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Synthesis and Biological Activity of Oxidation Products of the Antiprogestine Mifepristone

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Summary. Selenium dioxide oxidation allows the selective introduction of a hydroxyl group at position 6 of the steroid skeleton of the antihormone (11β, 17β)-11-(4-dimethylamino-phenyl)-17-hydroxyl-17-(1-propynyl)-estra-4,9-dien-3-one (mifepristone, RU 486) and leads in a one-step procedure to two diastereomeric oxidation products. Their structure (6α- and 6β-hydroxy-mifepristone) was determined by NMR spectroscopy. The antiprogestinic activity of the oxidation products is comparable to that of mifepristone.

Keywords. Anti-hormones; Mifepristone; NMR spectroscopy; Oxidation; Cancer therapy.

Introduction

(11β, 17β)-11-(4-Dimethylamino-phenyl)-17-hydroxyl-17-(1-propynyl)-estra-4,9-dien-3-one (**1**, mifepristone, RU 486) is a well known progesterone receptor antagonist, also exhibiting antiglucocorticoide activity [1, 2]. It has been applied with success to the control of human fertility [3, 4]. In Austria, its trade name is Mifegyne and it is used for early pregnancy termination since 1999 [5, 6]. In addition, **1** shows tumor inhibitory potential in hormone-dependent breast cancer varieties, and its use for the treatment of progesterone-dependent tumors has been discussed recently [6, 7]. In order to map the interaction sites of 11β-norsteroids with the receptor proteins and to establish structure-affinity relationships, many related compounds have been synthesized and evaluated in various biological systems. Therefore, there is a fundamental importance of new selective synthetic methods as a tool for the derivatization of such 11β-norsteroids. Since anti-glucocorticoid activity causes adverse effects, recent emphasis has been directed to a further increase of the dissociation between antiprogestine and antiglucocorticoid activity. For 6β-methyl analogues, a reduced affinity to the glucocorticoide receptor without affecting the antiprogestinic activity has been described [8]. According to Ref. [9] it is believed that polar substituents below the D-ring, contrary to

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hydrophobic groups, rather would lead to compounds with diminished antiprogestine and increased glucocorticoid receptor activity. To facilitate the synthesis of new antiprogestines with polar substituents at carbon 6, a simple procedure as an alternative to the total synthesis [10] would be appreciable. In the present work we describe the application of selenium dioxide oxidation for the selective introduction of a hydroxyl group at position 6 of the steroid skeleton of **1**. We could also show that the oxidation products have similar affinity to the progesterone receptor and can therefore be used as a lead for the synthesis of biologic active derivatives of **1**, which might be useful for the treatment of hormone dependent cancer [11, 12].

Results and Discussion

Selenium dioxide – introduced in 1932 as a selective oxidating agent for organic compounds [13] – is regarded as a standard reagent for oxidation reactions and used under various reaction conditions. Selenium dioxide enables the introduction of various functionalities, *e.g.* acetyl [14], aldehyde [15], or hydroxyl groups [13], in the allylic position of unsaturated compounds.

Mifepristone (**1**) was oxidized in dioxane with selenium dioxide at 80°C for 20 hours. Usual workup and purification by column chromatography gave a mixture of the two diastereomeric hydroxyl compounds **2** and **3** in 39% yield [16] (Fig. 1), the major product being **3**.

HPLC analysis of the product mixture showed that **2** and **3** were obtained in a ratio of 1:9. The separation of the diastereomers was carried out by isocratic preparative HPLC. This procedure allowed the separation of up to 150 mg of product in a single run. The purified compounds were obtained as colourless crystals after lyophilization.

