Synthesis and biological activity of 16β-morpholinosteroid derivatives

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Fusion of morpholine at the 16 position of the steroid nucleus has been carried out to prepare monoquaternary 6, 7 and 8 and bisquaternary ammonium compounds 13 and 14. Compounds 13 and 14 partly resemble chandonium iodide in structure. All the compounds have been evaluated for their neuromuscular blocking and ganglion blocking activities. The compounds 3, 4, 5, 11 and 12 have been screened for antineoplastic activity at NCI, Bethesda.

The principal use of neuromuscular blocking agents is to provide muscle relaxation to obviate the need for higher doses of volatile anaesthetic agents and making anaesthesia safer and pleasant. Clinically used suc-amethonium chloride, a depolarising neuromuscular blocking agent with transient duration of action, possess side effects believed to be inherent in its mode of action.

Nondepolarising neuromuscular blocking agents containing benzylisoquinolinium and steroid skeleton with rapid onset, short duration of action and highly specific for the neuromuscular junction have been developed. The discovery of pancuronium bromide resulted in the development of monoquaternary diaminosteroid drugs. Chandonium iodide (Candocuronium) is another bisquaternary heterosteroid. It has interonium distance of 1.029 nm, a powerful nondepolarising blocking activity with rapid onset and short duration of action. Structure-activity relationship has suggested that interonium distance plays a significant role to compete with acetylcholine at the neuromuscular junction. In earlier publications, many modifications of chandonium iodide have been reported. We report herein the synthesis and pharmacology of mono- and bisquaternary aminosteroids, partly resembling chandonium iodide. The interonium distance between two quaternary heads in compound 14 is 1.135 nm as measured by Drieding models.

17-Oxo-5-androsten-3β-ol 1 was brominated using cupric bromide in dry methanol to obtain 16α-bromo-17-oxo-5-androsten-3β-ol 2. Condensation of the compound 2 with dry morpholine was carried out at boiling temperature to obtain 16β-morpholinono-17-oxo-5-androsten-3β-ol 3 (Scheme 1). In the NMR spectrum compound 3 showed a multiplet centered at δ 3.05 (1H, 16α-H). The assignment of the configuration at 16-position in the compound 3 was done as previously reported. Sodium borohydride reduction of 3 in methanol at room temperature yielded 3β, 17β-diol 4. Its NMR spectrum showed signals at δ 2.82 (1H, q, J=9Hz, 16α-H), 3.45 (1H, d, J=9Hz, 17α-H) and 3.51 (1H, m, 3α-H). The acetylation of 4 was effected with acetic anhydride in pyridine at 100°C to obtain the diacetate 5. Its proton resonance signals appeared at δ 2.03 (3H, s, 3β-OCOCH3), 2.10 (3H, s, 17β-OCOCH3), 3.07 (1H, q, J=9Hz,16α-H), 4.58 (1H, m, 3α-H) and 4.80 (1H, d, J=9Hz,17α-H). IR bands at 1732 and 1239 cm⁻¹ indicated the acetoxy function. Quaternisation of the amines 3 and 4 with methyl iodide in ethanol yielded monoquaternary compounds 6 and 7. 16β-Morpholinono-5-androstene-3β, 17β-diol diacetate methiodide 8 was prepared from 16β-morpholinono-5-androstene-3β, 17β-diol diacetate 5 in dichloromethane containing methyl iodide at room temperature. The monoquaternary compound showed vibrational C=O stretching at 1730 cm⁻¹ in the IR spectrum.

Oppenauer oxidation of 3 using a cyclohexanone-toluene system afforded 16β-morpholinono-4-
androstene-3,17-dione 9 (UV maximum at 238.6 nm). Compound 9 was refluxed with freshly distilled pyrrolidine in methanol to obtain the enamine 10 (UV maximum at 274.6 nm). It showed a vinylic proton singlet at δ 4.70 (1H, 4-H) and 5.0 (1H, 6-H), respectively. Sodium borohydride reduction of 10 in methanol at room temperature gave 16β-morpholino-3β-pyrrolidino-5-androsten-17β-ol 11. A proton resonance signal appeared at δ 5.30 (1H, m, 6-H). The amine 11 was acetylated with acetic anhydride in pyridine at 100°C to give 16β-morpholino-3β-pyrrolidino-5-androsten-17β-yl acetate 12. ¹H NMR spectra of 12 showed the proton resonance signals at δ 2.20 (3H, s, -OCOCH₃), 3.03 (1H, q, J=9Hz, 16α-H) and 4.78 (1H, d, J=9Hz, 17α-H). IR spectra showed bands at 1725 and 1230 cm⁻¹ for the acetoxy function. Bisquaternary compounds 13 and 14 were prepared by treating the tertiary amines 11 and 12 with methyl
iodide in absolute ethanol at refluxing temperature and with dichloromethane at room temperature, respectively.

