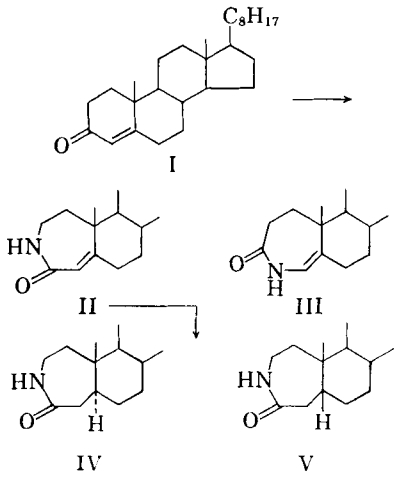
Steroids X

Preparation of Azasteroids from Cholestenone and Progesterone

By NORMAN J. DOORENBOS and HARKISHAN SINGH†

Cholestenone (I) was converted into 3-aza-A-homo-4a-cholesten-4-one (II) by means of the Schmidt reaction in polyphosphoric acid. The structure was assigned on the basis of its ultraviolet spectrum, resistance to hydrolysis, and hydrogenation to a mixture of 3-aza-A-homo-5α-cholestan-4-one (IV) and 3-aza-A-homo-5β-cholestan-4-one (V). By similar procedures, progesterone (VI) yielded either 17β-acetylamino-4-androsten-3-one (VII) or 17β-acetylamino-3-aza-A-homo-4a-androsten-4-one (VIII), depending upon the quantity of sodium azide used. The structure of VII was confirmed by preparing it from 17β-acetylamino-5-androsten-3β-ol (IX) by Oppenauer oxidation.

THE ANDROGENIC, progestational, and corticosteroid hormones each contain an α,β-unsaturated ketone system in ring A. As an extension of our investigation of azasteroids, we sought to synthesize A-homosteroid homologs of some of these steroids with a nitrogen in position 3 by means of the Schmidt reaction. An example of such a steroid is illustrated by structure II.



The initial studies were carried out with cholestenone (4-cholesten-3-one) (I). No crystalline products were isolated when the Schmidt reaction was carried out by the usual procedures with sulfuric acid as the catalyst (1). However, when polyphosphoric acid was used as the solvent and catalyst (2, 3), 3-aza-A-homo-4a-cholesten-4-one (II) was obtained as a white crystalline product. Chromatographic studies on II established that it was a single compound and not a mixture. Structure II was assigned to this product (*a*) because of its resistance to hydrolysis, (*b*) since its spectrum resembles that of a known steroidal α,β-unsaturated lactam, and differs from known steroidal enamine lactams, and (*c*) because it is hydrogenated to a mixture of 3-aza-A-homo-5α-cholestan-4-one (IV) and 3-aza-A-homo-5β-cholestan-4-one (V).

Enamine lactams of the type represented by structure III are relatively easy to hydrolyze as illustrated by the ease of hydrolysis of 3β-acetoxy-17-acetylamino-5,16-androstadiene (4) and 4-aza-5-cholesten-3-one (5). The product obtained by the Schmidt reaction on cholestenone was not hydrolyzed by refluxing in 5 per cent ethanolic potassium hydroxide or by refluxing in dioxane-concentrated hydrochloric acid mixture. This resistance to hydrolysis indicates that it is the α,β-unsaturated lactam represented by structure II.

Table I lists the major absorption peak in the ultraviolet spectrum for several enamine and α,β-unsaturated steroid lactams. The first four compounds listed are enamine lactams. The fifth is an α,β-unsaturated lactam. The spectrum of lactam II, the sixth compound in this list, is similar to that of the α,β-unsaturated lactam.

This unsaturated lactam (II) was reduced with platinum and hydrogen in glacial acetic acid at 70° and 60 lb. pressure. A reduced lactam was separated from some unreacted starting material by chromatography. This reduced lactam, m.p. 202–245°, was a mixture of the 5α-[m.p. 268–271° (6)] and 5β-[m.p. 166–174° (6)] isomers. Repeated crystallization from absolute ethanol yielded a sample, m.p. 263–269°, which was shown, by mixed melting points and a comparison of infrared spectra, to be 3-aza-A-homo-5α-cholestan-4-one (IV).