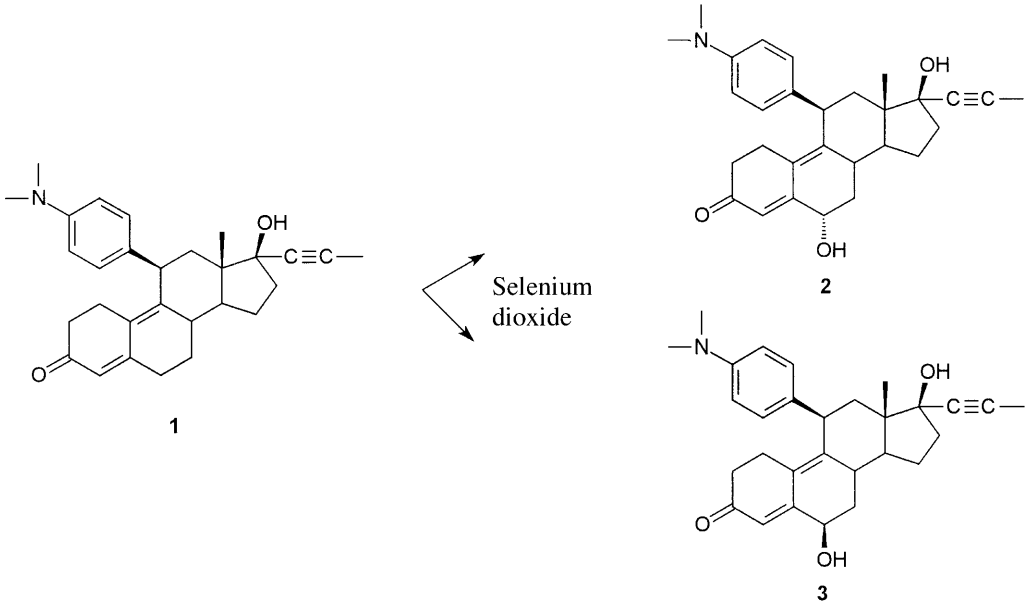

Fig. 1. Oxidation of mifepristone (**1**) with selenium dioxide to 6α- and 6β-hydroxymifepristone (**2**, **3**)

Table 1. NMR data of **2** and **3**

2				**3**			
δ/ppm (^{1}H)	Position	δ/ppm (^{13}C)	Position	δ/ppm (^{1}H)	Position	δ/ppm (^{13}C)	Position
0.54	13/CH_3	3.82	17/3′/propynyl	0.54	13/CH_3	3.79	17/3′/propynyl
1.36	15β	14.18	13′/methyl	1.36	15β	13.76	13′/methyl
1.70	7α	23.19	C-15	1.64	7α	23.18	C-15
1.74	15α	26.85	C-1	1.73	15α	26.01	C-1
1.77	14α	37.16	C-7	1.77	14α	33.83	C-7
1.86	17/CH_3	37.21	C-8	1.86	17/CH_3	34.21	C-8
1.94	16β	38.68	C-2	1.93	16β	37.28	C-2
2.22	7β	38.79	C-16, C-12	2.09	7β	38.92	C-16, C-12
2.23	16α	39.44	C-11	2.21	16α	39.80	C-11
2.27	12α	40.67	CH_3–N–CH_3	2.26	12α	40.80	CH_3–N–CH_3
2.32	2	46.77	C-13	2.27	2	47.20	C-13
2.36	12β	49.86	C-14	2.30	12β	49.48	C-14
2.40	2	68.52	C-6	2.36	1α	68.41	C-6
2.45	1α	80.13	C-17	2.43	2	80.14	C-17
2.60	8β	82.17	17/1′/propynyl	2.75	1β	82.37	17/1′/propynyl
2.78	1β	82.65	17/2′/propynyl	2.77	8β	82.49	17/2′/propynyl
2.91	CH_3–N–CH_3	112.85	11/C-ar/2′,6′	2.91	CH_3–N–CH_3	113.18	11/C-ar/2′,6′
4.32	11α	118.74	C-4	4.32	11α	122.24	C-4
4.58	6β	127.40	11/C-ar/3′,5′	4.48	6α	126.12	C-5
6.20	4	127.78	C-5	5.89	4	127.58	11/C-ar/3′,5′
6.67	ar/11	145.09	C-9	6.67	ar/11/2′,6′	146.46	C-9,
7.05	ar/11	148.46	C-10	7.05	ar/11	146.66	C-10
		159.26	11/C-ar/1′,4′			155.93	11/C-ar/1′,4
		199.57	C-3			200.22	C-3

