Antiandrogen. III.[1] 11-Oxapregnane Steroids

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11-Oxachlormadinone acetate (17-acetoxy-6-chloro-11-oxapregna-4,6-diene-3,20-dione) and 2,11-dioxachlormadinone acetate (17-acetoxy-6-chloro-2,11-dioxapregna-4,6-diene-3,20-dione) were prepared as potential antiandrogenic agents. The effect of the latter compound on antiandrogenic activity when tested in the castrated rat was shown to be more potent than that of the parent compound, chlormadinone acetate.

Key words antiandrogen; ventral prostate; 2,11-dioxachlormadinone acetate; 11-oxachlormadinone acetate

In the course of screening studies on antiandrogens, it has been found that the antiandrogenic activity of chlormadinone acetate[2] is increased by the replacement of the carbon atom at the C-2 position with an oxygen atom. Thus, we have directed our attention to other oxasteroids in the hope of finding more potent antiandrogens. The C-11 position of the steroid ring has a major effect on biological properties, such as corticoid activities. Chlormadinone acetate has been found to be metabolized to 11-oxygenated products in the rat,[3] and these metabolites may produce adrenal side effects. The replacement of a carbon atom of the C-11 position of an antiandrogen by an oxygen atom seemed, therefore, to be a reasonable approach to blocking its metabolism. The present paper describes the preparation and antiandrogenic activity of 11-oxa and 2,11-dioxachlormadinone acetate.

17-Acetoxy-11-oxapregn-4-ene-3,20-dione (**3**) and its 1-dehydro compound (**2**) have previously been prepared by Engel *et al.*[4] from hecogenin acetate (**1**), and the former showed progestational activity, having one-eightieth of the potency of 17-acetoxyprogesterone.

For the synthesis of 11-oxachlormadinone acetate (**6**) from **3**, the method[5] previously reported for the preparation of chlormadinone acetate was employed, as follows. Dehydrogenation of **3** with chloranil in *tert*-butanol[6] gave the 4,6-diene (**4**) in a reasonable yield as a crude product, which was converted with *m*-chloroperbenzoic acid (*m*-CPBA) in chloroform to the α-epoxide (**5**), as reported for a related compound.[7] Treatment of the epoxide with hydrogen chloride in 2-propanol afforded the target compound, **6**, in a good yield.

Next, our attention was directed to the preparation of 2,11-dioxachlormadinone acetate (**12**), which has greater hydrophilicity than the 2-oxa compound (**6**), with the aim of increasing bioavailability. The method[8] which we previously reported for the replacement of a carbon atom of the C-2 position by an oxygen atom gave a good result in this case. Dehydrogenation of **4** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxane furnished the trienone compound (**7**) in a satisfactory yield as a crude product. Compound **7** was treated with *m*-CPBA in chloroform to yield the epoxide (**8**), which was then submitted to ozonolysis[9] in pyridine to give the lactol (**9**) in a yield similar to that previously reported.[8] The crude product **9** was reduced with sodium borohydride to the lactone, which was treated with hydrochloric acid to furnish the chlorhydrin (**10**) in a reasonable yield as a crude product.

Acetylation of **10** and subsequent treatment with potassium acetate in dimethylformamide gave the desired compound, **12**, in a sufficient yield.

Biological Activities

The antiandrogenic activity of the compounds obtained was determined in immature male castrated rats treated with testosterone propionate. The ability to prevent testosterone propionate-stimulated weight gain of the seminal vesicles and ventral prostate was used as an index of the antiandrogenic activity.[10] These data are shown in Table 1.

Although 11-oxachlormadinone acetate (**6**) was evaluated as inactive at the dose tested, the 2,11-dioxa compound (**12**) displayed about ten times higher activity than chlormadinone acetate.

Experimental

Melting points were measured on a Mettler FPI melting point apparatus and are uncorrected. ^{1}H-NMR spectra were determined on a Hitachi R-90H instrument or a JEOL JNM GX-500 instrument in $CDCl_3$ solution using tetramethylsilane as an internal standard. Mass spectra were recorded on a Shimadzu GCMS-QP1000 spectrometer. Elemental analysis was performed on a Hitachi 026 CHN analyzer. Preparative thin-layer chromatography (TLC) was carried out on 20 × 20 cm plates with a 0.25 mm or 2 mm layer of Merck Silica gel 60 GF254. Ozone was generated with a Nippon Ozone 0-10-2 instrument.

