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Neurosteroids: Can a 2alpha,3alpha-epoxy ring make up for the 3alpha-hydroxyl group?

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ABSTRACT

Seven steroid epoxides were prepared from 5α-pregn-2-en-20-one and 5α-pregn-3-en-20-one and their side-chain derivatives. All compounds were tested *in vitro* for binding to γ-aminobutyric acid ($GABA_A$) receptor, some of them also *in vivo* for anticonvulsant action.

2α,3α-Epoxy-5α-pregnan-20-one inhibited the TBPS binding to the $GABA_A$ receptor and showed a moderate anticonvulsant action in immature rats. In contrast, its 3α,4α-isomer was inactive. More polar epoxide derivatives, modified at the side chain were less active or inactive.

Noteworthy, diol **20**, the product of *trans*-diaxial opening of the 2α,3α-epoxide **4**, was not able to inhibit the TBPS binding, showing that the activity of the epoxide is due to the compound itself and not to its hydrolytic product.

The 3α-hydroxyl group is known to be essential for the $GABA_A$ receptor binding. Despite the shortness of *in vivo* effects which are probably due to metabolic inactivation of the products prepared, our results show that the 2α,3α-epoxy ring is another structural pattern with ability to bind the $GABA_A$R.

1. Introduction

Anticonvulsant drugs are primarily developed for the treatment of epilepsy, a neurological condition that affects some 50 million people worldwide. The anticonvulsants reduce seizure frequency by suppressing neuronal excitability *via* various molecular targets in the synapse. The use of classical anticonvulsants is not free of complications, thus other means have been sought [1,2]. One venue is offered by the fact that some steroids also modulate γ-aminobutyric acid receptor type A ($GABA_A$). The key endogenous compound acting at the $GABA_A$ receptor is allopregnanolone (**1**, 3α-hydroxy-5α-pregnan-20-one, Fig. 1). This is a compound with dual origin and function; it is not only a metabolite of the female sexual hormone – progesterone, but also a neurosteroid, i.e., the compound produced in the brain for the brain. Its roles in the body include both the termination of the activity of progesterone in cell nuclei as well as the modulation of inhibitory neurotransmitter receptors in cell membranes [3]. These receptors actually are chloride channels whose frequency and length of opening are controlled by GABA and modulated by neurosteroids, especially in the case of extrasynaptic receptors containing the delta subunit [3,4]. The entry of a chloride anion into a neuron hyperpolarizes its membrane and makes the generation of action potentials less probable, or in other words, it stops the passage of neural signals.

The 3α-hydroxyl group in allopregnanolone is not metabolically stable. It is rapidly oxidised to the oxo group and then reduced to the 3β epimer, a product with a reverse activity, or eliminated upon the Phase II conjugation. The half-life of allopregnanolone (**1**) in the human body is ca. 16 min. Much greater stability towards oxidation of the secondary 3α-hydroxyl group was expected by its conversion to a tertiary hydroxyl in ganaxolone (**2**): surprisingly, its half-life in man is also relatively short (6 h) [5].

An epoxy ring was already found to suit to the $GABA_A$ receptor, see compound **3** [6]. In our study we expected that an oxirane moiety attached to the A ring would lend the compound (e.g., **4**, see Fig. 2) a much greater metabolic stability [7–11]. In this paper, we report on the preparation of several steroidal A ring epoxides (compounds **4** to **10**) and their neuronal activity.

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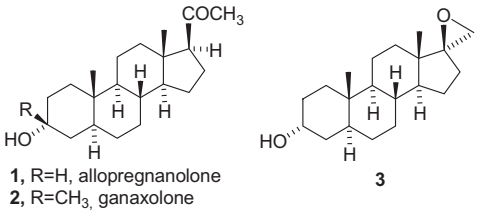

Fig. 1. Endogenous neurosteroid **1** and its analogues **2,3**.

