D-fructose 1,6-diphosphate and inorganic phosphate was a modification of Gutzeit's method.[11]

Herein the dried paper chromatogram is sprayed with a 1% hydrochloric acid solution which contains 2% molybdic acid. After drying, the paper is sprayed thoroughly with a 1% solution of benzidine in acetic acid. At this point phosphate ion gives a bright blue spot, phosphate ion with reducing sugar gives a yellow-green spot and reducing sugar alone gives a brown spot. To bring out the position of minute amounts of sugars, the dried paper is next sprayed with ammonium hydroxide solution and heated at 100° for 5 minutes. Phosphate ion is now seen as a green spot, phosphate ion with reducing sugar as a gray-green spot and reducing sugar alone as a dark brown spot. The test, sensitive enough to detect 0.3 microgram of D-glucose 1-phosphate, has been, with some modifications, previously used[12] for the detection of phosphorylated sugars but not for the simultaneous detection of phosphorylated and non-phosphorylated sugars.

These techniques did not indicate the presence of phosphorylated sugars as either intermediates or products in the hydrolysis of cellulose with cellobiose.

Acknowledgment.—The authors take pleasure in acknowledging their indebtedness to Professor A. K. Balls of this Department for helpful suggestions.

(11) G. Gutzeit, *Helv. Chim. Acta*, **12**, 829 (1929), quoted by F. Feigl, "Qualitative Analysis by Spot Tests," Third English Edition, Elsevier Publishing Co., New York, N. Y., 1946, p. 284.

(12) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, THIS JOURNAL, **72**, 1718 (1950).

LAFAYETTE, INDIANA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

"Enamine" Derivatives of Steroidal Carbonyl Compounds. II

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Steroidal C_3-ketones have been shown to condense readily with pyrrolidine to form 3-(N-pyrrolidyl) enamines. In those polyketonic steroids having carbonyl groups in the C_3-position and elsewhere in the molecule the reaction was selective on the three position leaving the other functional groups unprotected and available for further study. Testosterone was readily prepared by the lithium aluminum hydride reduction of the C_3-(N-pyrrolidyl)-3,5-androstadien-17-one obtained thus from 4-androstene-3,17-dione.

In an earlier paper[1] it was shown that α,β-unsaturated amines—"enamines"—were readily prepared by the reaction of piperidine with representative steroidal aldehydes. Although Mannich and Davidsen[2] converted not only aldehydes but also several ketones, *e.g.*, phenylacetone and cyclohexanone, into enamines, the reaction when conducted as previously described[1] on 3-ketobisnor-4-cholenaldehyde, was remarkably selective on the aldehyde group, thus forming an intermediate which was readily ozonized to progesterone. Likewise, significant yields of enamines were not obtained by the reaction of piperidine with stigmastadienone, cholestan-3-one and 4-cholesten-3-one under the conditions reported here.

Continued studies, however, indicated that C_3-carbonyl groups readily condensed with the secondary amine, pyrrolidine, in such a manner that the formation of 3-(N-pyrrolidyl) enamines, with the elimination of water, appeared to be a characteristic reaction of C_3-steroidal ketones. Pyrrolidine, however, failed to react with the C_{17}- and C_{20}-keto groups of dehydroepiandrosterone and 5-pregnen-3β-ol-20-one, respectively. This observed selectivity led to the ready preparation of 3-(N-pyrrolidyl)-3,5-androstadien-17-one (IX) and 3-(N-pyrrolidyl)-3,5-pregnadien-20-one (I).

To study further the characteristic nature and selective aspects of this reaction the following C_3-pyrrolidyl enamines were prepared in addition to those mentioned above: 3-(N-pyrrolidyl)-2-(or 3)-cholestene (IV), 3-(N-pyrrolidyl)-3,5-cholestadiene (II), 3-(N-pyrrolidyl)-3,5,22-stigmastatriene (III), 3-(N-pyrrolidyl)-3,5-pregnadien-11,20-dione (V), 3-(N-pyrrolidyl)-3,5-pregnadien-11α-ol-20-one (VI), methyl-3-(N-pyrrolidyl)-7,12-diketo-3(or 2)-cholenate (VI), 3-(N-pyrrolidyl)-3,5-androstadien-17β-ol (X) and 3-(N-pyrrolidyl)-17α-methyl-3,5-androstadien-17β-ol (VIII).

These C_3-enamines, when prepared from 3-keto-Δ^4-steroids, were in all instances light yellow highly crystalline compounds which gradually decomposed and darkened upon melting. Even after all traces of solvent had been removed they continued to have a characteristic amine odor unusual for steroidal compounds. In those few cases where apparently pyrrolidine did not react readily with C_3-carbonyl groups, *e.g.*, progesterone, the addition of a catalytic amount of *p*-toluenesulfonic acid caused the reaction to proceed smoothly.