17-Acetoxy-6α,7α-epoxy-11-oxapregn-4-ene-3,20-dione (5) A mixture of **3** (214 mg, 0.57 mmol), chloranil (990 mg, 4.03 mmol) and *tert*-BuOH (10 ml) was refluxed for 7 h. The excess chloranil was removed by filtration and the filtrate taken to dryness. The residue was taken up in $CHCl_3$ and the organic layer was washed with water, 4% NaOH, and then water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness. The crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 19 : 1) to give 17-acetoxy-11-oxapregna-4,6-diene-3,20-dione (**4**, 124 mg, 58.3%). ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, C18-H_3), 1.20 (3H, s, C19-H_3), 2.06 and 2.09 (each 3H, s, C21-H_3, 17α-OAc), 3.85 and 3.91 (2H, ABq, J = 11 Hz, C12-H_2), 5.74 (1H, s, C4-H), 6.02 (1H, br d, J = 10 Hz, C6-H), 6.13 (1H, dd, J = 2, 10 Hz, C7-H). MS *m/z*: 372 (M^+), 329, 312, 287, 269. This product was used in the next step without further purification.

A mixture of the crude product **4** (92 mg, 0.25 mmol), 70% *m*-CPBA (166 mg, 0.67 mol) and $CHCl_3$ (3 ml) was stirred for 4 h at room temperature. After addition of water, the product was extracted with EtOAc. The organic layer was washed with 5% $Na_2S_2O_3$, 4% NaOH and then water, and dried over anhydrous $MgSO_4$, and the crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 19 : 1) to give **5** (26 mg, 27.1%). mp 140—145 °C. *Anal.* Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.21; H, 7.20. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, C18-H_3), 1.20 (3H, s, C19-H_3), 2.05 and 2.11 (each 3H, s, C21-H_3,

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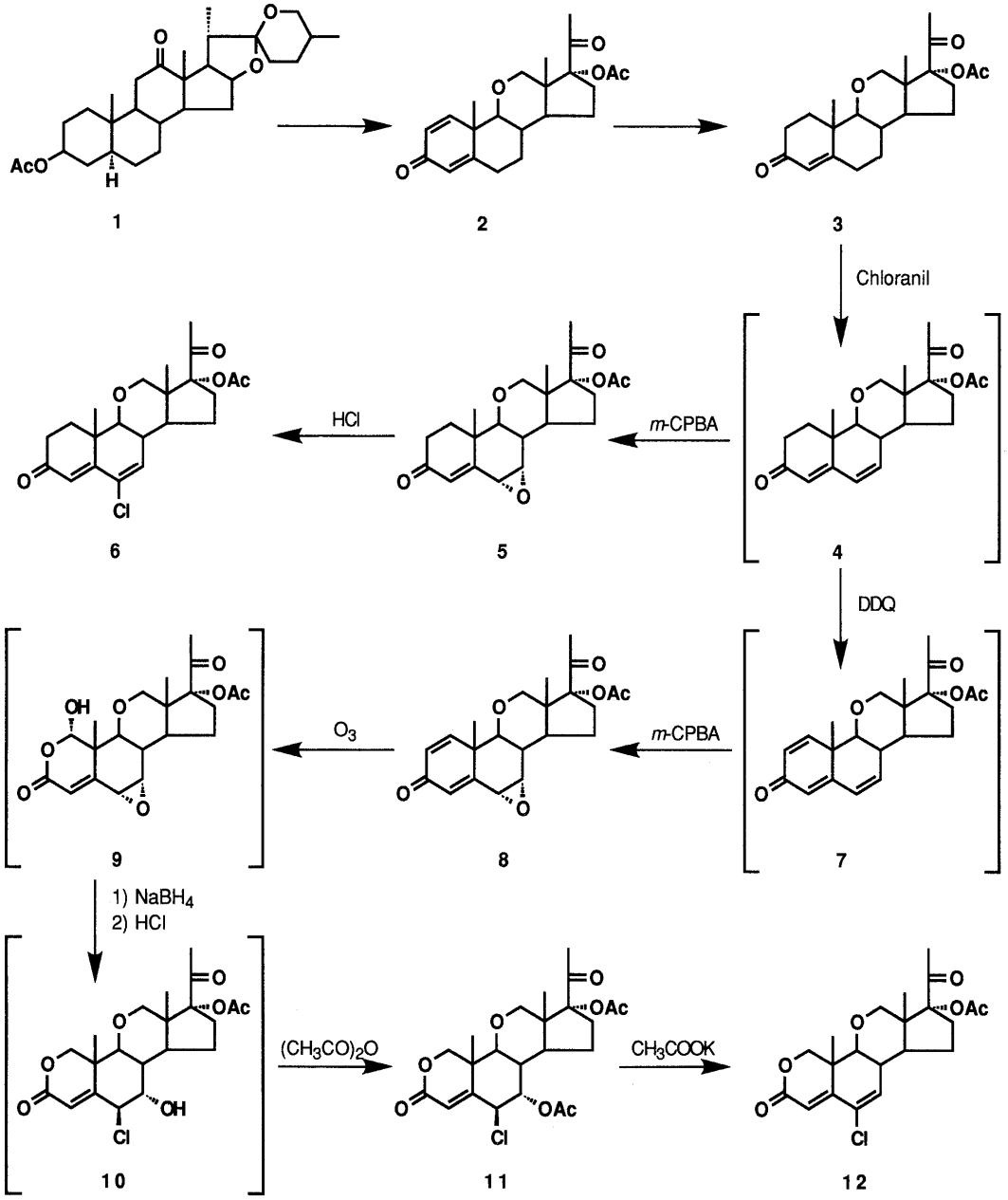

