Worcester Foundation for Experimental Biology

Heterocyclic Steroids. X. 2-Aza-4-oxa-steroid Analogs (1,2)

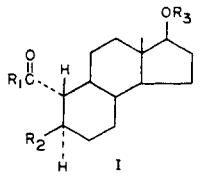
D. M. Piatak (3) and E. Caspi

Two 2-aza-4-oxa steroids have been synthesized from acid Ia. In addition the Beckmann rearrangement of the C-1 amide Ij has been explored.

The acetyl acid (Ia) has been employed in our laboratory for the synthesis of 2,3-diaza (4,5), 2,4-diaza (6), 2-oxa (7), A-*nor*-2-oxa (1) and 4-aza-2-oxa (8) steroids. In preparing the 4-aza-2-oxa products (8) the carboxylic moiety was used as the source of oxygen for position 2 and nitrogen was introduced at position 4 by a Beckmann rearrangement. It was clear that the acetyl acid (Ia) could also be used for the synthesis of 2-aza-4-oxa derivatives, where the heteroatom positions would be reversed. The devised route was to insert the oxygen at position four *via* Baeyer-Villiger oxidation of the acetyl and introduce a nitrogen at position two through the initial formation of an amide from the carboxylic acid. Subsequently, ring A could be reconstructed to yield the 2-aza-4-oxa analogs.

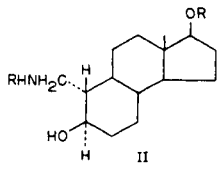
The previously described ester-acetate (6b) (Ib) was chosen as the starting material since it already contained the 4-oxygen. The ester-acetate (Ib) was first hydrolyzed to the hydroxy acid (Id), then acetylated to yield the acetoxy acid (Ie). The acid (Ie) was converted to its acid chloride which upon treatment with anhydrous ammonia gave amide If. Reduction with lithium aluminum hydride of If yielded the hydroxyamine (IIa), the key intermediate for the synthesis of 2-aza-4-oxa steroids. The amine (IIa) resisted crystallization and was acetylated with acetic anhydride-pyridine. Interestingly, only a diacetate (IIb) was formed. The structure of the diacetate (IIb) follows from its elemental analysis and spectroscopic data. An infrared spectrum exhibited bands at 3330 cm^{-1} for the C-5 hydroxyl and at 1635 and 1545 cm^{-1} for the acetamide moiety. In an n.m.r. spectrum signals were observed at 121.5 c.p.s. equivalent to 3 protons for the 17-acetate moiety, at 123 c.p.s. for the acetamide methyl, and at 360 c.p.s. for the nitrogen proton. Finding the 18 methyl signal at 48.5 c.p.s. is consistent with the hypothesis that the 17-hydroxyl and not the 5-hydroxyl was esterified. Our previous experience (8) indicated that the 18 methyl signal for a 17β-hydroxy steroid of this type would occur at about 45 c.p.s.

Construction of ring A was accomplished with two reagents, phosgene and paraformaldehyde, as was the case for the 4-aza-2-oxa steroids. The reaction of crude amine (IIa) with phosgene proceeded readily to give the urethane (III). The product had the correct elemental composition and showed infrared bands at 1700 cm^{-1} for the urethane moiety, at 1660 cm^{-1} for the amide II band, and at 3330 cm^{-1}



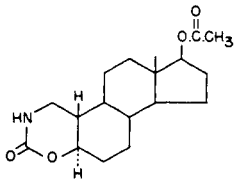
I

a. R_1 = HO; R_2 = CH_3CO; R_3 = H
b. R_1 = CH_3O; R_2 = CH_3COO; R_3 = NO_2
c. R_1 = CH_3O; R_2 = CH_3CO; R_3 = NO_2
d. R_1 = R_2 = HO; R_3 = NO_2
e. R_1 = HO; R_2 = CH_3COO; R_3 = NO_2
f. R_1 = H_2N; R_2 = CH_3COO; R_3 = NO_2
g. R_1 = H_2N; R_2 = HO; R_3 = NO_2
h. R_1 = CH_3NH; R_2 = HO; R_3 = NO_2
i. R_1 = HO; R_2 = R_3 = $COCH_3$
j. R_1 = H_2N; R_2 = R_3 = $COCH_3$
k. R_1 = HO; R_2 = CH_3CO; R_3 = NO_2
l. R_1 = H_2N; R_2 = CH_3CO; R_3 = NO_2