It should be noted that the α,β-unsaturated steroid lactam described in Table I was also

Received April 28, 1961, from the School of Pharmacy, University of Maryland, Baltimore 1.
Accepted for publication July 19, 1961.
This work was supported by Research Grant CY-4132 from the National Cancer Institute, U. S. Public Health Service.
For paper IX in this series see Doorenbos, N. J., and Dorn, C. P., Jr., THIS JOURNAL, 51, 414 (1962).
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.
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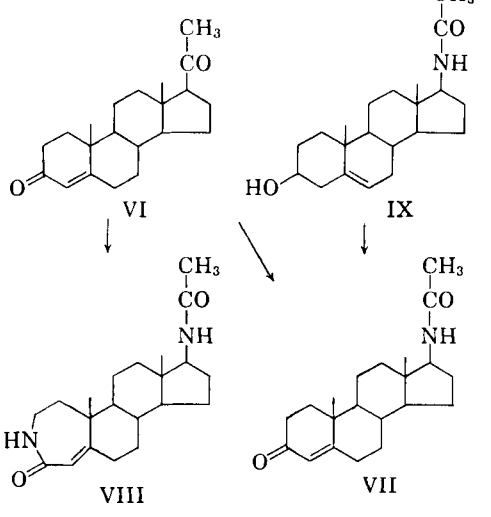
TABLE I.—MAJOR ABSORPTION PEAKS OF SOME UNSATURATED STEROID LACTAMS

Compound	Solvent	$\lambda_{max.}$	Log ϵ
2β-Acetoxy-17-acetylamino-5,16-androstadiene (4)	EtOH	240 mμ	3.82
4-Aza-5-cholesten-3-one (7)	EtOH	233 mμ	4.14
6-Aza-4-cholesten-7-one (8)	EtOH	234 mμ	4.11
4-Aza-4-methyl-5-cholesten-3-one (9)	EtOH	234 mμ	4.13
3β-Acetoxy-12a-aza-C-homo-5α,22α-spirost-9-(11)-en-12-one (10, 11)	MeOH	220 mμ	4.20
3-Aza-A-homo-4a-cholesten-4-one (II)	EtOH	220 mμ	4.05
17β-Acetylamino-3-aza-A-homo-4a-androsten-4-one (VIII)	EtOH	220 mμ	4.14

prepared from an α,β-unsaturated ketone. Mazur (10) prepared this compound from 3β-acetoxy-5α,22α-spirost-9(11)-en-12-one by means of the Beckmann rearrangement of its oxime. The structure which Mazur tentatively assigned to the product was confirmed by Bladon and McMeekin (11).

The Schmidt reaction was carried out with progesterone (VII) under conditions similar to those used for cholestenone (I). 17β-Acetylamino-4-androsten-3-one (VII) was obtained with 1 mole of sodium azide and 17β-acetylamino-3-aza-A-homo-4a-androsten-4-one (VIII) with an excess of sodium azide. The structural assignments were made on the basis of analysis and spectra.

Compound VII (12, 13) was prepared by Oppenauer oxidation of 17β-acetylamino-5-androsten-3β-ol (IX) (14) and shown by means of mixed melting points and a comparison of spectra to be identical with the sample prepared by the Schmidt reaction of progesterone.



