HETEROCYCLIC STEROIDS V. 2,4-DIAZA STEROIDS[1]

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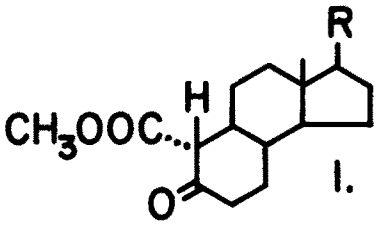
Abstract

A new route to methyl 17β-acetoxy-1,5-seco-2,3,4-trisnorestr-5-one-1-oate has been developed. This β-ketoester has been used for the synthesis of steroidal pyrimidines.

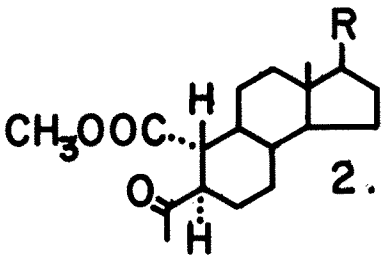
Since our publication of the first steroidal pyrimidine synthesis[1a], we have extended the project to include additional 2,4-diaza steroids. A novel, improved route to the key intermediate 1 has been evolved. Our approach for the preparation involved the acetyl esters 2, which can be synthesized from 1-hydroxy-4-methyl-1,3,5(10)-trienes in one operation[3] (40-60% yield). The removal of the acetyl function at C-5 in ester 2 and subsequent conversion to a C-5 ketone can be readily accomplished by (1) Baeyer-Villiger oxidation, (2) saponification, and (3) chromic acid oxidation. However, to avoid possible reactions involving the C-17 and C-20 ketones during the condensation with ureas[4], it was considered advisable to use a protecting group for the 17β- and 20β-hydroxyls. The nitro esters[5] appeared to fulfill the requirements, since they would withstand the projected reactions and can be removed under mild reductive conditions without affecting the expected product.

Treatment of the γ-ketoester with a mixture of fuming nitric acid and acetic anhydride easily provided the 17β-nitrate ester 2b. Oxidation



1.

a. R = ONO_2
b. R = OH
c. R = $OCOCH_3$
d. R = $CHONO_2(\beta)CH_3$
e. R = $CHOH(\beta)CH_3$

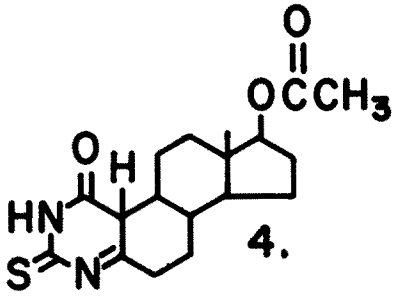


2.

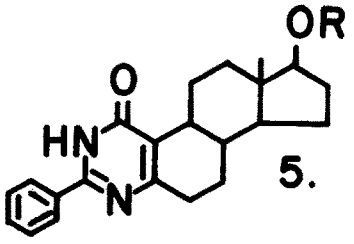
a. R = OH
b. R = ONO_2
c. R = $CHOH(\beta)CH_3$
d. R = $CHONO_2(\beta)CH_3$

3.

a. R_1 = CH_3CO; R_2 = ONO_2
b. R_1 = H; R_2 = ONO_2
c. R_1 = CH_3CO; R_2 = $CHONO_2(\beta)CH_3$



4.



5.

a. R = H
b. R = $COCH_3$

of 2b with trifluoroperacetic acid gave the 5β-acetate 3a, which was saponified, then treated with diazomethane, to yield the β-hydroxyester 3b. Chromic acid-acetic acid oxidation of 3b readily produced the β-ketoester 1a. Removal of the nitrate moiety with zinc-acetic acid[5] proceeded as expected to give methyl 17β-hydroxy-1,5-seco-2,3,4-trisnorestr-5-one-1-oate (1b). The latter upon acetylation yielded the 17β-acetate 1c which was identical to the previously described sample[1a, 3b]

