crystallization from ethanol gave colorless needles, m.p. 203.5–205° (lit.[8b] 205–206°), identical[7] with authentic N-methylcotarnimide.[8,9]

Anal. Calcd. for $C_{11}H_9O_5N$: C, 56.17; H, 3.86; N, 5.96. Found: C, 56.19; H, 3.97; N, 6.08.

3,3',4',5'-Tetramethoxy-4,5-methylenedioxybenzophenone (III).—A mixture of 284 mg. of the keto acid II, 0.7 g. of copper bronze (Schering–Kahlbaum "Naturkupfer C") and 7 ml. of quinoline was boiled vigorously for 30 minutes.[5] The mixture was cooled, diluted with ether and filtered. The filtrate was washed repeatedly with 2 *N* hydrochloric acid, then with 5% sodium hydroxide and dried with sodium sulfate. Vacuum sublimation, followed by recrystallization from dilute methanol, yielded 153 mg. (61%) of yellow needles, m.p. 162–164°. Further purification by chromatography on alumina, using chloroform, followed by recrystallization from methanol, gave colorless prismatic needles, m.p. 164–165°. The yields were somewhat lower (50–56%) in preparations employing 1.5- to 2-g. lots.

Anal. Calcd. for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24; OCH_3, 35.84. Found: C, 62.58; H, 5.12; OCH_3, 36.01.

Two isomeric oximes were prepared by refluxing a mixture of 689 mg. of the ketone, 435 mg. of hydroxylamine hydrochloride, 1.24 g. of potassium hydroxide, 0.73 ml. of water and 9.4 ml. of 95% ethanol for two hours. Addition of 10 ml. of water produced a clear solution, which was chilled and acidified with hydrochloric acid. The rapidly crystallizing oil was collected after standing in ice for one hour. The pale pink solid (672 mg., 94%) melted between 141 and 163° (sintering 130°). Recrystallization from 50% ethanol, then from methanol gave 290 mg. of almost colorless fine needles, m.p. 168–169°.

Anal. Calcd. for $C_{18}H_{19}O_7N$: C, 59.83; H, 5.30; N, 3.88. Found: C, 59.73; H, 5.49; N, 3.65.

The mother liquor from the first recrystallization of the oxime mixture was diluted with water to yield 62 mg. of tiny colorless prisms, m.p. 135–138° (sintering 130°). Recrystallization from dilute methanol gave material melting at 135–138°.

Anal. Found: C, 59.81; H, 5.53; N, 3.80.

Attempts to obtain known degradation products from the oximes by Beckmann rearrangement, followed by saponification, were unsuccessful. No crystalline compounds could be isolated.

Cleavage of 3,3',4',5'-Tetramethoxy-4,5-methylenedioxybenzophenone.—Swan's procedure[6] was employed with the modifications indicated. The benzophenone III (750 mg.) in 13 ml. of dioxane containing 40 mg. of water was refluxed under argon with potassium *t*-butoxide (from 265 mg. of potassium) for 18 hours. The resulting oily mixture of acids (500 mg.) was crystallized from dilute methanol, from water (Norit), then from methanol to yield 21 mg. of crystalline **myristicinic acid** (IV), m.p. 209.5–212° (lit.[16] 211–212°), identical[7] with an authentic sample prepared from myristicin aldehyde.[16] Another 18 mg., m.p. 185–201°, was isolated from the mother liquor of the last recrystallization. The mother liquor of the first crystallization gave, when treated with Norit and concentrated, a product which after recrystallization from dilute methanol formed colorless needles (33 mg.), m.p. 165–167°, identical[7] with **3,4,5-trimethoxybenzoic acid** (V).[3]

Dehydro-β-peltatin Methyl Ether (VIII).—A mixture of 1.0 g. of β-peltatin-B methyl ether, 1 g. of 10% palladium-on-charcoal, 0.6 g. of quinone[17] and 15 ml. of phenyl ether[18]

(13) W. M. Hearon, H. B. Lackey and W. W. Moyer, THIS JOURNAL, **73**, 4005 (1951).

(14) E. H. Price, Ph.D. thesis, University of Maryland, 1949.

(15) Melting points are corrected. Infrared and ultraviolet absorption spectra were determined, respectively, with the Perkin–Elmer model 21 spectrometer and the Beckman model DU spectrophotometer.

(16) K. N. Campbell, P. F. Hopper and B. K. Campbell, *J. Org. Chem.*, **16**, 1736 (1951).

(17) M. Pailer, *Monatsh.*, **77**, 45 (1947); P. Ruggli and E. Girod, *Helv. Chim. Acta*, **27**, 1464 (1944).

(18) E. C. Horning and M. G. Horning, THIS JOURNAL, **69**, 1359 (1947).

was refluxed vigorously under argon for 20 hours. The catalyst was removed with suction and washed with hot chloroform. The very dark filtrate was concentrated, chromatographed on alumina and the strongly fluorescent zone (ultraviolet light) eluted with chloroform. Concentrating with addition of ethanol gave 169 mg. (17%) of yellow prisms, m.p. 269–270°. Vacuum sublimation, followed by repeated chromatography and two recrystallizations from chloroform ethanol provided tiny faintly yellowish needles, m.p. 271–272°.

Anal. Calcd. for $C_{23}H_{30}O_8$: C, 65.09; H, 4.75; OCH_3, 29.25. Found: C, 64.85; H, 4.86; OCH_3, 29.46.

Attempts to prepare this compound by dehydrogenation with lead tetraacetate[11,19] were unsuccessful. Only a trace of material with blue fluorescence, indicative of a naphthalene derivative, was formed.