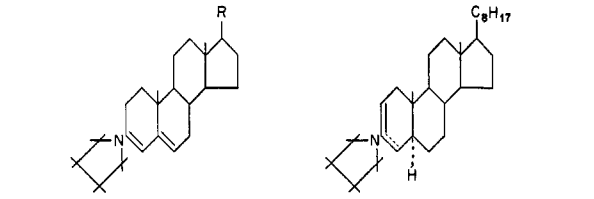
The ease of hydrolysis of the enamines to regenerate the ketones varied considerably between compounds of the single ethylenic double bond type IV and the conjugated ethylenic dienes type I, II and III. The enamine (IV) prepared from cholestan-3-one readily reverted to cholestan-3-one upon heating at reflux for 5 minutes in 95% ethanol whereas it was found better to heat compounds of the type prepared from stigmastadienone for 4 hours in a sodium acetate–acetic acid buffered solution in order to regenerate the ketone. Upon treatment with methanolic semicarbazide acetate, enamines were converted to semicarbazones.

The ultraviolet absorption spectra of the pyrrolidine enamines IV and VII, prepared from cholestanone and methyl dehydrocholate are in agreement with data reported by Bowden, *et al.*[3] Whether the position of the double bond is Δ^2 or Δ^3 has not been established.

(1) M. E. Herr and F. W. Heyl, THIS JOURNAL, **74**, 3627 (1952).

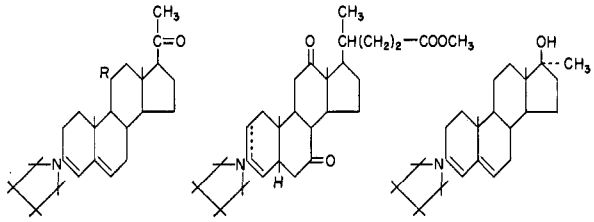
(2) C. Mannich and H. Davidsen, *Ber.*, **69**, 2106 (1936).

(3) K. Bowden, E. A. Braude, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 45 (1946). They report that the conjugation of a single ethylenic bond with an amino group results in light absorption equivalent to that exhibited by classical conjugated systems such as butadiene; thus for $C_2H_5\cdot CH{=}CH\cdot NC_5H_{10}$ (piperidyl) they report λ_{max}^{hexane} 228 mμ.



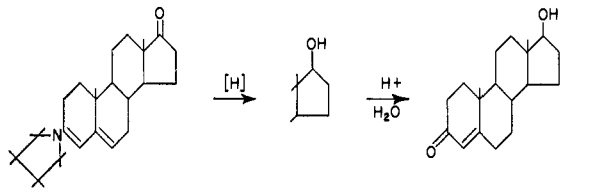
I, R = — $COCH_3$
II, R = — C_8H_{17}
III, R = — $C_{10}H_{19}$

IV



V, R = = O
VI, R = — — —OH

VII VIII



IX X XI

Pending further ultraviolet absorption studies the enamines prepared from 3-keto-Δ^4-steroids are tentatively represented as $\Delta^{3,5}$-dienes. They all exhibit absorption, λ_{max}^{ether} 279–282 mμ.[4]

The formation of 3-enol-ethyl ethers of the $\Delta^{3,5}$-dienes[5] by blocking the C_3-keto group which reacts with orthoformic ethyl ester is accompanied by a characteristic levo shift of the specific rotation. These heteroannular dienes are quite unstable in chloroform solution[6] and the rotation reverts rapidly to that of the original free ketone. The 3-(N-pyrrolidyl)-3,5-dienes reported here are stable when carefully dried but they show decomposition and a shift in rotation on standing in chloroform solution, gradually changing to the dextro side. The solutions of these enamines initially show a similar wide shift of specific rotation to the left above that of the ketones but the regeneration of the original ketones does not proceed smoothly as is the case with the enol-ethers.

It has previously been shown that lithium aluminum hydride reduction of 17-ketosteroids affords the 17β-hydroxyl configuration, *e.g.*, estrone gave the natural estradiol-17β.[7]

(4) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1949, p. 185, point out that in conjugated cyclic dienes, if two double bonds are in the same ring, absorption lies in the region 265–285 mμ, whereas if the bonds are in different rings the maxima are in the region 230–250 mμ, *e.g.*, 3,5-cholestadiene, λ_{max}^{alc} 234 mμ. We therefore attribute the difference of 46 mμ toward the longer wave length between 3,5-cholestadiene and these enamines as due to the bathochromic effect caused by the "extended conjugation" of the amino group with the $\Delta^{3,5}$-diene.