Fusion of 1c with thiourea provided 17β-acetoxy-2,4-diazaestr-4-en-1-one-3-thione (4). The product analyzed for $C_{18}H_{24}N_2O_3S$ and absorbed ultraviolet light at 219 and 280 mμ in methanol. As expected, the maxima were essentially unaffected by the addition of acid to the solution (220 and 282 mμ), whereas the addition of base shifted the absorption to 264 and 315 mμ. These changes are consistent with those reported for simple pyrimidines[6] An infrared spectrum showed bands at 3100 (NH), 1730 (CH_3CO), 1670 (C=O), 1615 (-C=N-), and 1565 cm^{-1} (C=S), further corroborating the assigned structure. The C-10 hydrogen is assigned the β-configuration on the basis of molecular models, since an α-configuration would require a highly strained molecule. In view of the non-enolic character of the parent β-ketoester[7], a non-enolic mechanism of formation can easily be rationalized.

Base catalyzed condensation of 1c with benzamidine provided 2,4-diaza-17β-hydroxy-3-phenylestra-3,5(10)-dien-1-one (5a). The pyrimidosteroid had an elemental analysis consistent with structure 5a and absorbed ultraviolet light in methanol at 238 and 295 mμ. The ultraviolet absorption in acidified methanol gave characteristic shifts to 245 and 282 mμ. Use of aqueous methanolic sodium hydroxide increased the aromatic character of the ring as evidenced by a strong increase in

end absorption with a shoulder at 258 mμ and a peak at 286 mμ. An infrared spectrum further supported the structure by indicating absorption at 3380 (17β-hydroxy), 3050 (NH), 1625 (-NH-CO-C=C-), 1590, 1550, 1530, and 1490 cm^{-1} (aromatic -C=C- and conjugated diene). Treatment of 5a with acetic anhydride-pyridine gave the 17β-acetate 5b, whose spectral data were essentially the same as those of the parent compound 5a. The proposed homoannular 3,5(10)-diene structure is favored because of the NH absorption observed in the infrared and the UV absorption consistent with an extended conjugation. The alternate structure with conjugation between positions 2-3 and 4-5 is less probable in view of the spectroscopic evidence.

Since pyrimidines are capable of existing as various tautomers, the structures presented represent the most probable form as deduced from infrared spectroscopy in the solid state and ultraviolet absorption in neutral solutions. However, changes in ultraviolet absorption in acid and alkaline media do indicate that these compounds are in effect pyrimidines and can be expressed in conventional pyrimidine forms.

The same sequence of degradation reactions was performed on methyl 20β-hydroxy-4-methyl-1,4-seco-2,3,19-trisnor-pregnan-4-one-1-oate[3b] (2c). The nitrate ester (2d) was readily formed by fuming nitric acid-acetic anhydride. Baeyer-Villiger oxidation of 2d was accomplished readily, and the product 3c was saponified, methylated, and oxidized to yield the β-ketoester nitrate 1d. Removal of the nitro moiety from 1d proceeded smoothly to afford 1e, which had a rotary dispersion curve similar to 1a and 1b.

The extension of our work to include other steroidal pyrimidines is now in progress.

EXPERIMENTAL

M.p.'s are corrected. Infrared spectra were taken in the solid state in potassium bromide blotters. Extracts were dried over anhydrous sodium sulfate before distillation. Optical rotary dispersion spectra were taken at 26° with a Rudolph Recording Spectropolarimeter. Ultraviolet spectra were taken on about 100 μg of sample in 5 ml. of methanol. The spectra in acidic or alkaline medium were taken on solutions after the addition of either one drop of 1N HCl or one drop of 1N NaOH. Yields are based on crystalline material, whose infrared spectra were identical to those of the corresponding analytical samples.

