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Original article

Synthesis and $GABA_A$ receptor activity of A-homo analogues of neuroactive steroids

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ABSTRACT

A procedure is described for the preparation of A-homo-5-pregnenes via an acid catalyzed rearrangement of cyclopropylcarbinols assisted by microwave irradiation. 3α-Hydroxy and 4α-hydroxy-A-homo-5-pregnen-20-one, analogues of the neuroactive steroid allopregnanolone, were obtained by means of a regioselective epoxidation of a double bond in the expanded A-ring, using a fructose-derived chiral ketone as catalyst and oxone as oxidant. Although both these compounds were marginally active in inhibiting TBPS binding to $GABA_A$ receptors, 3β-hydroxy-A-homo-5-pregnen-20-one was almost as active as allopregnanolone. Reduction of the double bond of the latter compound resulted in a ten fold loss of activity.

1. Introduction

The term "neuroactive steroid" (NAS) refers to steroids which, independent of their origin, are capable of modifying neural activities. It is now demonstrated that these neuroactive steroids positively or negatively modulate the function of members of the ligand-gated ion channel receptor superfamily [1–4]. Most of these studies have focused on their positive allosteric actions on γ-amino butyric acid type A receptor ($GABA_A$ receptor) as those elicited by the endogenous steroids allopregnanolone (**1**) and pregnanolone (**2**). The physiological and pharmacological actions of neuroactive steroids are topics of widespread interest, since they have shown to be potent anticonvulsants, anxiolytics, and antistress agents as well as to possess sedative, hypnotic, and anesthetic activities. Structure-activity relationship studies of neuroactive steroids at $GABA_A$ receptors [4] have established a pharmacophore for positive modulation of the receptor by steroids, consisting of a hydrogen bond accepting group (such as $COCH_3$ or CN) in a pseudoequatorial configuration at the 17β position and a hydrogen bond donating hydroxyl group in the 3α configuration. The importance of the steric constraint imposed on the 3α-hydroxyl group by the steroid A ring for GABAergic activities was examined by Covey and coworkers, who prepared a series of nonsteroidal analogues of **1** and **2** that mimicked parts of the steroid nucleus [5,6]. Among these analogues, perhydro benz[*e*]indenes as **3** and **4** were potent modulators of $GABA_A$ receptor function with certain analogues of **4** (with a modified side chain) displaying both potentiating and inhibitory actions [7]. Perhydro benz[*e*]indenes are steroid–like molecules in which the A-ring has been replaced by an open chain of appropriate length, giving the molecule considerable flexibility at the position originally occupied by the critical 3α hydrogen bond donor. The greater flexibility of benz[*e*]indenes would allow the 3-hydroxy group to mimic steroids having either a 3α or a 3β hydroxyl, thus being able to bind to the potentiating and the inhibitory sites on the $GABA_A$ receptor [3]. Consequently, those studies demonstrated that GABAergic activity does not require the 3α-hydroxyl group to be kept in a fixed position by a rigid A ring.

To further explore these effects, we envisaged that a more controlled conformational mobility of the allopregnanolone A ring could be obtained by its expansion to a seven membered ring. Also the additional carbon in the resulting A-homopregnanes would allow further variations in the position of the A ring hydroxyl (e.g. **5** and **6**).

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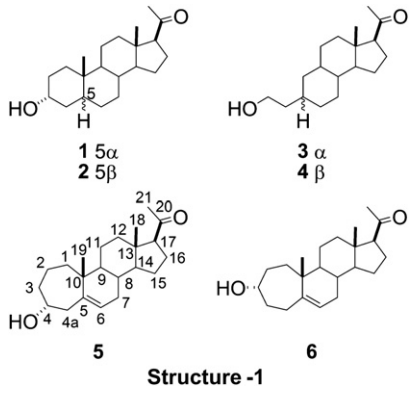


Structure -1

2. Results and discussion

2.1. Chemistry

In a previous publication we showed that treatment of cyclopropyl alcohol **7** with BF_3-Et_2O in dichloromethane gave A-homopregnadiene **8** in moderate yield (Scheme 1; Table 1, entry 1) [8]. Further attempts to improve the yield of this rearrangement using either zinc bromide or aluminum chloride as catalysts at room temperature were unsuccesful as the reaction was slow and gave complex mixtures of byproducts (Table 1, entries 3 and 4). However, when these reactions were carried out under microwave irradiation (MW) very good to excellent yields of the homosteroid (**8**) were obtained (entries 5 and 6). Treatment of the latter compound with $LiAlH_4$ in THF gave alcohol **9.**