The structure of the diastereomers was determined by NMR spectroscopy (see Table 1). Characteristic for the substitution in position 6 is the resonance of the olefinic protons at 6.20 ppm for **2** and at 5.89 ppm for **3**, both shifted to lower field as compared to the resonance of the corresponding proton in **1** (5.76 ppm). The signals of the protons at position 6 are found as 4.58(**2**) and 4.48(**3**) ppm. The configuration of the new stereogenic centre was deduced from the coupling constant between the proton at position 6 and the protons at position 7. The resonance of the equatorial hydrogen atom in **3** shows a small coupling constant of 5 Hz (equatorial-axial coupling), whereas the axial proton in **2** shows a broad resonance, indicating a large coupling constant of 15 Hz (axial-axial coupling). From this we conclude that **2** is 6α-hydroxy-mifepristone and **3** is 6β-hydroxy-mifepristone. The relative configuration of carbon 6 was confirmed by a 2D-ROESY experiment, where a correlation between the 6β-proton and the protons 7β and 8β was observed in **2**. For compound **3**, the 6α-proton showed a correlation exclusively to the neighbouring protons in positions 7α and 7β. Additional information concerning the configuration of C-6 was obtained from the ^{13}C NMR spectra. A comparison the chemical shifts of carbon 4 in **2** (118.7 ppm) and in **3** (122.2 ppm) shows a significant γ-effect induced by the equatorial hydroxyl group in **2** [17]. In **3**, the axial hydroxyl group induces a similar high field shift at C-8 (**2**: $\delta = 37.2$ ppm, **3**: $\delta = 34.2$ ppm).

The antiprogestinic activity of **2** and **3** was determined using the alkaline phosphatase (AP) assay of T47-D breast cancer cells. For progesterone concentrations between 10^{-10} and 10^{-8} mol/dm^3, the AP activity of this cell line increases with drug concentration. AP activation is diminished when progesterone and an antiprogestinic compound, *e.g.* mifepristone, are applied simultaneously. Thus, cells were co-incubated with 10^{-9} mol/dm^3 progesterone and increasing concentrations (10^{-11} to 10^{-5} mol/dm^3) of **2** or **3**. Cells co-incubated with progesterone and **1** served as control. In order to compare the antiprogestinic activity of the compounds quantitatively, the IC_{50} values (concentration of the antiprogestine which resulted in 50% inhibition of the AP activity caused by 10^{-9} mol/dm^3 progesterone) were determined. Although the IC_{50} values of **2** (50 n*M*) and **3** (100 n*M*) were significantly higher as compared with **1** (1 n*M*), the new compounds exhibited antiprogestinic activity in the nanomolar range, indicating a high affinity to the progesterone receptor. These results demonstrate that both **2** and **3** exhibit comparable antiprogestinic activity, but less pronounced than **1**. Compounds **2** and **3** are regarded as valuable biologically active intermediates for the preparation of novel 11β-substituted 19-norsteroids.

Experimental

General

M.p.: Büchi/Tottoli melting point apparatus; uncorrected; analytical TLC: precoated Al-backed 0.2 mm silicagel 60 F_{254} plates (E. Merck), mobile phase: cyclohexane:AcOEt = 1:1; UV/Vis: Shimadzu UV-160 A (MeOH); IR: Perkin-Elmer 1720-X (KBr); NMR: Varian Unity 600 ($CDCl_3$); MS: Varian MAT-711 (70 eV).

