available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/steroids

Syntheses of 19-[O-(carboxymethyl)oxime] haptens of epipregnanolone and pregnanolone

Ivan Černý[a], *Vladimír Pouzar*[a,*], *Martin Hill*[b], *Helena Havlíková*[b], *Richard Hampl*[b]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic*
[b] *Institute of Endocrinology, 116 98 Prague 1, Czech Republic*

ARTICLE INFO

Article history:
Received 29 June 2005
Received in revised form 16 August 2005
Accepted 2 September 2005
Available online 20 October 2005

Keywords:
Synthesis
Pregnanolone
O-(Carboxymethyl)oxime

ABSTRACT

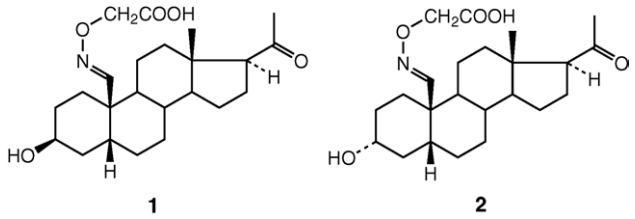
O-(Carboxymethyl)oximes **1** and **2** derived from two epimeric 5β-pregnanolones (3β-hydroxy-5β-pregnan-20-one and 3α-hydroxy-5β-pregnan-20-one) in position 19 were prepared. Two synthetic routes were employed, both using protection of the 20-keto group after reduction into the (20R)-alcohol in the form of acetate. In the first route, (20R)-19-hydroxy-5β-pregnan-3β,20-diyl diacetate (**3**) was transformed into the corresponding 19-[*O*-(carboxymethyl)oxime] methyl ester **6**, then deacetylated by acid and partially silylated with *tert*-butyldimethylsilyl chloride. The desired 3-*O*-silylated derivative **8** was separated, oxidized to the 20-ketone and protecting groups were sequentially removed to give the first title hapten **1**. The second route started from (20R)-19-hydroxy-3-oxopregn-4-en-20-yl acetate (**11**), which was hydrogenated in the presence of base to the 5β-pregnan-3-one derivative **12**, protected in position 19 with *tert*-butyldimethylsilyl group and reduced with borohydride. The prevailing 3α-alcohol **15** was separated, protected in position 3 with a methoxymethyl group, deprotected in position 19 and transformed into the 19-[*O*-(carboxymethyl)oxime] **19**. After deacetylation, esterification with diazomethane and oxidation in position 20, the pregnanolone skeleton was regenerated. Final deprotection steps gave the second title hapten **2**. Both haptens, i.e., (19E)-3β- and -3α-hydroxy-20-oxo-5β-pregnan-19-al 19-[*O*-(carboxymethyl)oxime], were designed for the development of immunoassays of the corresponding parent neuroactive steroids.

1. Introduction

Naturally occurring pregnanolone derivatives with a hydroxy group in position 3 and a ketone in position 20 exist as four stereoisomers, differing in configuration at positions 3 (orientation of hydroxyl) and 5 (annulation of rings A and B of the steroid skeleton). It is known that compounds of this type are potent endogenous neuromodulators with a variety of functions in the central nervous system and in the periphery [1–5]. They primarily act as modulators of membrane receptors regulating the permeability of ion channels [6–11].

Although the pharmacological effects of pregnanolone derivatives are mostly known, the information concerning levels and tissue concentrations of the steroids in human body is limited. The lack of information is particularly obvious in the isomers with hydrogen in the 5β-position. For instance, pregnanolone (3α-hydroxy-5β-pregnan-20-one), which is better known as eltanolone, is clinically used as a very efficient anesthetic with an extremely short half-life [12]. Our previous studies indicated [13] that the endogenous pregnanolone could be a stabilizing factor in pregnancy. To verify this assumption, it is necessary to know the neurosteroid

* *Corresponding author.* Tel.: +420 220 183111; fax: +420 220 183559.
E-mail address: pouzar@uochb.cas.cz (V. Pouzar).
0039-128X/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2005.09.002



Scheme 1 – Epimeric 19-CMO 5β-pregnanolones.

concentrations in various tissues and body fluids during different physiological situations in humans.

For monitoring of steroid levels, a combination of chromatographic methods with mass spectrometry or methods based on immunoassays are frequently used [14–16]. For the development of new immunoanalytical methods, haptens for the preparation of immunogens capable of producing sufficiently specific antisera are needed. To our knowledge, at present, there are no specific antisera available against 5β-pregnenolone isomers.

The present work focused on the synthesis of 5β-pregnanolone haptens **1** and **2** (Scheme 1), using the well-established *O*-(carboxymethyl)oxime connecting bridge (CMO) [17]. From the possible locations, position 19 was selected as it is sufficiently distant from characteristic groups required for recognition, i.e., from the hydroxy group in position 3 and from the ketone in position 20. We used a similar approach in our previous studies on other types of steroids (7α-/7β-hydroxydehydroepiandrosterone) and the resulting immunoassays displayed minimal cross reactions [18,19].