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

(6) E. Schwenk, G. Fleischer and B. Whitman, THIS JOURNAL, **60**, 1702 (1938).

(7) A. C. Ott and M. F. Murray, Abst. A. C. S. 108th Meeting, Chicago, Ill., 1948; reference 4, p. 326.

We have found that 3-(N-pyrrolidyl)-3,5-androstadien-17-one (IX), which is obtained in high yields, was likewise reduced using lithium aluminum hydride, and testosterone was readily prepared by subsequent hydrolysis.

The use of pyrrolidine in the capacity of a blocking agent for the C_3-ketone group is being investigated further in this Laboratory.

Acknowledgment.—The authors are indebted to Dr. J. L. Johnson and Mr. J. E. Stafford of our Physics Department for their determination of the absorption spectra, and to Mr. Wm. A. Struck and staff of our Microanalytical Laboratory for the analytical data. The 11α-hydroxyprogesterone was kindly supplied by Dr. D. H. Peterson and coworkers.

Experimental[8]

General Procedure for the Preparation of C_3-(N-Pyrrolidyl) Enamines.—The steroidal C_3-ketone was dissolved or suspended in 50 ml. of thiophene-free benzene per 0.01 mole of steroid and 4 mole equivalents of pyrrolidine was then added. Stirring was accomplished by means of a magnetic stirrer and the mixture was heated at reflux on a Glas-col mantle. The course of the reaction was followed by noting the amount of water collected in a Bidwell–Sterling moisture trap placed between the reaction flask and the condenser. The reaction was allowed to proceed until one mole equivalent of water was collected or until the amount was constant, at which point the mixture was concentrated to dryness *in vacuo*, using ordinary precautions to preclude moisture. The product was then triturated with methanol or acetone, cooled and recovered by filtration. This sufficed in most instances to give analytically pure material; however, for analyses the product was recrystallized from methylene chloride–methanol mixture, ethyl acetate, ether or benzene. The results obtained by this general procedure are shown in Table I.

Hydrolysis of 3-(N-Pyrrolidyl)-2(or 3)-cholestene (IV) to Regenerate Cholestan-3-one.—The enamine (IV) was heated at reflux for 5 minutes with 95% ethanol and the mixture cooled; cholestan-3-one, m.p. and mixed m.p. 128°, was recovered as the sole product.

Hydrolysis of 3-(N-Pyrrolidyl)-3,5,22-stigmastatriene (III) to Regenerate Stigmastadienone.—The enamine (III) (0.5 g.) was heated at reflux for 4 hours in a buffered solution of 1.5 g. of sodium acetate, 1.5 ml. of water, 1.0 ml. of glacial acetic acid and 10 ml. of methanol. Upon cooling, stigmastadienone separated in needles; after recrystallization from alcohol the product gave a melting point and mixed melting point, 123–124°.

Hydrolysis of Other C_3-(N-Pyrrolidyl) Enamines.—Under the conditions described in the above experiment 11α-hydroxyprogesterone and testosterone were recovered from the corresponding enamines VI and X, respectively.

Conversion of 3-(N-Pyrrolidyl)-3,5,22-stigmastatriene (III) to the Semicarbazone of Stigmastadienone.—When 0.5 g. of the enamine (III) was heated at reflux for 1 hour in a buffered solution of methanol, water, semicarbazide hydrochloride and sodium acetate, the known semicarbazone of stigmastadienone, m.p. 235° (dec.), was obtained.

Testosterone semicarbazone, m.p. 264° (dec.), was prepared from 3-(N-pyrrolidyl)-3,5-androstadien-17β-ol (X) as described above.

Shift of Specific Rotations of C_3-(Pyrrolidyl) Enamines in Chloroform Solution.—Chloroform solutions of this class of enamines initially showed a wide shift of specific rotation to the left above that of the parent ketones. They were quite unstable however and showed decomposition, and a shift in rotation to the right on standing in chloroform solution as indicated in Table II. This shift is much less pronounced in pyridine solution as shown by the examples in the table.

(8) Melting points are as read on a Fisher–Johns block which checked the melting point of standard compounds within ±1° over a range from 70 to 237°. Unless otherwise specified, specific rotations are in chloroform.