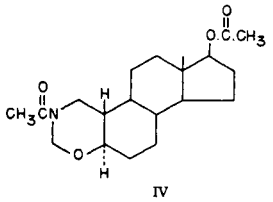


II

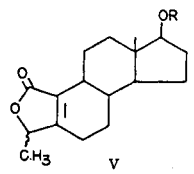
a. R = H
b. R = CH_3CO



III

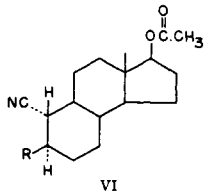


IV



V

a. R = $COCH_3$
b. R = NO_2



VI

a. R = CH_3CONH
b. R = CH_3NHCO

for the NH stretching frequency. Its n.m.r. spectrum (9) had singlets at 49.5 c.p.s. for the 18 methyl and at 122 c.p.s. for the 17 acetate. The C-5 proton was visible as a triplet at 174.5 c.p.s. (J = 10.5 c.p.s.), and the C-1 protons, as a multiplet centered at 208.5 c.p.s. equivalent to two protons. The signal for the nitrogen proton was found at 366 c.p.s. and vanished after an exchange with deuterium oxide.

When the amine (IIa) was condensed with paraformaldehyde, and the crude material acetylated, the oxazine (IV) was isolated. The product (IV) analyzed for $C_{20}H_{31}NO_4$ and exhibited the expected bands in the infrared. In an n.m.r. singlets were seen at 47.5, 121.5 and 128 c.p.s. for the methyls at C-18, 17-acetate and 3-acetamide, respectively. A broad signal at 187 c.p.s. was assigned to the C-5 proton. The rest of the spectrum was rather complex but a pair of doublets at 273.5 and 314.5 c.p.s. (J = 11 c.p.s.) was discernible. These doublets were tentatively assigned to the C-3 protons.

In exploring approaches to other 2-aza-4-oxa steroids the acid (Ie) was converted to its *N*-methyl amide (Ih). In this instance the C-5 acetate moiety was not retained, since the aqueous reagents used for the amide synthesis caused hydrolysis.

Having completed the synthesis of 2-aza-4-oxa analogs we turned our attention to exploring additional pathways to 1,5-diamino intermediates for the synthesis of 2,4-diaza steroids (6). It was hoped that the above described If could also be used for the preparation of a 1,5-diamino derivative. The projected route involved oxidation of the hydroxyl to a C-5 ketone and condensation with hydroxyl amine to a product which would be reduced to a diamine. For this purpose the acetate moiety of If was selectively hydrolyzed to yield hydroxyamide (Ig). However, attempts to oxidize the alcohol with various reagents, *e.g.*, pyridine-chromic oxide, acetic acid-chromic oxide, Jones' reagent, failed. The reaction either did not proceed at all, or the amide portion was hydrolyzed.

Since the oxidation of Ig proved fruitless, we investigated the introduction of the nitrogen at position four by Beckmann rearrangement of C-1 amides. The mixed anhydride method was employed for amide synthesis to minimize formation of enol lactones. Indeed, amide Ij was formed from acid (10) Ii quite easily, albeit in low yield. However, most of the starting acid (Ii) could be recovered and reused. A small amount of the expected A-*nor* lactone (Va) was also formed and was identified by comparison with an authentic sample (1). Apparently, the anhydrous conditions of the reaction were sufficient to form the angelica lactone analog (Va).

Since the yield of Ij was rather poor and formation of Va was not avoided, the synthesis of the C-1 amide *via* the acid chloride was investigated. In this instance the 17β-nitrate acid (Ik) was refluxed with oxalyl chloride, and the crude acid chloride was treated with ammonia to yield amide Il. The overall yield of amide (Il) was found to be better, even though the β-angelica lactone analog (Vb) was also formed.