Methyl 2,3-Bisnor-4-methyl-1,4-seco-5α-estr-4-one-17β-nitrate-1-oate (2b). To a stirred mixture of nitric acid (d=1.51; 1.0 ml.) and acetic anhydride (7.5 ml.) at -5 to -10° was added ester 2a (100 mg.). The reaction was stirred for 20 min., then decomposed by pouring onto ice. The product was collected by filtration, washed with water, and dried to give 100 mg. (87%). Recrystallization from methanol gave an analytical sample, m.p. 93-94°; ν_{max} 1730, 1700, 1615 cm^{-1}; R.D. in dioxane (C, 0.11) $[\phi]_{450}$ +296, $[\phi]_{400}$ +464, $[\phi]_{350}$ +925, $[\phi]_{300}$ +4010, $[\phi]_{298}$ +4035, $[\phi]_{285}$ +1820. Anal. Calcd. for $C_{18}H_{27}NO_6$: C, 61.17; H, 7.70: N, 3.96. Found: C, 61.30; H, 7.69; N, 4.08.

Methyl 5β-Acetoxy-1,5,seco-2,3,4-trisnor-5α-estrane-17β-nitrate-1-oate (3a). A solution of hydrogen peroxide (90%; 0.08 ml.) in trifluoroacetic anhydride (0.38 ml.) and anhydrous methylene chloride (2.5 ml.) was added over 15 min. to a solution of the nitrate ester 2b (100 mg.) in methylene chloride (2.5 ml.) containing dry disodium hydrogen phosphate (250 mg.). After the mixture had been stirred for 2.5 hr., it was decomposed with ice. The steroids were taken up in ether, washed with sodium bicarbonate and saline, and dried. Removal of the solvents gave 108 mg. (90%) of crystalline material. Recrystallization from methanol gave a sample which exhibited m.p. 168-170°; ν_{max} 1740, 1730, 1620 cm^{-1}. Anal. Calcd. for $C_{18}H_{27}NO_7$: C, 58.52; H, 7.37; N, 3.79. Found: C, 58.61; H, 7.46; N, 4.14.

Methyl 5β-Hydroxy-1,5-seco-2,3,4-trisnor-5α-estrane-17β-nitrate-1-oate (3b). A sample of 3a (100 mg.) was saponified in methanol (10.0 ml.) with a sodium hydroxide solution (2N; 3.0 ml.) by refluxing for 2 hr. Dilution of the solution and acidification with 2N hydrochloric acid gave an acid which was recovered by extraction with ether. Removal of the ether by distillation gave crude acid which was methylated in methanol by the addition of ethereal diazomethane. Evaporation of the solvents yielded 100% of crystalline hydroxyester. The product was recrystallized to m.p. 108-110° (from ethyl acetate-pentane), ν_{max} 3540, 1720, 1620 cm^{-1}. Anal. Calcd. for $C_{16}H_{25}NO_6$: C, 58.70; H, 7.70. Found: C, 58.57; H, 7.94.

Methyl 1,5-Seco-2,3,4-trisnorestr-5-one-17β-nitrate-1-oate (1a). A solution of chromium trioxide (2.0 g.) in acetic acid (90%; 50 ml.) was added over 30 min. to a solution of 3b (0.97 g.) in acetic acid (70 ml.) at 10°. The reaction was stirred at room temp. for 3.5 hr., then terminated with methanol. After dilution of the mixture with

water, the steroids were dissolved in ether and washed with sodium bicarbonate and water. Removal of the solvent yielded 1a (805 mg.; 83.5%). Recrystallization of a portion from methanol gave an analytical sample melting at 105-107°; ν_{max} 1740, 1710, 1620 cm^{-1}; R.D. in dioxane (c, 0.03). $[\phi]_{450}$ +405, $[\phi]_{400}$ +359, $[\phi]_{350}$ +270, $[\phi]_{325}$ -79.5, $[\phi]_{307.5}$ -1730, $[\phi]_{299-303}$ -1380, $[\phi]_{275}$ +2470. Anal. Calcd. for $C_{16}H_{23}NO_6$: C, 59.06; H, 7.13. Found: C, 59.23; H, 6.87.