Chart 1

Table 1. The Effect of 11-Oxapregnenes on Accessory Sex Organ Weights in Castrated Rats Given Testosterone Propionate (50 μg/rat, s.c.[a])

Compound	Dose (mg/kg/*p.o.*[a])	Organ weight[b] (mg/100 g body weight)	
		Ventral prostate	Seminal vesicle
6	0.22	29.0±1.7	37.5±1.9
6	0.67	25.4±2.3	41.2±2.8
6	2	25.1±2.3	34.1±2.1
12	0.22	25.7±1.5	34.4±2.2
12	0.67	23.5±2.0	31.9±3.0[e]
12	2	18.7±1.9[e]	26.9±3.2[d]
CMA[f]	5	23.4±1.2	37.8±1.2
CMA	15	20.6±1.5[e]	22.0±1.3[e]
CMA	45	15.9±0.6[d]	23.4±1.7[c]
Castrated control		6.1±0.8[c]	6.6±0.6[c]
T. P.[g] control		27.8±2.5	41.9±3.0

a) *p.o.*, *per os*; s.c., subcutaneous. *b*) Each value represents the mean±S.E. ($n=5$). *c*) Significantly different from the T. P. control ($p<0.001$). *d*) Significantly different from the T. P. control ($p<0.01$). *e*) Significantly different from the T. P. control ($p<0.05$). *f*) CMA, chlormadinone acetate. *g*) T. P., testosterone propionate.

17α-OAc), 3.47 (2H, br s, C6, 7-H×2), 3.79 and 3.87 (2H, ABq, $J=11$ Hz, C12-H_2), 6.16 (1H, s, C4-H). MS *m/z*: 388 (M^+), 372, 345, 303.