Oxidation procedure

107 mg **1** (0.243 mmol) were dissolved in 50 cm^3 dry dioxane in a 100 cm^3 three-neck flask equipped with a reflux condenser. After addition of 33 mg SeO_2 (0.303 mmol) the mixture was stirred at 80°C under an Ar atmosphere. The slightly yellow coloured reaction mixture became red. The reaction was stopped after 20 h by addition of 30 cm^3 5% aqueous KOH solution. Thereafter, the solution was extracted four times with AcOEt, and the combined organic layers were washed with H_2O to neutral *pH*, dried over Na_2SO_4, and evaporated. A mixture of **2** and **3** was obtained as a yellowish oil which was further purified by column chromatography on silica (mobile phase: cyclohexane:AcOEt = 3:7). Separation of the diastereomers was performed by preparative HPLC (Labomatic-HD-200/high pressure metering pump) on a Bischoff prep 3250, Prontoprep-120-10 C 18 HS 10 µm HPLC column (length 500 mm diameter 32 mm); mobile phase: water:acetonitril = 65:35, flow: 25 cm^3/min, detection: preparative UV-detector, 277 nm (Labocord 700 UV/VIS-spectrophotometer), pressure: 75 bar, injection volume: 5 cm^3, amount of substance: 150 mg. Fractions containing **2** or **3** were lyophilized, and the desired compounds were obtained as colourless crystals.

(11β, 17β)-11-(4-Dimethylamino-phenyl)-6α, 17β-dihydroxy-17-(1-propynyl)-estra-4,9-dien-3-one (**2**; $C_{29}H_{35}NO_3$)

Yield: 4%; m.p.: 158–161°C; $R_f = 0.15$; MS: 445 (100 M^+), 296 (50), 278 (20), 134 (18), 121 (72); IR (KBr): $\nu = 3425$ (b, OH-valence), 3021 (w), 2904 (w), 2846 (b, CH-valence), 1597 (s, CO-valence) cm^{-1}; UV/Vis (MeOH): $\lambda_{max} = 231, 277$ nm.

(11β, 17β)-11-(4-Dimethylamino-phenyl)-6β,17β-dihydroxy-17-(1-propynyl)-estra-4,9-dien-3-one (**3**; $C_{29}H_{35}NO_3$)

Yield: 35%; m.p.: 150–153°C; $R_f = 0.15$; MS: 445 (100 M^+), 296 (50), 278 (16), 134 (18), 121 (75); IR (KBr): $\nu = 3424$ (b, OH-valence), 3019 (w), 2904 (w), 2845 (b, CH-valence), 1594 (s, CO-valence) cm^{-1}; UV/Vis (MeOH): $\lambda_{max} = 231, 277$ nm.

Biological tests

Human T47-D breast cancer cells were cultivated in RPMI 1640 medium without phenol red (Life Technologies, Wiesbaden, Deutschland) supplemented with 5% dextran charcoal treated fetal calf serum and antibiotics. 24 h after seeding, cells were incubated with progesterone and antiprogestines **1–3** for another 48 h. After removal of the incubation medium, the alkaline phosphatase (AP) activity was determined according to Ref. [18]. Cells were briefly rinsed with physiological saline and stored for at least 15 min. at −80°C. After thawing, cells were incubated for 2 h with 4-nitrophenyl phosphate (*pNPP*, 5×10^{-3} mol/dm^3) dissolved in a buffer solution containing diethylamine (1 mol/dm^3), $MgCl_2$ (5×10^{-4} mol/dm^3), and $ZnSO_4$ (2×10^{-5} mol/dm^3) adjusted to *pH* 9.8. Quantitative assessments were performed at 405 nm (reference: 695 nm) using an microtiterplate photometer. At least three independent experiments were performed in quadruplicates; values are given as the arithmetic mean. Standard errors of the mean (SEM) were usually below ±15%. Concomitantly, cytotoxicity of the antiprogestines has been evaluated using the neutral red assay [19]. For each compound, no inhibition of cell growth was observed at incubation concentrations below 10^{-6} mol/dm^3.

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