Methyl 17β-Hydroxy-1,5-seco-2,3,4-trisnorestr-5-one-1-oate (1b). A sample of 1a (770 mg.) was dissolved in glacial acetic acid (80 ml.) and cooled to 10-20°. Zinc dust (4.0 g.) was then added portionwise with stirring over 15 min. Stirring was continued for 45 min. The reaction was diluted with chloroform, and the excess zinc dust was removed by filtration through Celite. The filtrate was diluted with ether, washed with sodium bicarbonate and water, and dried over sodium sulfate. Removal of the solvents in vacuo gave a sample (400 mg.; 60.5%) which was recrystallized from ethyl acetate-pentane to m.p. 155-157°, ν_{max} 3540, 1735, 1710 cm^{-1}; R.D. in dioxane (c, 0.09) $[\phi]_{450}$ -43, $[\phi]_{400}$ -125, $[\phi]_{350}$ -451, $[\phi]_{325}$ -1050, $[\phi]_{307}$ -2760, $[\phi]_{301.5}$ -2525 (infl.), $[\phi]_{285}$ +512. Anal. Calcd. for $C_{16}H_{24}O_4$: C, 68.54; H, 8.63. Found: C, 68.44; H, 8.65.

Acetylation of a portion of 1b gave crystalline 1c, which was identical by a mixture m.p. and comparison of infrared spectra to a sample obtained by an alternate route[1a, 3b].

17β-Acetoxy-2,4-diazaestr-4-en-1-one-3-thione (4). A ground mixture of the β-ketoester 1c (100 mg.) and thiurea (60 mg.) was fused at 180° for 30 min. The mixture first became a viscous liquid, then resolidified. The solids were dissolved in ether-chloroform (3:1), washed with water, and dried. Evaporation of the solvents gave a semi-solid residue, which was submitted to thin layer chromatography (silica gel HF_{254}; 50% ethyl acetate-chloroform). The ultraviolet absorbing zone was recovered and the steroid isolated; yield, 33 mg. Recrystallization from chloroform-ethanol gave colorless crystals; m.p. above 300°; λ_{max} (in MeOH) 219 mμ (ϵ12000), 280 mμ (ϵ21000); λ_{max} (in HCl-MeOH) 220 mμ (ϵ13400), 282 mμ (ϵ21600); λ_{max} (in NaOH-MeOH) 264 mμ (ϵ14300), 315 mμ (ϵ12900); ν_{max} 3100, 1730, 1670, 1615, 1565, 1235 cm^{-1}. Anal. Calcd. for $C_{18}H_{24}N_2O_3S$: C, 62.06; H, 6.94. Found: C, 62.18; H, 6.85. Positive sulphur test.

17β-Hydroxy- and 17β-Acetoxy-2,4-diaza-3-phenyl-estra-3,5(10)-dien-1-one (5a and 5b). To a mixture of β-ketoester 1c (100 mg.) and benzamidine hydrochloride (80 mg.) in absolute ethanol (8.0 ml.) was added sodium methoxide (80 mg.). After the mixture had been refluxed for 14 hr., it was cooled and diluted with water. The steroids were isolated from the basic solution by extraction with ether. Removal of the solvents gave 44 mg. of a solid residue, which recrystallized from chloroform-ethanol as colorless needles; m.p. 270-277° (dec.); λ_{max} (in MeOH) 239 mμ (ϵ12500), 295 mμ (ϵ10400); λ_{max} (in HCl-MeOH) 245 mμ (ϵ16600), 282 mμ (ϵ12800); λ_{max} (in NaOH-MeOH) shoulder 258 mμ (ϵ10000), 286 mμ (ϵ8800); ν_{max} 3380, 3050, 1625, 1600, 1590, 1550, 1530, 1490 cm^{-1}. Anal. Calcd. for $C_{22}H_{26}N_2O_2$: C, 75.40; H, 7.48; N, 7.99. Found[8]: C, 74.10, 73.98; H, 7.81, 7.50; N, 7.33.

Acetylation of the 2,4-diaza-3-phenyl steroid 5a with acetic anhydride-pyridine in the usual manner provided the corresponding 17β-acetate 5b. A sample was crystallized to m.p. 270-273 (from methylene chloride-ethyl acetate); λ_{max} (in MeOH) 240 mμ (ε13100); 295 mμ (ε10900); ν_{max} 3060, 1730, 1630, 1590, 1585, 1550, 1530, 1490 cm^{-1}. Anal. Calcd. for $C_{24}H_{28}N_2O_3$: C, 73.44; H, 7.19. Found: C, 73.54; H, 7.37.

