Preparation of 20-(Hydroxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnan-3-one as an Inhibitor of Testosterone 5α-Reductase

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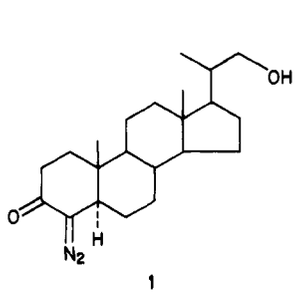
20-(Hydroxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnan-3-one and the corresponding 3-thione were synthesized with use of 20-(hydroxymethyl)-4-methyl-4-aza-5α-pregnan-3-one as the starting material. The compounds were tested in vitro for inhibition of testosterone 5α-reductase and found to be weak inhibitors with K_i's in the 10^{-7} range.

We have found from previous work in our laboratories on testosterone 5α-reductase inhibitors that diazo ketone **1** is a potent, time-dependent inhibitor of this enzyme and that the bisnorcholane side chain is compatible with high enzyme affinity.[1,2] Amide **2** is the most potent inhibitor of testosterone 5α-reductase reported in the literature,[3] but it is short acting.[4] Replacement of carbon 2 in the steroid nucleus by oxygen is not expected to cause steric perturbations. This simple transformation in the case of a 4-aza 3-one steroid would convert it to an urethane from a lactam, thereby enhancing the polarity of the C-3 carbonyl and perhaps its affinity for the enzyme active site. Substitution of a 2-oxa for a methylene group is not without precedent as several 2-oxa steroids are about equivalent in their biological properties to their 2-carba analogues.[5] We hoped the activity of amide **2** would be enhanced by incorporation of the bisnorcholane side chain and a 2-oxa function as in **3** and report here the results of this study.



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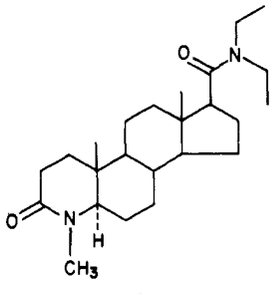

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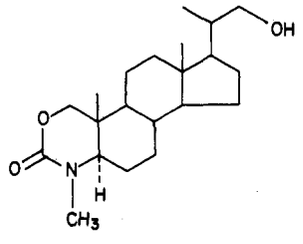

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Results and Discussion

The synthetic sequence started from lactam **4** (Scheme I).[6] Direct phenylsulfenation was not successful on **4** because its derived dianion was too insoluble. Therefore, phenylsulfenation was carried out on the silyl ether **5**. Oxidation of the sulfide **6** gave the sulfoxide, which was not isolated but converted by heating in toluene to the olefin **8**. Ozonolysis of **8** followed by borohydride reduction of the intermediate gave the diol **9**, which was saponified to the amino alcohol **10**. Cyclization of **10** wtih carbonyldiimidazole gave the title compound **3**, which was acetylated to give **11**.

Reaction of amino alcohol **10** with thiocarbonyldiimidazole gave the thiocarbonyl analogue **12**, which was similarly acetylated to **13**.

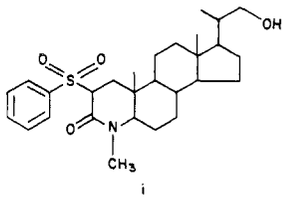
The K_i's for **2–4** and **12** were determined by using a Dixon plot and are listed in Table I. The K_i's for compounds **11** and **13** were not determined as their activity was too low. It can readily be seen that **3** and **12** are 30–100 times less active than are **2** and **4**. These results were not encouraging enough to warrant continued study.

Biochemical Assay

Rat prostate microsomes, prepared as previously described,[2b] were used as the source of 5α-reductase. 5α-Reductase assays were conducted in 0.05 M phosphate buffer, pH 6.6, containing 0.1% bovine serum albumin; NADPH, 10^{-3} M; glucose 6-phosphate, 5×10^{-3} M; glucose-6-phosphate dehydrogenase, 0.5 IU/mL. Inhibitors were added in dimethyl sulfoxide (Me_2SO) (final Me_2SO concentration <5%); control incubates received Me_2SO only. Assays typically contained 0.6 mg of microsomal protein in a total volume of 200 mL. Extraction, chromatographic separation, and radioassay of 5α-reduced products of the [4-^{14}C]testosterone or [2,3-^{3}H]testosterone substrate have been described previously.[8]