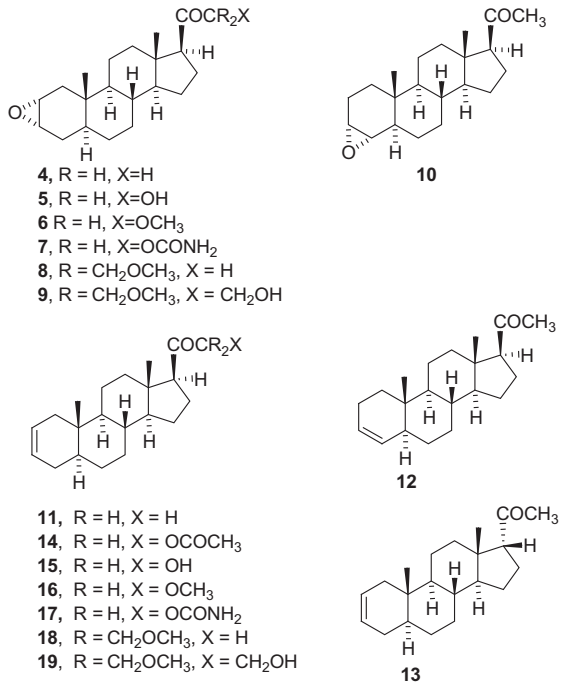

Fig. 2. Epoxides and olefins for their synthesis.

2. Experimental

2.1. Chemical synthesis

2.1.1. General methods

Melting points were determined on a melting point micro apparatus Boetius (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured in chloroform using an Autopol IV (Rudolf Research Analytical, Flanders, USA), $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra of chloroform solutions were recorded on a Bruker IFS 88 spectrometer, wave numbers are given in cm^{-1}. NMR spectra were measured on FT-NMR spectrometers AVANCE-600 (^{1}H at 600 MHz; ^{13}C at 150.9 MHz), Bruker AVANCE-500, and Bruker Avance III (^{1}H at 400 MHz; ^{13}C at 100 MHz) in $CDCl_3$, CD_3COCD_3 and/or C_6D_6. Chemical shifts (^{1}H referenced to TMS; ^{13}C related to the solvent peak – $\delta(CDCl_3)$ 77.0 or δ (C_6D_6) 128.0) are given in ppm (δ-scale), coupling constants in Hz. Homonuclear and heteronuclear 2D-NMR experiments (H,H-COSY, H,H-ROESY, H,H-J-resolved, H,C-HSQC and H,C-HMBC) were used for complete structure assignments of proton and carbon signals. Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals) or alumina containing 5% of gypsum. Preparative TLC (PLC) was carried out on 200 × 200 mm plates coated with a 0.7 mm thick layer of the same material; before use, the plates were kept in atmosphere of ammonia for 2 h. Before evaporation on a rotary evaporator in vacuum (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous magnesium sulphate. Whenever solutions of potassium carbonate, potassium hydrogen carbonate or hydrochloric acid were used, their concentration was always 5%. For column chromatography, 60–120 μm silica gel was used. Reversed-phase preparative HPLC was carried out on Separon SGX RPS C-18 in linear water–methanol gradient (40–60% during 20 min).

General procedure for epoxidation of 5α-pregn-2-ene derivatives. To a solution of olefins **11**, and **15** to **19** (150 mg, 0.5 mmol) in chloroform (5 mL), 3-chloroperoxybenzoic acid (100 mg, 0.66 mmol) was added while stirring, keeping the temperature at 0 °C. After consumption of the starting material (TLC, usually 2 h), the mixture was sequentially washed with aqueous potassium hydrogen sulphite, potassium carbonate and water. After drying with sodium sulphate, the solvent was evaporated under reduced pressure, and the residue was subjected to thin layer chromatography and crystallization.

2.1.2. 2α,3α-Epoxy-5α-pregnan-20-one (**4**)