Biological activities

Neuromuscular blocking activity

Compounds 7, 8, 13 and 14 were tested for their neuromuscular blocking activity. Monoquaternary compound 7 was less potent than tubocurarine. Compound 13, a bisquaternary compound was found to be more potent than the corresponding 17-mononitrogen analogue. The bisquaternary 17-acetoxy compound 14 was found to be more potent than the corresponding 17-monohydroxy analogue. The compound 14 has been found to be less potent than tubocurarine. The purity of the compounds was established by TLC and by elemental analyses (C,H,N). Elemental analyses were carried out on a Perkin-Elmer-2400. Anhydrous sodium sulphate was used as a drying agent.

16β-Morpholino-17-oxo-5-androsten-3β-ol 3. Compound 2 (0.5 g) was refluxed with morpholine for 1 hr. After removal of majority of the morpholine by distillation under reduced pressure, the product was precipitated with water. The product was crystallised from acetone to afford 3 (0.2 g, 49.2%); mp 200-204° (lit 200°); IR: 1200, 1075, 1725 (C=O); 'H NMR: 0.93 (3H, s, 18-CH3), 1.06 (3H, s, 19-CH3), 2.46-2.83 (4H, m, J=5Hz, N-methylene of morpholino function), 3.05 (IH, m, 16α-CH3), 3.63 (5H, t, J=9Hz, 17α-methylene of morpholino function), 3.63 (5H, t, J=9Hz; O-methylene of morpholino function, 3α-CH3), 5.4 (IH, m, 6-CH3) (Found : C, 73.50; H, 9.40; N, 3.75). C23H17NO3 requires C, 73.95; H, 9.44; N, 3.75%.

16β-Morpholino-5-androsten-3β-ol -diol 4. Sodium borohydride (1.0 g) was added to a stirred suspension of 3 (1.0 g) in methanol (100 mL) in small quantities at room temperature. Stirring was continued for 2 hr, the reaction mixture was poured into ice-cold water (500 mL) and the aqueous suspension was extracted with chloroform (4x 50 mL). The combined chloroform extract was washed with water, dried and solvent removed under reduced pressure to give a solid residue which was crystallised from methanol to afford 4 (0.7 g, 65.7%); mp 245-248°; IR: 3473 (O-H), 1070 (C-O stretching); 'H NMR: 0.70 (3H, s, 18-CH3), 1.01 (3H, s, 19-CH3), 2.55-2.79 (4H, m, J=5Hz, N-methylene of morpholino function), 2.82 (1H, q, J=9Hz, 16α-CH3), 3.45(1H, d, J=9Hz, 17α-CH3), 3.51 (IH, m, 3α-CH3), 3.65-3.77 (4H, m, J=5Hz, O-methylene of morpholino function), 5.30 (IH, d, 6-CH3) (Found : C, 73.30; H, 9.88; N, 3.54. C23H19NO3 requires C, 73.56; H, 9.93; N, 3.73%).

Experimental Section

Melting points reported are uncorrected. 'H NMR spectra were recorded on AC-300F, 300 MHz, Varian EM-390, 90 MHz and EM-360, 60 MHz NMR instruments using TMS as an internal standard (chemical shifts in δ, ppm); IR spectra in KBr (νmax in cm⁻¹) on Perkin-Elmer 882 and UV spectra in methanol on a Lambda 15 spectrophotometer models respectively; and mass spectra on a V6-11-250 J 70S. The purity of the compounds was screened for their antineoplastic activity for further studies.

The compounds was established by TLC and by elemental analyses (C,H,N). Elemental analyses were carried out on a Perkin-Elmer-2400. Anhydrous sodium sulphate was used as a drying agent.

16β-Morpholino-17-oxo-5-androsten-3β-ol 3. Compound 2 (0.5 g) was refluxed with morpholine for 1 hr. After removal of majority of the morpholine by distillation under reduced pressure, the product was precipitated with water. The product was crystallised from acetone to afford 3 (0.2 g, 49.2%); mp 200-204° (lit 200°); IR: 1200, 1075, 1725 (C=O); 'H NMR: 0.93 (3H, s, 18-CH3), 1.06 (3H, s, 19-CH3), 2.46-2.83 (4H, m, J=5Hz, N-methylene of morpholino function), 3.05 (IH, m, 16α-CH3), 3.63 (5H, t, J=9Hz; O-methylene of morpholino function, 3α-CH3), 5.4 (IH, m, 6-CH3) (Found : C, 73.50; H, 9.40; N, 3.75). C23H17NO3 requires C, 73.95; H, 9.44; N, 3.75%.