For determination of inhibitor constants, two substrate concentrations were used: 3×10^{-7} and 1×10^{-6} M testosterone. Four to six inhibitor concentrations were used

(1) Benson, H. D.; Blohm, T. R. U.S. Patent 4 088 760, 1978.
(2) (a) Metcalf, B. W.; Jund, K.; Burkhart, J. P. *Tetrahedron Lett.* **1980**, *21*, 15. (b) Blohm, T. R.; Metcalf, B. W.; Laughlin, M. E.; Sjoerdsma, A.; Schatzman, G. L. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 273.
(3) Liang, T.; Heiss, C. E. *J. Biol. Chem.* **1981**, *256*, 7998.
(4) Brooks, J. R.; Berman, D.; Glitzer, M. S.; Gordon, L. R.; Primka, R. L.; Reynolds, G. F.; Rasmusson, S. H. *Prostate* **1982**, *3*, 35.
(5) Pappo, R. *Intra-Sci. Chem. Rep.* **1969**, *3*, 105 and references cited therein.
(6) Subsequent to the start of this work, a patent for 4 was issued: Rasmusson, G. H.; Johnston, G. B. R. U.S. Patent 4 377 584, 1983.
(7) Compound i [mp 216–218 °C; ^{1}H NMR δ 0.69 (3 H, s, C_{18}-CH_3), 0.82 (3 H, s, C_{19}-CH_3), 1.04 (d, C_{21}-CH_3), 2.84 (3 H, s, N-CH_3), 3.16 (d, $C_{5\alpha}$-H), 3.28 + 3.58 (pr q, C_{22}-CH_2), 4.03 + 4.16 (1 H, pr d, C_2-CH), 7.34–7.77 (3 H, m, arom), 7.78–7.97 (2 H, m, arom). Anal. ($C_{28}H_{41}NO_4S$) C, H, N, S] was isolated in one experiment and compound ii, although not isolated, was identified in a column fraction.



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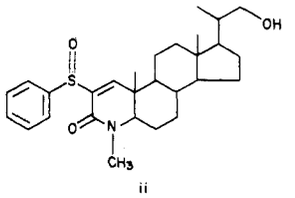

ii

(8) Blohm, T. R.; Laughlin, M. *J. Steroid Biochem.* **1978**, *9*, 603.