Acknowledgments.—The authors wish to thank Mrs. Iris J. Siewers of the National Heart Institute for the infrared spectra and Dr. W. C. Alford and his co-workers for the microanalyses.

(19) H. Erdtman, *Ann.*, **513**, 229 (1934); R. D. Haworth and G. Sheldrick, *J. Chem. Soc.*, 636 (1935); R. D. Haworth and D. Woodcock, *ibid.*, 809 (1938).

BETHESDA, MARYLAND

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

"Enamine" Derivatives of Steroidal Carbonyl Compounds. III. The Synthesis of C_{11}-Oxygenated Testosterones

BY M. E. HERR AND F. W. HEYL

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Selective aspects of the reaction of polycarbonyl steroids with secondary amines have been investigated further. 11α- and 11β-hydroxytestosterone have been prepared from 11α-hydroxy-4-androstene-3,17-dione and adrenosterone, respectively, by the formation of the C_3-enamine, reduction of the free carbonyl groups with lithium aluminum hydride and subsequent hydrolysis to regenerate the 3-keto-Δ^4-system. 11β-Hydroxytestosterone thus prepared was readily acylated at the C_{17}-hydroxyl position and the resulting 11β-hydroxy-17-ester upon oxidation yielded the 11-keto-17-ester which was saponified to 11-ketotestosterone. Under certain conditions adrenosterone gave a $C_{3,17}$-bispyrrolidyl enamine which upon reduction and subsequent hydrolysis yielded 11β-hydroxyandrostene-3,17-dione.

In view of the critical importance of C_{11}-oxygen, either as a β-hydroxyl or ketone, for some of the principal adrenal cortical hormone properties it was of interest to investigate the biological properties of C_{11}-oxygenated compounds in the androstane series.

The preparation of C_{11}-oxygenated testosterone derivatives and similar compounds here reported is based on securing as starting material, 11α-hydroxy-4-androstene-3,17-dione (I), by the microbiological oxygenation of 4-androstene-3,17-dione.[1]

The reaction of 3-ketobisnor-4-cholenaldehyde with secondary amines to selectively form C_{22}-enamines and of C_3-carbonyl steroids to form enamines at carbon atom 3 have previously been described.[2] This reaction has been found to be especially useful in the present work as a blocking agent for the C_3-carbonyl group, while conducting a metal hydride reduction elsewhere in the molecule. Thus when 11α-hydroxy-4-androstene-3,17-dione (I) was allowed to react with pyrrolidine, 3-(N-pyrrolidyl)-3,5-androstadien-11α-ol-17-one (II) was readily formed. The C_{17}-carbonyl group of this compound was reduced with lithium aluminum hydride and subsequent hydrolysis of the reduced enamine gave 11α-hydroxytestosterone (IV) which had previously been prepared by the microbiological oxygenation of testosterone[1]; the synthetic compound was identical with the one produced microbiologically.

Adrenosterone (V), readily prepared by the chromic acid oxidation of 11α-hydroxy-4-androstene-3,17-dione, reacted with pyrrolidine to form the $C_{3,17}$-bispyrrolidyl enamine (VI) or the C_3-mono enamine (IX) depending upon the conditions employed. A large excess of pyrrolidine and a more concentrated reaction mixture resulted in reaction at both the C_3- and C_{17}-carbonyl groups, whereas when approximately one molecular equivalent of the secondary amine was employed the reaction was selective at the C_3-ketone. In either instance it was found expedient to catalyze the reaction by the addition of a small amount of *p*-toluenesulfonic acid.

This reactivity of the C_{17}-carbonyl group with pyrrolidine was in striking contrast to the unreactivity of the C_{17}-ketone of 4-androstene-3,17-dione, which formed only the C_3-pyrrolidyl compound under similar conditions.[2] It is apparent that the C_{11}-ketone group has influenced the reactivity of the C_{17}-carbonyl group.

Lithium aluminum hydride reduction of the $C_{3,17}$-di-(N-pyrrolidyl)-11-one (VI) and subsequent hydrolysis led to the formation of 11β-hydroxy-4-androstene-3,17-dione (VIII).[3] Aside from being different from the 11α-hydroxy isomer I[1] this compound was further characterized by the preparation of a disemicarbazone and by chromic acid oxidation to adrenosterone (V).

When the C_3-(N-pyrrolidyl)-11,17-dione (IX) was reduced with lithium aluminum hydride followed by hydrolysis, 11β-hydroxytestosterone (XI) was readily obtained.

The 11β-hydroxyl group was unreactive to acylating agents[4] under the conditions used and the 17-acetate XII, 17-propionate XIII and 17-benzoate XIV of 11β-hydroxytestosterone were obtained in good yield from the selective acylation of the C_{17}-

(1) S. H. Eppstein, P. D. Meister, H. Marian Leigh, D. H. Peterson, H. C. Murray, L. M. Reineke and A. Weintraub, Abst. A.C.S., 123rd Meeting, Los Angeles, Calif., 1953; U. S. Patent 2,602,769.

(2) M. E. Herr and F. W. Heyl, THIS JOURNAL, **74**, 3627 (1952); **75**, 1918 (1953).

(3) T. Reichstein, *Helv. Chim. Acta*, **20**, 978 (1937), has obtained this compound from the periodic acid oxidation of 3-keto-4-pregnene-11β,17α,20β,21-tetraol and from the lead tetraacetate oxidation of 17-hydroxycorticosterone.

(4) L. F. and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Edition, Reinhold Publishing Corp., New York, N. Y., 1949, p. 408.