EXPERIMENTAL[1]

3-Aza-A-homo-4a-cholesten-4-one (II).—A mixture of 5.0 Gm. of 4-cholesten-3-one (I), 160 Gm. of polyphosphoric acid, and 1.0 Gm. of sodium azide was heated at 50–60° for 10 hours with frequent stirring, following a previously described procedure for the Schmidt reaction of steroids in polyphosphoric acid (3). The mixture was poured onto crushed ice, made alkaline with cold 50% potassium hydroxide, extracted with chloroform, and washed with water. Removal of the solvent, after drying over sodium sulfate, yielded 5.1 Gm. of a brown solid residue. The residue was chromatographed with 60 Gm. of neutral Woelm alumina, activity grade I, which had been prepared with benzene. Elution with benzene (3 × 150 ml.) yielded a sticky material which melted completely by 63°. A series of elutions extending from chloroform-benzene (1:19) through chloroform yielded 3.1 Gm. (60%), m.p. 220–240°. An analytical sample was prepared by crystallization from benzene-acetone; m.p. 255–260°; $[\alpha]_D^{27}$ +20.0° (c, 1.0 $CHCl_3$); $\lambda_{max.}^{EtOH}$ 220 mμ, log ε 4.05; $\lambda_{max.}^{CHCl_3}$ 291 mμ (free NH); 6.05 μ (lactam C=O); 6.22 μ (C=C).

Anal.—Calcd. for $C_{27}H_{45}NO$: C, 81.14; H, 11.35; N, 3.51. Found: C, 80.89; H, 11.40; N, 3.70.

During the course of subsequent preparations it was established that the product of this reaction could be purified by repeated digestion with petroleum ether.

The initial attempts to prepare this product by the Schmidt reaction using chloroform as the solvent and sulfuric acid as the catalyst yielded an oily product which could not be separated by chromatography.

Attempted Hydrolysis of 3-Aza-A-homo-4a-cholesten-4-one (II).—A 1-Gm. sample of II was added to 100 ml. of 5% potassium hydroxide in 95% ethanol and the mixture was refluxed 1½ hours. The lactam was recovered unchanged.

A mixture of 1 Gm. of II, 150 ml. of dioxane, and 50 ml. of concentrated hydrochloric acid was refluxed 3 hours. The lactam was recovered unchanged.

Hydrogenation of 3-Aza-A-homo-4a-cholesten-4-one (II).—3-Aza-A-homo-4a-cholesten-4-one, 4.0 Gm., was dissolved in 200 ml. of glacial acetic acid. Platinum oxide, 700 mg , was added and the mixture was shaken in a Parr hydrogenator at 72° with a hydrogen pressure of 60 lb. for 9 hours. The mixture was cooled, filtered, diluted, and saturated with sodium chloride. The mixture was extracted with methylene chloride. The extracts were washed with sodium carbonate solution and water and then dried over sodium sulfate. The residue, 3.8 Gm., obtained by evaporating the solvent was chromatographed on a column prepared with 125 Gm. of neutral Woelm alumina, activity grade I. The column was prepared with benzene. No product was obtained by elution with benzene or methylene chloride. Elution with absolute ethanol-methylene chloride, 1:99, (6 × 500 ml.) yielded 2.6 Gm. (frac-

[1] Melting points are uncorrected. Analyses were obtained from Drs. Weiler and Strauss, Oxford, England. Spectra were obtained on Perkin-Elmer Infracord and Spectracord spectrophotometers. The authors wish to express their thanks to the Cancer Chemotherapy National Service Center for the steroid intermediates used in this investigation.

tion A), m.p. 202–245°. There was no absorption which could be attributed to an olefinic double bond. A series of fractions were combined as fraction A since they had similar melting points and identical infrared spectra.

Repeated crystallizations from absolute ethanol raised the melting point to 263–269° without changing the infrared spectrum. A mixed melting point with an authentic sample of 3-aza-A-homo-5α-cholestan-4-one, m.p. 270–272°, gave a m.p. of 264–269°, establishing that these samples are identical.

Further elution of the column with ethanol yielded 800 mg. of starting material, II.