0022-2623/85/1828-0831$01.50/0

Scheme I

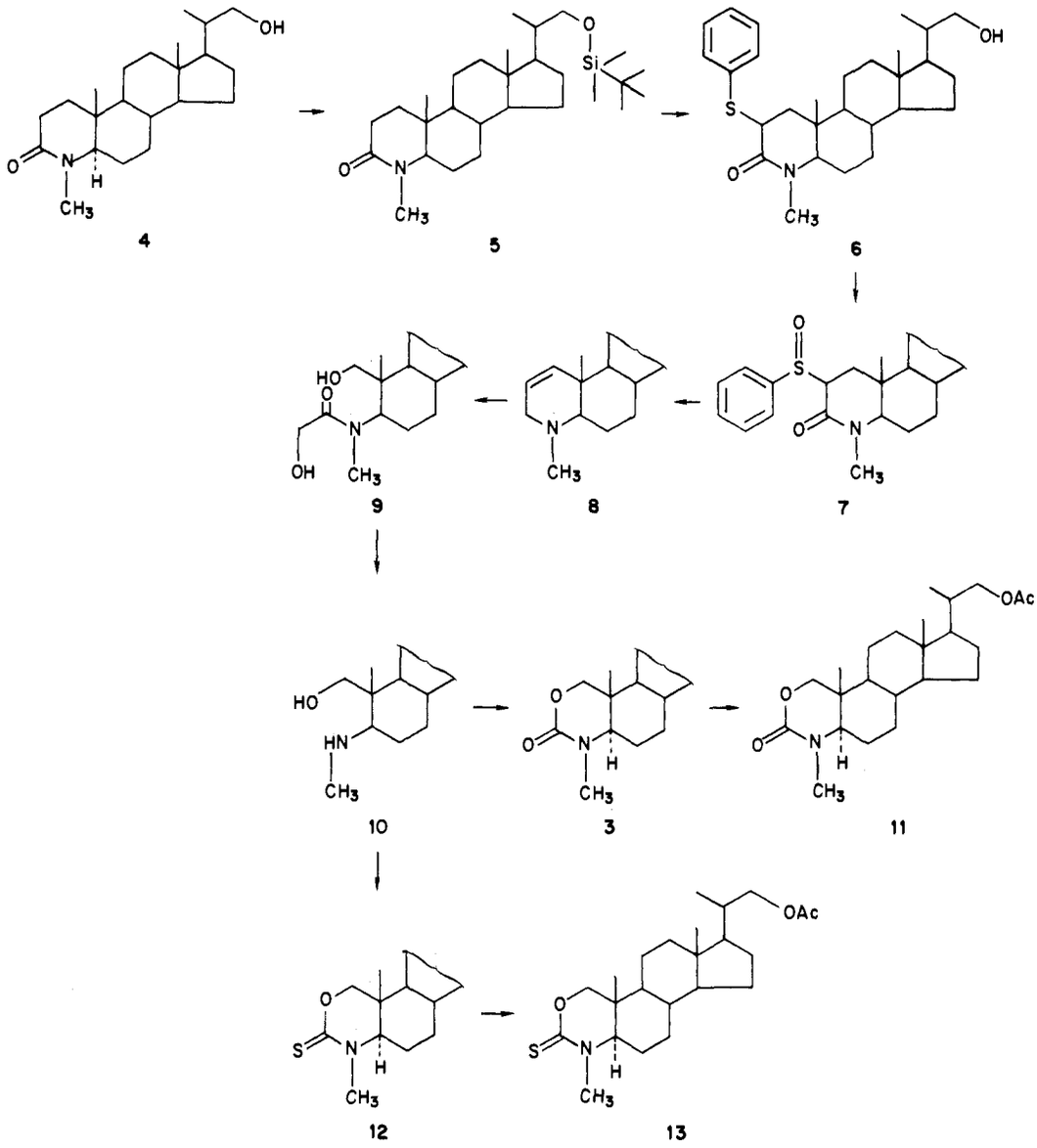

Table I. In Vitro Biological Activity[a]

compd	K_i (× 10^{-8} M)[b]	rel act.[c]	compd	K_i (× 10^{-8} M)[b]	rel act.[c]
2	0.73	1/2	4	0.38	1
3	12	1/32	**12**	40	1/105

[a] In our hands testosterone has a K_m of 6×10^{-7}. [b] [S] = 3×10^{-7} M. [c] Comparative activity setting the K_i of 4 at 1.

for each substrate concentration; K_i's were obtained from Dixon plots.[9]

Experimental Section

All melting points were determined in open capillary tubes on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (potassium bromide) were recorded on a Perkin-Elmer 521 grating spectrophotometer. Nuclear magnetic resonance spectra ($CDCl_3$ with tetramethylsilane as internal standard) were run on a Varian FT-80A spectrometer. The standard drying agent was magnesium sulfate, and solvents were removed in vacuo on a rotary evaporator.

20-[[[Dimethyl(1,1-dimethylethyl)silyl]oxy]methyl]-4-methyl-4-aza-5α-pregnan-3-one (5). A solution of the alcohol **4** (3.6 g, 10.4 mmol), imidazole (0.87 g, 12.4 mmol), *tert*-butyldimethylchlorosilane (1.87 g, 12.4 mmol), and dimethylformamide (30 mL) was heated at 55 °C under an argon atmosphere for 18 h. The cooled solution was diluted with water (240 mL) and the resulting precipitate collected by filtration, washed with water, and air-dried. Purification was achieved by flash chromatography to give **5** (3.8 g, 79.5%). An analytical sample obtained from acetone had the following: mp 141–144 °C; IR ν 1620 cm^{-1}; ^1H NMR δ 0.03 (6 H, s, 2 $SiCH_3$), 0.67 (3 H, s, C_{18}-CH_3), 0.87 (s, 3 CCH_3), 2.38 (2 H, q, C_2-CH_2), 2.84 (s, NCH_3), 3.22 + 3.50 (pr q, C_{22}-CH_2). Anal. ($C_{28}H_{51}NO_2Si$) C, H, N.

20-(Hydroxymethyl)-4-methyl-2-(phenylthio)-4-aza-5α-pregnan-3-one (6). A solution of diisopropylamine (9.57 mL, 68.4 mmol) in tetrahydrofuran (200 mL), cooled in a dry ice–acetone bath, was treated dropwise with 1.6 M butyllithium in hexane (43.3 mL, 68.4 mmol). To this stirred solution was added the amide **5** (7.90 g, 17.1 mmol) in tetrahydrofuran (50 mL) and the temperature was raised to 0 °C for 1 h. After the solution was cooled to −78 °C, a solution of diphenyl disulfide (4.11 g, 18.8 mmol) in tetrahydrofuran (45 mL) was added. The solution was warmed slowly to room temperature and stirred overnight. The reaction mixture was poured into saturated aqueous ammonium chloride and extracted with ether (500 mL). The organic layer was dried and concentrated to a yellow liquid, which was filtered through silica gel with ethyl acetate–50% hexane to remove some nonpolar material (silanes) and starting material.