16β-Morpholino-5-androsten-3β-ol -diol 4. Sodium borohydride (1.0 g) was added to a stirred suspension of 3 (1.0 g) in methanol (100 mL) in small quantities at room temperature. Stirring was continued for 2 hr, the reaction mixture was poured into ice-cold water (500 mL) and the aqueous suspension was extracted with chloroform (4x 50 mL). The combined chloroform extract was washed with water, dried and solvent removed under reduced pressure to give a solid residue which was crystallised from methanol to afford 4 (0.7 g, 65.7%); mp 245-248°; IR: 3473 (O-H), 1070 (C-O stretching); 'H NMR: 0.70 (3H, s, 18-CH3), 1.01 (3H, s, 19-CH3), 2.55-2.79 (4H, m, J=5Hz, N-methylene of morpholino function), 2.82 (1H, q, J=9Hz, 16α-CH3), 3.45(1H, d, J=9Hz, 17α-CH3), 3.51 (IH, m, 3α-CH3), 3.65-3.77 (4H, m, J=5Hz, O-methylene of morpholino function), 5.30 (IH, d, 6-CH3) (Found : C, 73.30; H, 9.88; N, 3.54. C23H19NO3 requires C, 73.56; H, 9.93; N, 3.73%).
16β-Morpholino-5-androstene-3β,17β-diol diacetate 5. A mixture of 4 (0.5 g) and acetic anhydride (3 mL) in pyridine (2 mL) was heated on a steam-bath for 1 hr. The contents were then poured into ice-cold water (100 mL) and basified with ammonia. The precipitate obtained was filtered, washed with water, dried and crystallised from methanol to afford 5 (0.4 g, 65.4%); mp 208-209°; IR: 1732 (OCOCH3); 1239 [C-C(O)-O]; 1160 (C-O stretch); 1H NMR: 0.81 (3H, s, 18-CH3); 1.03 (3H, s, 19-CH3); 2.03 (3H, s, 3β-OCOOCH3); 2.10 (3H, s, 17β-OCOOCH3); 3.07 (IH, q, J = 9Hz, 16α-CH); 3.61-3.71 (4H, m, O-methylene of morpholino function), 4.58 (IH, m, 3α-CH3); 4.80 (IH, d, J = 9Hz, 17α-CH); 5.30 (IH, m, 6-CH3); MS: m/z 459 (M+). (Found: C, 55.40; H, 8.99; N, 3.05%).

16β-Morpholino-17-oxo-5-androsten-3-β-ol methylidide 6. Methyl iodide (3 mL) was added to a refluxing solution of 3 (0.5 g) in absolute ethanol (20 mL) and stirred for 30 min. The solvent was removed under reduced pressure and the residue obtained was crystallised from ethanol to afford 6 (0.4 g, 58.0%); mp 255-256° (Found: C, 55.72; H, 7.39; N, 2.56. C22H31NO3 requires C, 55.92; H, 7.43; N, 2.72%).

16β-Morpholino-5-androstene-3β,17β-diol methylidide 7. Methyl iodide (0.5 mL) was added to a refluxing solution of 4 (0.2 g) in absolute ethanol (15 mL) and the solution refluxed for 30 min, processed as usual and the residue obtained was crystallised from acetone to afford 7 (0.2 g, 58.1%); mp 235-236° (Found: C, 55.40; H, 7.80; N, 3.08. C22H31NO3 requires C, 55.70; H, 7.79; N, 2.71%).

16β-Morpholino-5-androstene-3β,17β-diol diacetate methylidide 8. Methyl iodide (0.4 mL) was added to a solution of 5 (0.2 g) in dichloromethane (5 mL) and allowed to stand at room temperature for two days. The solvent was removed under reduced pressure to obtain a solid residue which was crystallised from acetone to afford 8 (0.2 g, 57.25%), mp 238-239° (Found: C, 55.66; H, 7.23; N, 2.53. C22H34NO3 requires C, 55.90; H, 7.37; N, 2.33%).

16β-Morpholino-4-androstene-3,17-dione 9. 16β-Morpholino-17-oxo-5-androsten-3β-ol 3 (0.1 g) was dissolved in a mixture of cyclohexanone (10 mL) and dry toluene (150 mL) and the solution was subjected to azeotropic distillation to remove traces of moisture. The distillation was continued at a slow rate during dropwise addition of a solution of aluminium isopropoxide (1.0 g) in dry toluene (20 mL). The reaction mixture was refluxed for 4 hr and allowed to stand at room temperature for 12 hr. The slurry was filtered and the filtrate was steam distilled until the complete removal of organic solvents was effected. The residual aqueous suspension was allowed to stand at room temperature and extracted with chloroform (4 x 25 mL). The combined chloroform extract was washed with water, dried and the solvent removed to obtain 9 (0.5 g, 50.3%); UV 238.6 (4.36); IR: 1726 (C=O), 1674 (C=C=C=O) (Found: C, 74.01; H, 8.85; N, 3.51. C22H18NO2 requires C, 74.36; H, 8.95; N, 3.77%).