17β-Acetylamino-4-androsten-3-one (VII).—*Method A, from Progesterone.*—Progesterone (VI), 2.0 Gm., was allowed to react with 0.4 Gm. of sodium azide in polyphosphoric acid in a procedure similar to that used for cholestenone. After repeated crystallizations from acetone the crude product yielded 220 mg. of an analytical sample of 17β-acetylamino-4-androsten-3-one (VII), m.p. 275–278°; $[\alpha]_D^{27}$ + 58.0° (c, 1.0 $CHCl_3$); $\lambda_{max.}^{EtOH}$ 239 mμ, log ε 4.11; $\lambda_{max.}^{CHCl_3}$ 2.92 μ (free NH), 3.00 μ (associated NH), 6.01 μ (C=O), and 6.20 μ (C=C). A second crop (500 mg.) was obtained giving a total yield of 720 mg. (35%).

Anal.—Calcd. for $C_{21}H_{31}NO_2$: C, 76.55; H, 9.48; N, 4.25. Found: C, 76.42; H, 9.39; N, 4.69.

Method B, from 17β-Acetylamino-5-androsten-3β-ol (IX).—To a refluxing suspension of 681 mg. of 17β-acetylamino-5-androsten-3β-ol (IX) (14) in a mixture of 100 ml. of dry benzene and 40 ml. of acetone, was added a solution of 4.1 Gm. of aluminum isopropoxide in 20 ml. of benzene. The mixture was refluxed 44 hours. After cooling, 10 ml. of water was added and the mixture was shaken vigorously. Then 40 ml. of dilute sulfuric acid was added and the mixture was again shaken. The benzene layer was separated, washed with water, and dried over sodium sulfate. The residue obtained by evaporating the solvent was crystallized from acetone to yield 345 mg. of 17β-acetylamino-4-androsten-3-one, m.p. 272–277° [reported 270° (12) and 284–286° (13)]. This product was shown to be identical to that prepared by method A by a comparison of infrared spectra and a mixed melting point.

17β-Acetylamino-3-aza-A-homo-4a-androsten-4-one (VIII).—The Schmidt reaction was carried out by the procedure described for cholestenone using 1.6 Gm. of progesterone, 60 Gm. of polyphosphoric acid, and 800 mg. of sodium azide. The residue obtained by the usual workup was triturated with cold acetone and filtered to yield 500 mg. (29%) of a white powder, m.p. 270–280°. A mixed m.p. with VII showed a marked depression, m.p. 230–250°. An analytical sample was prepared by crystallization from methanol-acetone; m.p. 289–291°; $[\alpha]_D^{27}$ − 2.0; $\lambda_{max.}^{EtOH}$ 220 mμ, log ε 4.14; $\lambda_{max.}^{CHCl_3}$ 3.07 μ, 3.20 μ, 5.98 μ, 6.05 μ, 6.30 μ.

Anal.—Calcd. for $C_{21}H_{32}N_2O_2$: N, 8.13. Found: N, 7.43.

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Studies on Sciatic Stimulated Rat Brain Sulfhydryl Levels

By JOHN H. MENNEAR, TOM S. MIYA, and GEORGE K. W. YIM

Rat brain nonprotein sulfhydryl levels were increased by electrical stimulation of the sciatic nerve. Pretreatment with morphine sulfate, meperidine hydrochloride, chlorpromazine hydrochloride, or meprobamate blocked the rise in cerebral levels of nonprotein sulfhydryl. The compounds had no effect on nonstimulated cerebral nonprotein sulfhydryl.

UNGAR AND ROMANO (1) demonstrated that electrical stimulation of the sciatic nerve of unanesthetized rats induces increased brain levels of free and bound sulfhydryl groups. Earlier work in our laboratories (2) showed that stimulation of the sciatic nerve of pentobarbitalized rats increased brain levels of nonprotein sulfhydryl (NPSH). Only the NPSH levels of the cerebrum were increased by sciatic stimulation.

Glutathione, a sulfhydryl-containing tripeptide, has been implicated as playing some role in conditions of mental aberration. The levels of NPSH detected by the amperometric titration

Received April 28, 1961, from Purdue University, Department of Pharmacology, Lafayette, Ind.
Accepted for publication July 31, 1961.
Supported by NIH Grant M-2405 (C2).
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.