The partially purified material was dissolved in methanol (300 mL) and stirred with 10% HCl (50 mL) for 2 h. The methanol

(9) Dixon, M. *Biochem. J.* **1953**, *55*, 170.

was removed and the residue dissolved in CH_2Cl_2 (1 L) and extracted with water (200 mL), dried, and concentrated. The resulting material was purified by flash chromatography to give **6** (5.9 g, 75.4%): mp 170–171 °C; IR ν 3480, 1630 cm^{-1}; ^1H NMR δ 0.66 (3 H, s, C_{18}-CH_3), 0.88 (s, C_{19}-CH_3), 1.02 (d, C_{21}-CH_3), 2.90 + 3.02 (s + 1/2 d, NCH_3 + $C_{5\alpha}$-H), 3.27 + 3.57 (pr q, C_{22}-CH_2), 3.72 + 3.84 (pr d, C_2-CH), 7.12–7.56 (5 H, m, arom). Anal. ($C_{28}H_{41}NO_2S$) C, H, N, S.

20-(Hydroxymethyl)-4-methyl-4-aza-5α-pregn-1-en-3-one (8). A solution of the sulfide **6** (0.8 g, 1.7 mmol) in methylene chloride (50 mL) was treated with 85% *m*-chloroperoxybenzoic acid (0.38 g, 1.1 equiv) and the mixture stirred at room temperature for 2.5 h. The solution was extracted with saturated aqueous sodium bicarbonate (50 mL), dried and concentrated to a solid foam, which was dissolved in toluene (50 mL) and heated at reflux for 2.5 h. Removal of the solvent gave crude **8**, which was purified by flash chromatography to give **8** (0.36 g, 60%) (7): mp 184–186 °C; IR ν 3430, 1650, 1590 cm^{-1}; ^1H NMR δ 0.71 (3 H, s, C_{18}-CH_3), 0.91 (3 H, s, C_{19}-CH_3), 1.03 (d, C_{21}-CH_3), 2.91 (3 H, s, NCH_3), 3.28 + 3.56 (m, C_{22}-CH_2), 5.77 (1 H, d, C_2-H), 6.61 (1 H, d, C_1-H). Anal. ($C_{22}H_{35}NO_2$) C, H, N.

20-(Hydroxymethyl)-4-methyl-4-aza-*A*-dinor-1,3-seco-pregnan-1-ol (10). A solution of the enamide **8** (5.5 g, 15.9 mmol) in methylene chloride (85 mL)–methanol (67 mL) was cooled to −78 °C and saturated with ozone. After the solution was purged with nitrogen and warmed to room temperature, the solvents were removed. The residue was dissolved in 95% ethanol (85 mL)–methylene chloride (10 mL) and treated carefully with sodium borohydride (1.53 g, in portions). After 3 h, the excess hydride was decomposed with 10% aqueous HCl. The mixture was diluted with water (2 L), made basic with concentrated NH_4OH, and extracted with ethyl acetate (1 × 500 mL + 2 × 300 mL). The combined extract was washed with saturated brine, dried, and concentrated to a solid foam.

The foam was treated with methanol (450 mL), water (50 mL), and potassium hydroxide (4.0 g) and the mixture heated at reflux for 4 h. Most of the methanol was removed and the residue diluted with water (ca. 1 L). The resulting solid was filtered off, washed with water, and dried to give **10** (4.4 g, 85.4%), which can be used directly in the next step.

The product can be crystallized from chloroform to give **10** as a white solid: mp 202–204 °C; IR ν 3370, 3270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (3 H, s, C_{18}-CH_3), 0.97 (s, C_{19}-CH_3), 1.01 (d, C_{21}-CH_3), 2.38 (3 H, s, N-CH_3), 3.28 + 3.56 (pr q, C_{22}-CH_2), 3.41 + 3.69 (pr d, C_1-CH_2). Anal. ($C_{20}H_{37}NO_2$) C, H, N; C: calcd, 74.25; found, 73.65.