With the 2-nitrogen potentially available we attempted the use of the Beckmann rearrangement for the insertion of nitrogen at position four. The amide (Ij) was condensed with hydroxylamine to give a mixture of *syn* and *anti* oximes (8). The crude oximes were rearranged with phosphorus oxychloride in pyridine. However, the rearrangement proceeded concommitant with the conversion of the amide to a nitrile. The major product, acetamide nitrile (VIa), was identified by its elemental analysis and infrared spectrum (a peak at 2240 cm^{-1} for a nitrile group was evident). The other product (VIb) also had an infrared nitrile band at 2240 cm^{-1}. Its structure was fully confirmed by an n.m.r. spectrum which had, among others, a doublet at 170 c.p.s. (J = 5.0 c.p.s.) for the *N*-methyl and a broad signal at 376 c.p.s. for the nitrogen proton. After exchanging the nitrogen proton for deuterium the 376 c.p.s. signal vanished and the doublet collapsed into a singlet.

EXPERIMENTAL (11)

5β-Hydroxy-1,5-seco-2,3,4-trisnorestran-17β-nitrate-1-oic Acid (Id).

The ester acetate (6b) (Ib) (370 mg.) was saponified in methanol (25 ml.) with 2*N* sodium hydroxide (10 ml.) by refluxing for 3 hours. The methanol was removed *in vacuo*, and the basic solution was acidified with 1*N* hydrochloric acid. The steroids were recovered by extraction with ethyl acetate to yield acid (Id) (315 mg.). Repeated recrystallizations from methanol-methylene chloride gave colorless crystals; m.p. 206-208°; ν max, 3270, 2610, 1700, 1615 cm^{-1}.

Anal. Calcd. for $C_{15}H_{23}NO_6$: C, 57.49; H, 7.40. Found: C, 57.44; H, 7.26.

5β-Acetoxy-1,5-seco-2,3,4-trisnorestran-17β-nitrate-1-oic Acid (Ie).

The acid (Id) (300 mg.) was acetylated as usual (acetic anhydride-pyridine) to yield the acetate (Ie) (300 mg.). The product was recrystallized from ethyl acetate-pentane to m.p. 195-199°; ν max, 3250, 1750, 1695, 1620 cm^{-1}.

Anal. Calcd. for $C_{17}H_{25}NO_7$: C, 57.45; H, 7.09. Found: C, 58.09; H, 7.10.

5β-Acetoxy-1,5-seco-2,3,4-trisnorestran-17β-nitrate-1-oic Amide (If).

A mixture of acetoxyacid (Ie) (100 mg.) and oxalyl chloride (3.0 ml.) was refluxed for 0.5 hours. The excess oxalyl chloride was removed *in vacuo*, and the product was dissolved in anhydrous methylene chloride (3.0 ml.). A stream of anhydrous ammonia was bubbled into the solution for 5 minutes then it was stored at room temperature for 3.5 hours. The mixture was diluted with ether and washed with 2*N* hydrochloric acid and water. Removal of the solvents and crystallization of the residue from acetone-pentane gave 70 mg. of amide (If); m.p. 176-178°; ν max, 3400, 3190, 1730, 1660, 1620 cm^{-1}.

Anal. Calcd. for $C_{17}H_{28}N_2O_6$: C, 57.61; H, 7.40; N, 7.91. Found: C, 57.59; H, 7.41; N, 7.79.

5β-Hydroxy-1,5-seco-2,3,4-trisnorestrane-17β-nitrate-1-oic Amide (Ig).

The acetoxy amide (If) (100 mg.) was heated at reflux in methanol (5 ml.) and 2*N* sodium hydroxide (0.5 ml.) for 0.75 hour. The reaction was diluted with water, and the product was collected by filtration. Repeated recrystallization of Ig from methanol-benzene gave fine

needles; m.p. 202-204°; ν max, 3400, 3160, 1650, 1620 cm^{-1}.