17-Acetoxy-6-chloro-11-oxapregna-4,6-diene-3,20-dione (6) A mixture of **5** (14 mg, 0.04 mmol) and 18% HCl in 2-propanol (1 ml) was stirred for 30 min at room temperature. After addition of water, the product was extracted with EtOAc. The organic layer was washed with 5% Na_2CO_3 and then water, dried over anhydrous $MgSO_4$, and concentrated to dryness. The crude product was subjected to preparative TLC (C_6H_6 : EtOAc = 4 : 1) to give **6** (13 mg, 89%). An analytical sample was obtained by recrystallization from Me_2CO–hexane as colorless prisms, mp 223—227 °C. *Anal.* Calcd for $C_{22}H_{27}ClO_5$: C, 64.94; H, 6.69. Found: C, 65.07; H, 6.62. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, C18-H_3), 1.23 (3H, s, C19-H_3), 2.06 and 2.09 (each 3H, s, C21-H_3, 17α-OAc), 3.85 and 3.91 (2H, ABq, $J=11$ Hz, C12-H_2), 6.23 (1H, d, $J=2$ Hz, C7-H), 6.33 (1H, s, C4-H). MS *m/z*: 406 (M^+), 363, 346, 321, 303.

17-Acetoxy-6α,7α-epoxy-11-oxapregna-1,4-diene-3,20-dione (8) A mixture of the crude product **4** (100 mg, 0.27 mmol) and DDQ (90 mg, 0.40 mmol) in dioxane (1.5 ml) was refluxed for 6 h and then cooled. The mixture was concentrated to dryness and the residue was chromatographed on an alumina column using CH_2Cl_2 as an eluent. The crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 19 : 1) to give 17-acetoxy-11-oxapregna-1,4,6-triene-3,20-dione (**7**, 86 mg, 86.5%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, s, C18-H_3), 1.30 (3H, s, C19-H_3), 2.06

(6H, s, C21-H_3, 17α-OAc), 3.86 and 3.92 (2H, ABq, J=11 Hz, C12-H_2), 5.8—6.4 (4H, m, C2, 4, 6, 7-H×4), 7.27 (1H, d, J=10 Hz, C1-H). MS m/z: 370 (M^+), 327, 310, 285, 267. This product was used in the next step without further purification.

A mixture of the crude product **7** (48 mg, 0.13 mmol), 70% *m*-CPBA (135 mg, 0.55 mmol) and $CHCl_3$ (1 ml) was stirred for 7 h at room temperature. After addition of water, the product was extracted with EtOAc. The organic layer was washed with 5% $Na_2S_2O_3$, 4% NaOH and then water, dried over anhydrous $MgSO_4$, and concentrated to dryness. The crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 19 : 1) to give **8** (44 mg, 87.9%). mp 145—150 °C. *Anal.* Calcd for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.57; H, 6.75. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, C18-H_3), 1.31 (3H, s, C19-H_3), 2.06 and 2.09 (each 3H, s, C21-H_3, 17α-OAc), 3.46 (1H, br d, J=4 Hz, C7-H), 3.65 (1H, d, J=4 Hz, C6-H), 3.82 and 3.88 (2H, ABq, J=11 Hz, C12-H_2), 6.25 (1H, dd, J=2, 10 Hz, C2-H), 6.51 (1H, d, J=2 Hz, C4-H), 7.25 (1H, d, J=10 Hz, C1-H). MS m/z: 386 (M^+), 343, 326, 301, 283.

7α,17-Diacetoxy-6-chloro-2,11-dioxapregn-4-ene-3,20-dione (11) A stream of ozone (1.0 mmol/min, 8 min) was passed into a solution of **8** (109 mg, 0.28 mmol) in pyridine (2.3 ml) at −28 °C. The progress of the reaction was followed by TLC. The resulting mixture was stirred for 15 min at room temperature and poured into 10% $NaHSO_3$, and the product was extracted with EtOAc. The organic layer was washed with 10% H_2SO_4 and water, dried over anhydrous $MgSO_4$, and then concentrated to dryness. The crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 9 : 1) to give 17-acetoxy-6α,7α-epoxy-1α-hydroxy-2,11-dioxapregn-4-ene-3,20-dione (**9**, 90 mg, 78.5%). ^{1}H-NMR ($CDCl_3$) δ: 0.79 (3H, s, C18-H_3), 1.27 (3H, s, C19-H_3), 2.05 and 2.12 (each 3H, s, C21-H_3, 17α-OAc), 3.46 (1H, d, J=4 Hz, C7-H), 3.53 (1H, d, J=4 Hz, C6-H), 3.70 and 3.90 (2H, ABq, J=11 Hz, C12-H_2), 5.54 (1H, s, C1-H), 6.22 (1H, s, C4-H). MS m/z: 406 (M^+), 388, 363, 321, 303. This product was used in the next step without further purification.