TABLE I

C_3-(N-PYRROLIDYL) ENAMINES PREPARED FROM STEROIDAL C_3-KETONES

Compd. No.	Parent C_3-ketone	Reflux time, hr.	Yield, %	M.p., °C. (dec.)	$[\alpha]_D$	λ_{max}^{ether}, mμ	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
I	Progesterone	1.5[a]	97	170–175	− 22	281	$C_{25}H_{37}NO$	81.69	81.96	10.15	10.39	3.81	3.83
II	4-Cholestene-3-one	4	79	138–140	−110	280	$C_{31}H_{51}N$	85.06	85.47	11.74	11.93	3.20	3.25
III	Stigmastadienone[b]	1	94	153–155	−121	280	$C_{33}H_{53}N$	85.45	85.56	11.52	11.52	3.02	3.07
IV	Cholestan-3-one	3	94	105–110	+ 45	229	$C_{31}H_{53}N$	84.67	84.62	12.15	11.92	3.19	3.20
V	11-Ketoprogesterone[c]	4	94	180–185	+ 34	282	$C_{25}H_{35}O_2$	78.72	78.99	9.25	9.12	3.67	3.68
VI	11α-Hydroxyproges-terone	2	86	145–152	−126	281	$C_{25}H_{37}NO_2$	78.29	78.10	9.73	9.75	3.91	3.93
VII	Methyl dehydrocholate	3	86	160–165		233	$C_{29}H_{43}NO_4$	74.17	73.95	9.22	8.96	2.98	2.71
VIII	17α-Methyltesto-sterone	3	91	160–170	− 93	281	$C_{24}H_{37}NO$	81.69	81.57	10.49	10.49	3.94	3.93
IX	4-Androstene-3,17-dione	2	90	200–205	−135	281	$C_{23}H_{33}NO$	81.35	81.31	9.80	9.55	4.13	4.33
X	Testosterone	5	86	133–137	−116	280	$C_{23}H_{35}NO$	80.88	80.81	10.33	10.18	4.10	4.07

[a] After 1 hour, no water had collected; upon the addition of 20 mg. of *p*-toluenesulfonic acid, water began to collect immediately. [b] E. Fernholz and H. E. Stavely, THIS JOURNAL, **61**, 2956 (1939). [c] Prepared by the oxidation of 11α-hydroxyprogesterone; D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952).

TABLE II

SHIFT IN SPECIFIC ROTATION ($CHCl_3$) OF C_3 STEROIDAL ENAMINES

Enamine IX[a] Time, hr.	Enamine IX[a] $[\alpha]_D$	Enamine X[b] Time, hr.	Enamine X[b] $[\alpha]_D$
0	−135°	0	−116°
.08	−122	2.5	− 83
.75	−108	4.5	− 53
2	− 98	7.3	− 44
24	− 19	24	+ 4
48	+ 15	49	+ 22
150	+ 59	168	+ 56

[a] In pyridine solution an original determination, $[\alpha]^{24}D$ −138°, was found at 100 hours to show $[\alpha]^{24}D$ −116°. [b] In pyridine solution an original determination, $[\alpha]^{24}D$ −131°, was found at 168 hours to show $[\alpha]^{24}D$ −116°.

Reduction of 3-(N-Pyrrolidyl)-3,5-androstadien-17-one (IX) to Form 3-(N-Pyrrolidyl)-3,5-androstadien-17β-ol (X) and Hydrolysis to Testosterone.—A solution of 3.4 g. (0.01 mole) of the ketoenamine (IX) in 70 ml. of tetrahydrofuran was added over 4 minutes to a stirred mixture of 1.9 g. of lithium aluminum hydride in 800 ml. of anhydrous ether and the reaction mixture brought to reflux. After 10 minutes the heating mantle was replaced by an ice-bath and the metal complex decomposed by the cautious addition of 10 ml. of water. The organic layer was decanted through a fluted filter paper and the solid residue extracted with benzene. The combined filtrate and extracts were washed with water, dried over sodium sulfate, and the solvent evaporated under reduced pressure. The solid yellow residue of 3-(N-pyrrolidyl)-3,5-androstadien-17β-ol (X) was heated at reflux in a mixture of 7.5 g. of sodium acetate, 8 ml. of water, 4 ml. of glacial acetic acid and 100 ml. of methanol for 4 hours. Upon concentration and dilution with water a crystalline precipitate separated. After cooling, the product was collected by filtration, washed with water, and dried; yield 2.19 g. (76%), m.p. 148–150°. Admixture with an authentic sample of testosterone did not depress the melting point and the ultraviolet absorption spectra were identical, λ_{max}^{EtOH} 241 mμ, log ε 4.20.

In other runs, after the reduction with lithium aluminum hydride, the reduced enamine (X) was isolated in 86% yields. Recrystallized from methylene chloride–methanol it was identical with that prepared directly from testosterone. It melted at 133–137° (dec.).

Anal. Calcd. for $C_{23}H_{35}NO$: N, 4.13. Found: N, 4.07.

KALAMAZOO, MICHIGAN