Methyl 4-Methyl-1,4-seco-2,3,19-trisnor-5α-pregnane-4-one-20β-nitrate-oate (2d). A sample of the γ-ketoester 2c (100 mg.) was added to a cooled (-5 to -10°), stirred solution of fuming nitric acid (d=1.51; 1.0 ml.) in acetic anhydride (7.5 ml.). The reaction was worked up as described for 2b to give a syrup, which was chromatographed on thin layer (with 10% ethyl acetate-chloroform). After elution of the product (90 mg.; 80%) from the silica, it was crystallized from methanol to m.p. 135-137°; ν_{max} 1730, 1700, 1620 cm^{-1}. Anal. Calcd. for $C_{20}H_{31}NO_6$: C, 62.97; H, 8.19. Found: C, 62.98; H, 7.92.

Methyl 5β-Acetoxy-1,5-seco-2,3,4,19-tetranor-5α-pregnane-20β-nitrate-1-oate (3c). A solution of 2d (1.60 g.) in dry methylene chloride (40 ml.) containing anhydrous disodium hydrogen phosphate (4.0 g.) was stirred and cooled (ice bath). Over 15 min. a solution of hydrogen peroxide (90%; 1.3 ml.) and trifluoroacetic anhydride (6.1 ml.) in dry methylene chloride (40 ml.) was added. The reaction was terminated after 3 hr. and processed as described for 3a to yield 1.65 g. (98%) of crystals.

A portion was recrystallized from methanol to yield colorless crystals; m.p. 127-129°; ν_{max} 1730, 1620 cm^{-1}. Anal. Calcd. for $C_{20}H_{31}NO_7$: C, 60.44; H, 7.86. Found: C, 60.84; H, 7.76.

Methyl 1,5-Seco-2,3,4,19-tetranorpregnane-5-one-20β-nitrate-1-oate (1d). A solution of 3c (1.40 g.) in 2N sodium hydroxide (40 ml.) and methanol (50 ml.) was refluxed for 2 hr. The solution was diluted with water and acidified with 2N hydrochloric acid. After the steroids had been isolated with ether, they were esterified with ethereal diazomethane.

The crude methylated product was dissolved in glacial acetic acid (20 ml.) and oxidized with chromium trioxide (1.60 g.) in 90% acetic acid (50 ml.) for 3 hr. The reaction was terminated with methanol, and the steroids were obtained as described for 1a to yield 800 mg. (64%) of crystals.

Recrystallization of a portion from methanol gave a pure sample; m.p. 156-158°; ν_{max} 1740, 1705, 1620 cm^{-1}. Anal. Calcd. for $C_{18}H_{27}NO_6$: C, 61.17; H, 7.70; N, 3.96. Found: C, 61.15; H, 7.50; N, 4.04.

Methyl 20β-Hydroxy-1,5-seco-2,3,4,19-tetranorpregnane-5-one-1-oate (1c). Zinc dust (800 mg.) was added portionwise over 15 min. to a solution of 1d (160 mg.) in glacial acetic acid (16 ml.) at 10-20°. The reaction was then carried out as described for 1b resulting in 104 mg. (74%). Recrystallization from ethyl acetate-pentane gave a sample

melting at 96-98°; ν_{max} 3560, 1735, 1710; R.D. in dioxane (c, 1.07) $[\phi]_{450}$ - 68.5, $[\phi]_{400}$ - 180, $[\phi]_{350}$ - 150, $[\phi]_{325}$ - 1135, $[\phi]_{307.5}$ -3020, $[\phi]_{301}$ - 2710 (infl.), $[\phi]_{300}$ - 2705, $[\phi]_{285}$ + 487. Anal. Calcd. for $C_{18}H_{28}O_4$: C, 70.10; H, 9.15. Found: C, 70.15; H, 9.04.

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