The synthetic strategy adopted the known approach to 19-hydroxy substituted steroids [20,21] and combined it with transformation into the 5β-pregnane series [22,23]. The selection of suitable protecting groups conformed the limitations, following from the bulk syntheses of starting compounds. A different approach to the protection of position 20 was used that was comparable to the syntheses of 19-CMO pregnanolone derivatives of the 5α-pregnane series [24]. Instead of using ethylene glycol ketals, which did not turn out to be sufficiently stable, we employed acetate protection after reduction of the 20-ketone into a (20R)-alcohol.

2. Experimental

2.1. General

Melting points were determined on a Boetius micro melting point apparatus (Germany). Optical rotations were measured at 25 °C on an AUTOPOL IV polarimeter (Rudolph Research Analytical, USA), and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^{1}H NMR spectra were taken on Brucker AVANCE-400 and AVANCE-500 instruments (400 and 500 MHz, FT mode) at 23 °C in $CDCl_3$ (unless stated otherwise) with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (*J*) and widths of multiplets (*W*) are given in Hz. ^{13}C NMR spectra were taken on Bruker AVANCE-500 instruments (^{13}C at 125.7 MHz) under the above conditions; secondary referencing was performed using the solvent signal at position $\delta(CDCl_3) = 77.0$ and $\delta(CD_3OD) = 49.0$; 2D heteronuclear experiments (HSQC, HMBC) were used for the structural assignment of signals. The electron impact mass spectrum of **24** was recorded on a VG Analytical ZAB-EQ spectrometer.

Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biochemicals). Detection was accomplished by spraying with concentrated sulfuric acid followed by heating. For column chromatography, neutral silica gel Kieselgel 60 (Merck) was used. Solutions in organic solvents were dried over anhydrous magnesium sulfate and concentrated on a rotary evaporator in vacuo (0.25 kPa, bath temperature 40 °C).

(20R)-19-hydroxy-5β-pregnane-3β,20-diyl diacetate (**3**) and (20R)-19-hydroxy-3-oxo-pregn-4-en-20-yl 20-acetate (**11**) were prepared from pregnenenolone acetate (Steraloids) according to standard procedures [20–22].

2.2. Derivatives of epipregnanolone

2.2.1. (20R)-19-(tert-Butyldimethylsilyl)oxy-5β-pregnane-3β,20-diyl diacetate (4)

Diacetate **3** (1.02 g, 2.43 mmol) and imidazole (4.4 g, 64.6 mmol) were dissolved in DMF (4.9 ml) and *tert*-butyldimethylsilyl chloride (1.80 g, 11.94 mmol) was added. After 3 h of standing at room temperature, the mixture was diluted with ether (100 ml) and washed sequentially with 5% aqueous citric acid (2×), saturated aqueous $KHCO_3$ and water. After drying, the solvents were evaporated, crude **4** (2 g) was dissolved in a mixture of petroleum ether/benzene (5:2, 5 ml), poured on the column of alumina (20 ml) and eluted with the same solvent mixture. The yield of foamy **4** was 1.20 g (93%). IR spectrum ($CHCl_3$): 1723 (C=O, acetate); 1472, 1462 (CH_3, *tert*-butyl); 1379 (CH_3); 1369 (CH_3, acetate); 1259, 1034, 1023 (C—O, acetate); 1088, 1075 (C—OSi). ^{1}H NMR (400 MHz): 5.16 (1 H, m, $W \approx 7$, H-3α); 4.95 (1 H, dq, $J = 10.4$, $J' = 6.1$, H-20); 3.94 (1 H, d, $J = 9.6$, H-19); 3.47 (1 H, d, $J = 9.6$, H-19′); 2.02 (3 H, s, CH_3COO); 2.00 (3 H, s, CH_3COO); 1.15 (3 H, d, $J = 6.1$, 3 × H-21); 0.91 (9 H, s, 3 × CH_3, *tert*-butyl); 0.62 (3 H, s, 3 × H-18); 0.05 (3H, s, CH_3–Si); 0.04 (3H, s, CH_3–Si). Analysis calculated for $C_{31}H_{54}O_5Si$ (534.8): C, 69.62; H, 10.18. Found: C, 69.41; H, 9.97.

2.2.2. (19E,20R)-19-Oxo-5β-pregnane-3β,20-diyl diacetate O-[(methoxycarbonyl)methyl]oxime (6)

Pyridine (4.8 ml, 59.3 mmol) was added dropwise at 0 °C under an argon atmosphere to a stirred suspension of chromium(VI) oxide (2.0 g, 20.0 mmol) and anhydrous magnesium sulfate (2.7 g) in dichloromethane (45 ml) and the stirring was continued at 0 °C for 20 min. Subsequently, a solution of compound **3** (1.4 g, 3.33 mmol) in dichloromethane (8 ml) was added, and the reaction mixture was stirred under argon at 0 °C for 2 h. After dilution with ether (45 ml), the mixture was filtered through a column of alumina (30 ml), which was washed with an ether/dichloromethane (1:1) mixture. The filtrate was concentrated, and pyridine was removed from the residue by coevaporation with toluene. The crude aldehyde **5** (1.3 g, 3.11 mmol) was dissolved in pyridine (20 ml), *O*-(carboxymethyl)hydroxylamine hemihydrochloride (2.2 g, 10.1 mmol) was added, and the mixture was stirred at room