Anal. Calcd. for $C_{15}H_{24}N_2O_5$: C, 57.67; H, 7.74. Found: C, 57.51; H, 7.56.

17β-Acetoxy-5β-hydroxy-2-aza-2,5-seco-3,4-bisnor-estrane (IIb).

Lithium aluminum hydride (400 mg.) was added to a solution of amide (If) (200 mg.) in tetrahydrofuran (15 ml.). The mixture was refluxed for 41 hours, then cautiously decomposed with ice. The product was isolated with ether and washed with 2*N* sodium hydroxide and water. Removal of the solvents gave 190 mg. of amine (IIa) which resisted crystallization.

A portion of the reduced material (IIa) (100 mg.) was acetylated as usual to give IIb, which was recrystallized from ethyl acetate, m.p. 182-191°; ν max, 3330, 1725, 1635, 1545 cm^{-1}; n.m.r. 48.5 (18 methyl), 121.5 (17-acetate), 123 (acetamide), 360 c.p.s. (nitrogen proton).

Anal. Calcd. for $C_{19}H_{31}NO_4$: C, 67.62; H, 9.26. Found: C, 67.61; H, 9.19.

17β-Acetoxy-2-aza-4-oxa-3-oxo-5α-estrane (III).

To crude amine (IIa) (210 mg.) in chloroform (8.0 ml.) and anhydrous pyridine (3.0 ml.) was added benzene saturated with phosgene (3.0 ml.). The mixture was stored overnight at room temperature, then decomposed with ice. The steroids were taken up in ethyl acetate and washed with 2*N* hydrochloric acid, water, sodium bicarbonate, and water. The solvent was removed to give a residue (110 mg.), which was acetylated as usual. The acetate was chromatographed on a silica TLC plate (1-1 benzene-ethyl acetate) to give 55 mg. of the urethane (III).

Repeated recrystallization from ethyl acetate-pentane gave an analytical sample; m.p. 219-222°; ν max, 3330, 1730, 1700, 1660 cm^{-1}, n.m.r. 49.5 (18 methyl), 122 (17-acetate methyl), 174.5 (triplet; J = 10.5 c.p.s.; C-5 proton), 208.5 (multiplet; C-1 proton), 366 c.p.s. (nitrogen proton).

Anal. Calcd. for $C_{16}H_{27}NO_4$: C, 67.26; H, 8.47. Found: C, 66.89; H, 8.33.

17β-Acetoxy-2-acetyl-2-aza-4-oxa-5α-estrane (IV).

A solution of amine (IIa) (100 mg.) and paraformaldehyde (11 mg.) in benzene (10 ml.) was refluxed for 16 hours. The water formed during the reaction was collected in a Dean-Stark tube. Evaporation of the solvent gave a residue, which was acetylated as usual. Chromatography of the acetate on a silica TLC plate (1-1 benzene-ethyl acetate) yielded 55 mg. of the oxazine (IV). Recrystallization of the product from ethyl acetate-pentane gave colorless crystals; m.p. 144-147°; ν max, 1725, 1630 cm^{-1}; n.m.r. 47.5 (18 methyl), 121.5 (17-acetate methyl), 128 (3-acetamide methyl), 187 (broad; C-5 proton), 273.5 and 314.5 (pair of doublets; J = 11 c.p.s.).

Anal. Calcd. for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94; N, 4.01. Found: C, 69.16; H, 8.91; N, 4.18.

2-Aza-5β-hydroxy-3,5-seco-4-nor-1-oxoestrane-17β-nitrate (Ih).

The acid (Ie) (300 mg.) was converted into its acid chloride with thionyl chloride (3.0 ml.). After removal of the excess thionyl chloride *in vacuo*, the steroid was dissolved in dioxane (10 ml.) and aqueous methyl amine (30%; 15 ml.) was added. The reaction was stored for 6 hours, then diluted with water. The steroids were recovered in ethyl acetate and washed with 2*N* hydrochloric acid, water, sodium bicarbonate, and water. The dried solvents were evaporated, and the residue crystallized from acetone-pentane to give 250 mg. of *N*-methylamide (Ih). Repeated recrystallizations from the same solvent gave colorless needles; m.p. 160-164°; ν max, 3280, 3130, 1635, 1620, 1555 cm^{-1}.