Olefin **11** gave white crystals of compound **4** (yield: 73%), mp 158–160 °C (acetone/heptane; Refs. [12,13] give 154–155 °C and 158–160 °C); $[\alpha]_D$ + 113 (*c* 0.2) (Ref. [11] gives + 110). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.59 s (3H, H-18); 0.68 ddd (1H, *J* = 14.5, 13.1, 4.0, H-9); 0.76 s (3H, H-19); 0.86 m (1H, H-7α); 1.11 ddd (1H, *J* = 12.5, 10.6, 6.6, H-14); 1.17 m (1H, H-6β); 1.18 m (1H, H-15β); 1.22 m (1H, H-8); 1.32 m (2H, H-5 and H-6α); 1.34 m (1H, H-11β); 1.39 m (1H, H-12α); 1.44 m (1H, H-1α); 1.54 m (2H, H-4β and H-11α); 1.63 m (1H, H-16α); 1.65 m (1H, H-7β); 1.67 m (1H, H-15α); 1.89 m (1H, H-4α); 1.92 dd (1H, *J* = 15.2, 6.0, H-1β); 2.00 ddd (1H, *J* = 12.0, 4.9, 2.8, H-12β); 2.11 s (3H, H-21); 2.14 m (1H, H-16β); 2.52 t (1H, *J* = 9.1); 3.12 dd (1H, *J* = 6.0, 4.0, H-2); 3.16 dt (1H, *J* = 4.0, 2.0, 2.0, H-3); ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 12.92 (C-19); 13.30 (C-18); 20.86 (C-11); 22.74 (C-16); 24.37 (C-15); 28.27 (C-6); 28.96 (C-4); 31.53 (C-21); 31.58 (C-7); 33.63 (C-10); 35.61 (C-8); 36.20 (C-5); 38.26 (C-1); 38.92 (C-12); 44.03 (C-13); 50.95 (C-2); 52.37 (C-3); 53.55 (C-9); 56.47 (C-14); 63.74 (C-17); 209.62 (C-20).

2.1.3. 2α,3α-Epoxy-21-hydroxy-5α-pregnan-20-one (**5**)

Olefin **15** (338 mg, 1.1 mmol) led to compound **5** (290 mg, 82%) as white crystals, mp. 156–158 °C (acetone/heptane). $[\alpha]_D$ + 96.2 (*c* 0.3). IR ($CHCl_3$, cm^{-1}) 3484 (OH); 1705 (C=O). ^{1}H NMR: δ 0.62 s (3H, H-18); 0.75 s (3H, H-19); 2.45 t (1H, *J* = 9.1); 3.12 m (1H, *W* = 13.6, H-3); 3.16 m (1H, *W* = 10.8, H-2); 3.27 (1H, *J* = 4.6, OH); 4.15 dd, 1H and 4.20 dd, 1H (CO–CH_2–O). ^{13}C NMR: δ 12.90 (C-19); 13.38 (C-18); 20.76 (C-11); 22.88 (C-16); 24.45 (C-15); 28.22 (C-6); 28.95 (C-4); 31.57 (C-7); 33.63 (C-10); 35.61 (C-8); 36.18 (C-5); 38.26 (C-1); 38.62 (C-12); 44.72 (C-13); 50.86 (C-2); 52.28 (C-3); 53.50 (C-9); 56.53 (C-14); 59.27 (C-17); 69.36 (C-21); 210.27 (C-20). Anal. Calcd. for $C_{21}H_{32}O_3$ (332.5) 75.86% C, 9.70% H; found: 75.69% C, 9.80% H.

2.1.4. 2α,3α-Epoxy-21-methoxy-5α-pregnan-20-one (**6**)

Olefin **16** (238 mg, 0.72 mmol) gave compound **6** (230 mg, 92%) as white crystals, mp. 107.5–108.5 °C (acetone/heptane). $[\alpha]_D$ + 104.2 (*c* 0.35). IR ($CHCl_3$, cm^{-1}) 2970, 1448, 1100 (OCH_3); 1718 (C=O); 1264 (epoxide). ^{1}H NMR: δ 0.62 s (3H, H-18); 0.75 s (3H, H-19); 2.55 t (1H, *J* = 9.0); 3.11 m (1H, *W* = 13.3, H-3); 3.15 m (1H, *W* = 10.2, H-2); 3.41 s (3H, OMe); 3.97 d, 1H and 4.02 d, 1H (CO–CH_2–O). ^{13}C NMR: δ 12.93 (C-19); 13.49 (C-18); 20.87 (C-11); 22.82 (C-16); 24.54 (C-15); 28.28 C-6); 28.99 (C-4); 31.63 (C-7); 35.67 (C-8); 36.22 (C-5); 38.32 (C-1); 38.81 (C-12); 50.92 (C-2); 52.34 (C-3); 53.59 (C-9); 56.66 (C-14); 58.85 (OMe); 59.21 (C-17); 78.72 (C-21); 208.21 (C-20). Anal. Calcd. for $C_{22}H_{34}O_3$ (346.5) 76.26% C, 9.89% H; found: 76.31% C, 9.90% H.