20-(Hydroxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnan-3-one (3). A solution of the amino alcohol **10** (1.62 g, 5 mmol) in tetrahydrofuran (75 mL) was treated with carbonyldiimidazole (0.81 g, 5.0 mmol) and the mixture stirred overnight at room temperature. The solvent was removed and the residue was dissolved in methylene chloride, extracted with 10% aqueous HCl (100 mL), dried, and concentrated to a white solid, which was purified by flash chromatography to give **3** (1.1 g). Recrystallization from acetone gave pure **3** (0.83 g, 47.4%): mp 239–241 °C; mass spectrum, *m/e* 249 (parent, 10%), 57 (base peak); IR ν 3440, 1685 cm^{-1}; ^1H NMR δ 0.69 (3 H, s, C_{18}-CH_3), 1.02 (d, C_{21}-CH_3), 1.02 (s, C_{19}-CH_3), 2.90 (3 H, s, NCH_3), 3.25 + 3.58 (2 H, pr q, C_{22}-CH_2). 3.81 + 4.03 (2 H, pr d, C_1-CH_2). Anal. ($C_{21}H_{35}NO_3$) C, H, N.

20-(Acetoxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnan-3-one (11). The alcohol **3** (0.42 g, 1.2 mmol) was dissolved in acetic anhydride (3 mL) and pyridine (6 mL). After 24 h, water (25 mL) was added and the mixture stirred for 2 h. The mixture was diluted with more water (55 mL) and the solid removed by filtration. The solid was purified by flash chromatography to give **3** (0.37 g, 78%) after crystallization from aqueous acetone: mp 162–163 °C; IR ν 1735, 1695, 1230 cm^{-1}; ^1H NMR δ 0.69 (3 H, s, C_{18}-CH_3), 0.97 (d, C_{21}-CH_3), 1.01 (s, C_{19}-CH_3), 2.01 (s, C(=O)CH_3), 2.82 (3 H, s, NCH_3), 3.06 (1 H, q, $C_{5\alpha}$-H), 3.22–4.13 (4 H, m, CH_2O + CH_2O). Anal. ($C_{23}H_{37}NO_4$) C, H, N.

20-(Hydroxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnane-3-thione (12). A solution of the amino alcohol **10** (2.13 g, 6.68 mmol) in tetrahydrofuran (100 mL) was reacted with thiocarbonyldiimidazole (1.32 g, 6.68 mmol) and worked up as above for **3** to give **12** (0.99 g, 40%): mp 249–251 °C; IR ν 3390, 1490, 1260 cm^{-1}; ^1H NMR δ 0.69 (3 H, s, C_{18}-CH_3), 0.99 (s, C_{19}-CH_3), 1.03 (d, C_{21}-CH_3), 2.97–3.72 + 3.41 (6 H, m + s, C_{22}-CH_2 + $C_{5\alpha}$-H + NCH_3), 3.88 + 4.16 (2 H, pr d, C_2-CH_2). Anal. ($C_{21}H_{35}NO_2S$) H, N; C, S: calcd, 68.99; found, 68.39; calcd, 8.77; found, 8.32.

20-(Acetoxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnane-3-thione (13). The alcohol **12** (0.9 g, 2.46 mmol) was acetylated as above to give **13** (0.32 g, 32%): mp 184–186 °C; IR ν 1730, 1240 cm^{-1}; ^1H NMR δ 0.68 (3 H, s, C_{18}-CH_3), 0.97 (d, C_{21}-CH_3), 1.00 (s, C_{19}-CH_3), 2.01 (s, C(=O)CH_3), 3.12 + 3.23 (1 H, pr d, $C_{5\alpha}$-H), 3.41 (3 H, s, NCH_3), 3.53–4.25 (4 H, m, CH_2O + CH_2O). Anal. ($C_{23}H_{37}NO_3S$) C, H, N, S.

Registry No. **3**, 96000-11-2; **4**, 92542-38-6; **5**, 96000-12-3; **6**, 96000-13-4; **7**, 96000-14-5; **8**, 96000-15-6; **9**, 96000-16-7; **10**, 96000-17-8; **11**, 96000-18-9; **12**, 96000-19-0; **13**, 96000-20-3; **i**, 96021-28-2; **ii**, 96021-29-3; diphenyl disulfide, 882-33-7; carbonyldiimidazole, 530-62-1; thiocarbonyldiimidazole, 6160-65-2; testosterone 5α-reductase, 9036-43-5.