Antiandrogen. III. 1) 11-Oxapregnane Steroids
Shigehiro TAKEGAWA,* Naoyuki KOIZUMI, Mamoru MIEDA, and Kenyu SHIBATA
Research Department, Teikoku Hormone Mfg. Co., Ltd., 1604, Shimosakunobe, Takatsu-ku, Kawasaki 213, Japan.
Received September 1, 1995; accepted December 6, 1995

11-Oxachromadimadine acetate (17-acetoxy-6-chloro-11-oxapregna-4,6-diene-3,20-dione) and 2,11-dioxachromadimadine acetate (17-acetoxy-6-chloro-2,11-dioxapregna-4,6-diene-3,20-dione) were prepared as potential anti-androgenic 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, chromadimadine acetate.

Key words: antiandrogen; ventral prostate; 2,11-dioxachromadimadine acetate; 11-oxachromadimadine acetate

In the course of screening studies on antiandrogens, it has been found that the antiandrogenic activity of chromadimadine 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. Chromadimadine 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-dioxachromadimadine acetate.

17-Acetoxy-11-oxapregna-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-eightheth of the potency of 17-acetoxyprogesterone.

For the synthesis of 11-oxachromadimadine acetate (6) from 3, the method 5) previously reported for the preparation of chromadimadine 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-dioxachromadimadine 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 triene 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-oxachromadimadine acetate (6) was evaluated as inactive at the dose tested, the 2,11-dioxa compound (12) displayed about ten times higher activity than chromadimadine acetate.

Experimental
Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. 1H-NMR spectra were determined on a Hitachi R-90H instrument or a JEOL JNM GX-500 instrument in CDCl3 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 x 20 cm plates with a 0.25 mm or 2 mm layer of Merck Silica gel 60 GF254. Ozone was generated with a Nitron Ozone 0-10-2 instrument.

17-Acetoxy-6,7α-epoxy-11-oxapregna-4-ene-3,20-dione (5) A mixture of 3 (214 mg, 0.57 mmol), chloranil (990 mg, 4.03 mmol) and tert-ButOH (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 CHCl3 and the organic layer was washed with water, 4% NaOH, and then water. The organic layer was dried over anhydrous MgSO4 and concentrated to dryness. The crude product was subjected to preparative TLC (CHCl3:Me2CO=19:1) to give 17-acetoxy-11-oxapregna-4,6-diene-3,20-dione (4, 124 mg, 58.3%). 1H-NMR (CDCl3) δ: 0.84 (3H, s, C18-CH3), 1.20 (3H, s, C19-CH3), 2.06 and 2.09 (each 3H, s, C31-H3), 17α-Oac), 3.85 and 3.91 (2H, ABq, J=11 Hz, C12-H2), 5.74 (1H, s, C4-H), 6.02 (1H, brd, 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 mmol) and CHCl3 (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% Na2SO4, 4% NaOH and then water, and dried over anhydrous MgSO4 and the crude product was subjected to preparative TLC (CHCl3:Me2CO=19:1) to give 5 (26 mg, 27.1%). Rf 0.140-0.145. C31H21O5. Anal. Calcd. For C31H21O5: C, 82.6%, H, 7.27. Found: C, 82.62; H, 7.20. 1H-NMR (CDCl3) δ: 0.80 (3H, s, C18-CH3), 1.20 (3H, s, C19-CH3), 2.05 and 2.11 (each 3H, s, C21-H3). 1996 Pharmaceutical Society of Japan
Table 1. The Effect of 11-Oxapregnones on Accessory Sex Organ Weights in Castrated Rats Given Testosterone Propionate (50 μg/rat, s.c.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Organ weight(^{a})(mg/100 g body weight)</th>
<th>Ventral prostate</th>
<th>Seminal vesicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.22</td>
<td>29.0 ± 1.7</td>
<td>37.5 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.67</td>
<td>25.4 ± 2.3</td>
<td>41.2 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>25.1 ± 2.3</td>
<td>34.1 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.22</td>
<td>25.7 ± 1.5</td>
<td>34.4 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.67</td>
<td>23.5 ± 2.0</td>
<td>31.9 ± 3.0(^{a})</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>18.7 ± 1.9(^{a})</td>
<td>26.9 ± 3.2(^{a})</td>
<td></td>
</tr>
<tr>
<td>CMA(^{a})</td>
<td>5</td>
<td>23.4 ± 1.2</td>
<td>37.8 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>CMA</td>
<td>15</td>
<td>20.6 ± 1.5(^{a})</td>
<td>22.0 ± 1.3(^{a})</td>
<td></td>
</tr>
<tr>
<td>CMA</td>
<td>45</td>
<td>15.9 ± 0.6(^{a})</td>
<td>23.4 ± 1.7(^{a})</td>
<td></td>
</tr>
<tr>
<td>Castrated control</td>
<td>6.1 ± 0.8(^{a})</td>
<td>6.6 ± 0.6(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. P.(^{a}) control</td>
<td>27.8 ± 2.5</td>
<td>41.9 ± 3.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) 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<0.05). \(^{e}\) CMA, chlormadinone acetate. \(^{f}\) T. P., testosterone propionate.