Anal. Calcd. for $C_{16}H_{26}N_2O_5$: C, 58.88; H, 8.03; N, 8.58. Found: C, 58.86; H, 7.73; N, 8.82.

17β-Acetoxy-1,3-seco-4-oxo-2-nor-5α-estran-1-oic Amide (Ij).

To a solution of acid (Ii) (550 mg.) in anhydrous chloroform (10 ml.) and anhydrous pyridine (3.0 ml.) was added ethyl chloroformate (3.0 ml.). The mixture was stirred at room temperature overnight, then anhydrous ammonia was bubbled in for 30 minutes. After 16 hours the solution was diluted with ether and water. The organic layer was washed with 2*N* hydrochloric acid, water, sodium bicarbonate, and water. Removal of the solvent gave a residue which was chromatographed on silica plates (1-1 benzene-ethyl acetate). Elution of the least mobile zone gave 200 mg. of material, which was recrystallized from ethyl acetate-pentane to yield 120 mg. of the amide (Ij); m.p. 173-176°; ν max, 3440, 3310, 1735, 1700, 1680, 1615 cm^{-1}; n.m.r. 49.5 (18 methyl), 122 (17-acetate), 130 (C-5 acetyl), 331 c.p.s. (nitrogen protons).

Anal. Calcd. for $C_{19}H_{29}NO_4$: C, 68.03; H, 8.71. Found: C, 67.79; H, 8.57.

Starting material was recovered from the sodium bicarbonate washings after acidification.

17β-Acetoxy-3ξ-methyl-2-oxa-1-oxo-A-norestr-5(10)-ene (Va).

The most mobile zone from the above chromatography was eluted to yield 10 mg. of unsaturated lactone (Va) which was identical to a previously prepared (1) sample.

1,3-Seco-4-oxo-2-nor-5α-estrane-17β-nitrate-1-oic Acid (Ik).

The previously described (6b) ester (Ic) (300 mg.) was saponified with 2*N* sodium hydroxide (5 ml.) in methanol (20 ml.) as usual. The acid (Ik) (250 mg.) was recrystallized from ethyl acetate to give an analytical sample; m.p. 178-184° (dec.); ν max, 3200, 1735, 1675, 1620 cm^{-1}.

Anal. Calcd. for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.43; N, 4.13. Found: C, 60.12; H, 7.55; N, 3.99.

1,3-Seco-4-oxo-2-nor-5α-estrane-17β-nitrate-1-oic Amide (Il).

A mixture of acid (Ik) (290 mg.) and oxalyl chloride (3.0 ml.) was heated at reflux for 1 hour. After the oxalyl chloride had been removed *in vacuo*, an ammonia saturated dioxane solution (5 ml.) was added, and anhydrous ammonia was bubbled in for 20 minutes. The solution was stored at room temperature for 16 hours, then diluted with water. The steroids were dissolved in ethyl acetate, and the organic layer was washed with sodium bicarbonate and water. Removal of the dried solvent gave a residue, which was separated on a silica TLC plate (1-1; chloroform-ethyl acetate). The most mobile zone was eluted to yield 20 mg. of the lactone (Vb) (see below). The least mobile zone gave 120 mg. of the amide (Il).

Repeated recrystallization of Il from ethyl acetate-pentane gave an analytical sample, m.p. 157-160°; ν max, 3420, 3210, 1700, 1635, 1620 cm^{-1}.

Anal. Calcd. for $C_{17}H_{26}N_2O_5$: C, 60.34; H, 7.74. Found: C, 59.96; H, 7.74.

3ξ-Methyl-2-oxa-1-oxo-A-norestr-5(10)-ene-17β-nitrate (Vb).