A solution of $NaHCO_3$ (18 mg) in water (1.5 ml) was added to a solution of the crude product **9** (85 mg) in THF (1.5 ml) and MeOH (1.5 ml). After addition of $NaBH_4$ (30 mg), the mixture was stirred for 50 min at room temperature, and then concentrated HCl (0.2 ml) was added. The reaction mixture was stirred for 1.5 h at room temperature and poured into water. The product was extracted with EtOAc. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated to give 17-acetoxy-6β-chloro-7α-hydroxy-2,11-dioxapregn-4-ene-3,20-dione (**10**, 58 mg, 65.0%). ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, s, C18-H_3), 1.54 (3H, s, C19-H_3), 2.06 and 2.10 (each 3H, s, C21-H_3, 17α-OAc), 3.79 and 3.86 (2H, ABq, J=11 Hz, C12-H_2), 4.0—4.5 (4H, m, C1-H_2, C6, 7-H×2), 6.06 (1H, s, C4-H). MS m/z: 383 ($M^+ - COCH_3$), 366, 341, 323, 305. This product was used in the next step without further purification.

A mixture of the crude product **10** (54 mg), acetic anhydride (5 ml) and pyridine (4 ml) was stirred for 18 h at room temperature. After addition of water, the product was extracted with EtOAc. The organic layer was washed with 5% HCl, 5% $NaHCO_3$ and then water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness. The crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 19 : 1) to give **11** (50 mg, 84.3%). mp 215—220 °C. *Anal.* Calcd for $C_{23}H_{29}ClO_8$: C, 58.91; H, 6.23. Found: C, 59.06; H, 6.18. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, s, C18-H_3), 1.56 (3H, s, C19-H_3), 2.08, 2.09 and 2.12 (each 3H, s, C21-H_3, 7α, 17α-OAc×2), 3.81 (2H, s, C12-H_2), 4.13 and 4.36 (2H, ABq, J=11 Hz, C1-H_2), 4.46 (1H, d, J=2 Hz, C6-H), 5.17 (1H, t, J=2 Hz, C7-H), 6.02 (1H, s, C4-H). MS m/z: 425 ($M^+ - COCH_3$), 408, 383, 365, 323.

17-Acetoxy-6-chloro-2,11-dioxapregna-4,6-diene-3,20-dione (12) A mixture of **11** (54 mg), potassium acetate (27 mg) and DMF (2 ml) was stirred at 70 °C under N_2 for 1.5 h. After addition of 5% HCl, the product was extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ and water, dried over anhydrous $MgSO_4$, and concentrated to dryness. The crude product was subjected to preparative TLC ($CHCl_3$: Me_2CO = 19 : 1) to give **12** (39 mg, 82.8%). An analytical sample was obtained by recrystallization from Me_2CO as colorless prisms, mp 255—258 °C. *Anal.* Calcd for $C_{21}H_{25}ClO_6$: C, 61.69; H, 6.16. Found: C, 61.77; H, 6.11. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, s, C18-H_3), 1.30 (3H, s, C19-H_3), 2.07 and 2.08 (each 3H, s, C21-H_3, 17α-OAc), 3.83 and 3.88 (2H, ABq, J=11 Hz, C12-H_2), 4.15 and 4.39 (2H, ABq, J=11 Hz, C1-H_2), 6.23 (2H, m, C4, 7-H×2). MS m/z: 408 (M^+), 365, 348, 323, 305.

Antiandrogenic Assay Wistar strain male rats weighing 160—180 g were castrated at about 4 weeks of age. After two weeks, testosterone propionate (50 μg/rat) was administered daily by the subcutaneous route in 0.1 ml of sesame oil to all groups except the controls. The test compounds were given *per os* daily for 5 d. On day 6, the animals were sacrificed, and seminal vesicles and ventral prostates were secured and weighed.

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