16β-Morpholino-3-pyrrolidino-3,5-androstan-17-one 10. Freshly distilled pyrrolidine (2 mL) was added to a refluxing solution of 9 (2.5 g) in methanol (15 mL). Refluxing was further continued for 10 min and the solution was concentrated to induce crystallisation. The crystalline material was filtered, washed with methanol and dried to afford 10 (2.5 g, 87.4%); mp 178-179°; UV: 274 (4.13); IR: 1741 (C=O); 1H NMR: 0.90 (3H, s, 18-CH3); 1.0 (3H, s, 19-CH3); 2.93-3.16 (5H, m, N-methylene of morpholino function), 4.70 (1H, s, 4-CH3), 5.0 (1H, m, 6-CH3) (Found: C, 75.90; H, 9.32; N, 6.57. C22H34N2O2 requires C, 76.37; H, 9.50; N, 6.60%).

16β-Morpholino-3-pyrrolidino-5-androstan-17β-ol 11. Sodium borohydride (1.0 g) was added to a stirred suspension of 10 (1.0 g) in methanol (40 mL) in small quantities at room temperature. Stirring was continued for 2 hr, the reaction mixture was poured into ice-cold water (900 mL) and the aqueous suspension was extracted with chloroform (4 x 100 mL). The combined chloroform extract was washed with water, dried and the solvent removed under reduced pressure to obtain a solid residue which was crystallised from acetone to afford 11 (0.5 g, 49.6%); mp 188-190°; 1H NMR: 0.73 (3H, s, 18-CH3); 1.03 (3H, s, 19-CH3); 2.33-2.74 (8H, m, N-methylene of morpholino and pyrrolidino functions), 2.80 (1H, m, 16α-CH3); 3.40 (1H, q, J = 9Hz, 17α-CH3); 3.50-3.83 (4H, t, J = 5Hz, O-methylene of morpholino function), 5.30 (1H, m, 6-CH3); [α]D20 20.4° (c 0.07, CHCl3); MS: m/z 428 (M+). (Found: C, 75.21; H, 10.47; N, 6.50. C22H34N2O2 requires C, 75.65; H, 10.35; N, 6.54%).

16β-Morpholino-3-pyrrolidino-5-androstan-17β-yl acetate 12. A mixture of 11 (0.3 g) in pyridine (0.5 mL) and acetic anhydride (1 mL) was heated on a steam-bath for 1 hr. The reaction mixture was poured into ice-cold water and basified with ammonia. The precipitated material was filtered and the residue
crystallised from acetone to afford 12 (0.20 g, 60.79%); mp 198-200°; IR: 1725 (OOCCH3), 1230, [C-C(=O)-Ostretch), 1H NMR: 0.86 (3H, s, 18-CH3), 1.06 (3H, s, 19-CH3), 2.20 (3H, s, -OOCCH3). 2.40-2.73 (8H, m, N-methylene of morpholino and pyrrolidino functions), 3.03 (1H, q, J=9Hz, 16α-CH3), 3.63 (4H, m, J=5Hz, O-methylene of morpholino function), 4.78 (1H, d, J=9Hz, 17α-CH2), 5.30 (1H, m, 6-CH) (Found: C, 48.88; H, 7.07; N, 3.93%).

16β-Morpholino-3β-pyrrolidino-5-androsten-17β-ol dimethiodide 13. Methyl iodide (0.4 mL) was added to a solution of 11 (0.2 g) in absolute ethanol (10 mL) and refluxing was continued for 30 min. The reaction mixture was cooled, filtered and concentrated to induce crystallisation. The crystalline material was filtered, washed and dried to afford 13 (0.2 g, 45.2%); mp 282-85° (Found: C, 48.60; H, 7.14; N, 3.51. C29H46N2O2I requires C, 48.88; H, 7.07; N, 3.93%).

16β-Morpholino-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide 14. Methyl iodide (1 mL) was added to a solution of 12 (1.0 g) in dichloromethane (50 mL) and allowed to stand at room temperature for four days. The solvent was removed and residue washed with ether, filtered and crystallised from acetone-ethanol to yield 14 (0.1 g, 75.0%); mp 278-80° (Found: C, 48.23; H, 6.87; N, 3.49. C31H45N2O2I requires C, 49.34; H, 6.95; N, 3.71%).

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