The lactone (Vb) from the most mobile zone of the above TLC was recrystallized from ethyl acetate-pentane to m.p. 126-131°; ν max, 1740, 1660, 1620 cm^{-1}; λ max, 219 mμ (ε, 11,000).

Anal. Calcd. for $C_{17}H_{23}NO_5$: C, 63.53; H, 7.21. Found: C, 63.79; H, 7.23.

Beckmann Rearrangement of Amide (Ij).

A solution of hydroxylamine hydrochloride (100 mg.) and sodium acetate trihydrate (200 mg.) in water (1.0 ml.) was added to a solution of the amide (Ij) (120 mg.) in methanol (3.0 ml.). After 16 hours at room temperature the solution was diluted with water and the oxime was isolated by extraction with ether.

The total crude oxime was dissolved in anhydrous pyridine (1.5 ml.) and added to a mixture of phosphorous oxychloride (0.6 ml.) and pyridine (1.0 ml.) at 0°. The reaction was stirred at 0° for 4 hours, then cautiously decomposed with ice. The steroids were dissolved in ethyl acetate and washed with 2*N* hydrochloric acid and water. The residue (110 mg.) was separated on a silica TLC plate (1-1; ethyl acetate-chloroform). The least mobile zone was eluted to yield 90 mg. of the acetamide (VIa), which was recrystallized from ethyl acetate-pentane to m.p. 179-181°; ν max, 3300, 3070, 2240, 1735, 1650, 1560 cm^{-1}.

Anal. Calcd. for $C_{19}H_{28}N_2O_3$: C, 68.64; H, 8.49; N, 8.43. Found: C, 68.75; H, 8.39; N, 8.34.

The zone preceding the zone for VIa gave 7 mg. of the amide (VIb), m.p. sublimation above 241°; ν max, 3430, 2240, 1725, 1670, 1530 cm^{-1}; n.m.r. 49.5 (18 methyl), 122.5 (17-acetate), 170 (doublet; J = 5.0 c.p.s., *N*-methyl), 376 c.p.s. (nitrogen proton).

REFERENCES

(1) Part IX. D. M. Piatak and E. Caspi, *J. Org. Chem.*, 31, 4225 (1966).

(2) This work was supported by Public Health Service Grants A-5326 and CA-07137 and Career Program Award CA-K3-16614 from the National Cancer Institute.

(3) Present address: Department of Chemistry, Northern Illinois University, DeKalb, Illinois.

(4) E. Caspi, P. K. Grover, and D. M. Piatak, *Chem. and Ind.*, 1495 (1963).

(5) D. M. Piatak, R. I. Dorfman, D. Tibbetts, and E. Caspi, *J. Med. Chem.*, 7, 590 (1964).

(6a) E. Caspi and D. M. Piatak, *Experientia*, 19, 465 (1963); (b) D. M. Piatak and E. Caspi, *Steroids*, 3, 631 (1964).

(7) D. M. Piatak and E. Caspi, *Tetrahedron*, 22, 2823 (1966).

(8) D. M. Piatak and E. Caspi, *J. Org. Chem.*, in press.

(9) Some interesting observations on the n.m.r. spectra of urethanes, ureas, etc. have recently been made by A. J. Bloodworth and A. G. Davies, *J. Chem. Soc.*, (B), 125 (1966).

(10) D. M. Paitak and E. Caspi, *Chem. Commun.*, 501 (1966).

(11) All m.p.'s were taken on a micro hot stage and are corrected. Infrared spectra were taken of solids incorporated in potassium bromide blotters. N.m.r. spectra, on deuterio-chloroform solutions, unless otherwise noted, were recorded with a Varian Associates DP-DA-60 spectrometer. For thin layer chromatography (TLC) silica gel HF_{254} and alumina GF_{254} (Merck, A. G., Darmstadt) was used with the appropriately indicated developing solvents. All solvents in extractions were dried over anhydrous sodium sulfate before removal.

Received September 28, 1966 Shrewsbury, Massachusetts 01545