17α-OAc, 3.47 (2H, brs, C6, 7-H × 2), 3.79 and 3.87 (2H, ABq, J=11 Hz, C12-H2), 6.16 (1H, s, C4-H). MS m/z: 388 (M\(^{+}\)), 372, 345, 303.

17α-Acetoxy-6-chloro-11-oxapregn-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\(_2\)CO\(_3\) and then water, dried over anhydrous MgSO\(_4\), and concentrated to dryness. The crude product was subjected to preparative TLC (C\(_8\)H\(_4\), EtOAc = 4: 1) to give 6 (13 mg, 89%). An analytical sample was obtained by recrystallization from Me\(_2\)CO-hexane as colorless prisms, mp 223–227°C. Anal. Caled for C\(_{28}\)H\(_{35}\)O\(_3\): C, 76.41; H, 6.62. Found: C, 75.07; 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\(_3\)), 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.

17α-Acetoxy-6α,7α-epoxy-11-oxapregn-4,6-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\(_2\)Cl\(_2\) as an eluent. The crude product was subjected to preparative TLC (CHCl\(_3\), Me\(_2\)CO = 19: 1) to give 8-α-acetoxy-11-oxapregn-4,6,13-triene-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 (3H, s, C21-H\(_3\)).
A mixture of the crude product 7 (48 mg, 0.13 mmol), 70% m-CPBA (135 mg, 0.55 mmol) and CHCl₃ (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% Na₂S₂O₅, 4% NaOH and then water, dried over anhydrous MgSO₄, and concentrated to dryness. The crude product was subjected to preparative TLC (CHCl₃:Me₆CO = 19:1) to give 8 (44 mg, 87.5%) mp 145–150°C. Anal. Caled for C₁₂H₁₂O₃C₆: H, 68.38; H, 6.78. Found: C, 68.37; H, 6.75.

7-H-NMR (CDCl₃) δ: 0.83 (1H, s, C₁₈-H₃), 1.31 (1H, s, C₁₉-H₂), 2.06 and 2.09 (each 3H, s, C₂₁-H₂, 17α-OAc), 3.46 (1H, brd, 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-H₂), 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.

7a,17-Diacetoxy-6-chloro-2,11-dioxapregnen-4-ene-3,20-dione (11) A stream of oxygen (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% NaH₂SO₄, and the product was extracted with EtOAc. The organic layer was washed with 10% H₂SO₄ and water, dried over anhydrous MgSO₄, and then concentrated to dryness. The crude product was subjected to preparative TLC (CHCl₃:Me₆CO = 19:1) to give 10 (18 mg, 85.5%) mp 150–155°C. Anal. Caled for C₁₂H₁₀O₃C₆: H, 68.38; H, 6.78. Found: C, 68.37; H, 6.75.

7a,17-Diacetoxy-6-chloro-2,11-dioxapregnen-4-ene-3,20-dione (11) A mixture of 11 (54 mg), potassium acetate (27 mg) and DMF (2 ml) was stirred at 70°C under N₂ for 1.5 h. After addition of 5% HCl, the product was extracted with EtOAc. The organic layer was washed with 5% NaHCO₃ and water, dried over anhydrous MgSO₄, and concentrated to dryness. The crude product was subjected to preparative TLC (CHCl₃:Me₆CO = 19:1) to give 12 (39 mg, 82.8%). An analytical sample was obtained by recrystallization from Me₆CO as colorless prisms, mp 255–258°C. Anal. Caled for C₁₉H₂₂O₄C₆: H, 61.69; H, 6.16. Found: C, 61.77; H, 6.11.

7-H-NMR (CDCl₃) δ: 0.82 (1H, s, C₁₈-H₂), 1.30 (3H, s, C₁₉-H₂), 1.30 (3H, s, C₁₉-H₂), 2.07 and 2.08 (each 3H, s, C₂₁-H₂, 17α-OAc), 3.38 and 3.88 (2H, Abq, J = 11 Hz, C₁₂-H₂), 4.15 and 4.39 (2H, Abq, J = 11 Hz, C₁₂-H₂), 6.23 (2H, m, C₄, 7-H x 2). MS m/z: 408 (M⁺), 365, 348, 325, 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.

Acknowledgements The authors are indebted to Drs. H. Mori and K. Yasuda (of this company) for their support and encouragement throughout this work. The authors are also grateful to Mr. H. Takahashi for his cooperation in the biological experiments, and to Mrs. C. Watanabe for her technical assistance.

References