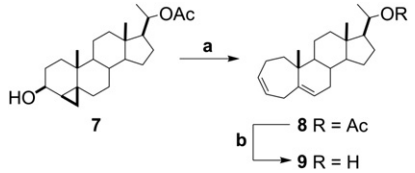
With the A-homosteroid **9** in hand, we focused our attention on the regio and stereoselective functionalization of the Δ^3-double bond. Epoxidation with *m*-chloroperbenzoic acid (MCPBA) gave a mixture of the undesired 5,6-epoxide and the 3,4:5,6-diepoxide. Since this reaction was electronically favored at the most substituted Δ^5-olefin, we turned to dioxirane mediated epoxidation, a highly efficient and stereospecific method towards both electron-rich and electron-deficient olefins [9,10]. Taking into account that the Δ^5-olefin was sterically hindered, we used the chiral and bulky fructose-derived ketone **10** as catalyst and potassium monopersulfate (Oxone) as oxidant. Under these conditions, the 3β,4β-epoxide **11** was obtained as the only product in 56% yield after recovery of unreacted material (Scheme 2). The stereospecific β-epoxidation of diene **9** may be explained by the bent conformation of the ring A towards the α face in this compound [8]. Confirmation of the stereochemistry of epoxide **11** came from NMR data and molecular modelling of the A ring alcohols obtained by reductive cleavage. Treatment of epoxide **11** with $LiAlH_4$ gave a 9:2 mixture of 3β-hydroxy (**12**) and 4β-hydroxy (**13**) A-homopregnenes (77% yield) that could be separated by flash chromatography. The ^{1}H NMR spectrum of diol **13** showed the resonance of H-4 at δ 3.54 as a triplet of triplets ($J = 10.2$ and 5.0 Hz) indicating an axial orientation of this hydrogen. A strong correlation between H-4 and H-6 was observed in the NOESY spectrum; molecular modelling of all conformers of the seven membered A-ring in **13** showed that this was only possible in the most stable conformer of the 4β-alcohol (Fig. 1a). The β orientation of the 4-hydroxyl confirmed the β stereochemistry of epoxide **11** and hence of the hydroxyl at C-3 in **12**. The ^{1}H NMR spectrum of alcohol **12** showed an unresolved multiplet at δ 4.05 ($W_{1/2} = 10.8$ Hz) for H-3, typical of an equatorial hydrogen.

To convert the 3β-oriented axial alcohol in **12** into the neurosteroid analogue **6**, we first attempted an oxidation reduction sequence (Scheme 2). Thus diol **12** was oxidized with pyridinium

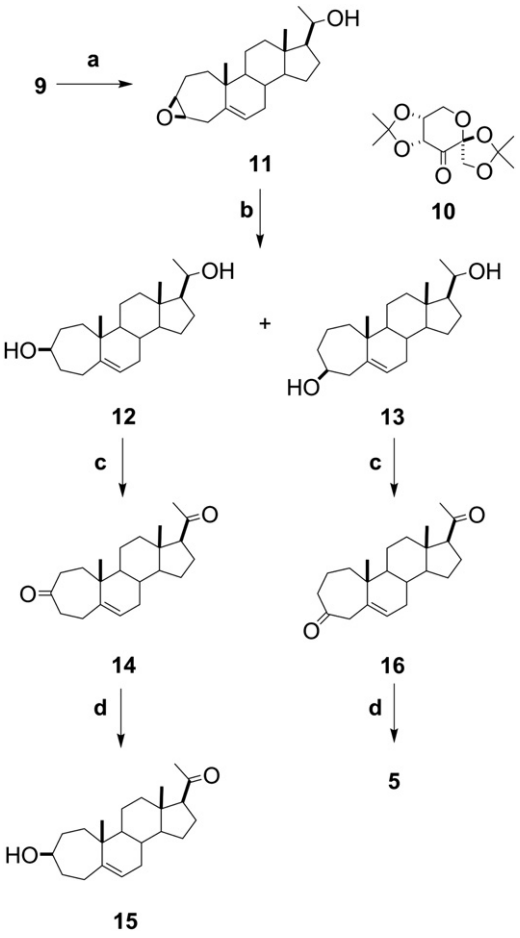
Table 1
Formation of the A-homo steroid **8** from the cyclopropyl alcohol **7** *via* cationic rearrangement.

Entry	Catalyst	Conditions	Yield (%)[a]
1	BF_3-éter/CH_2Cl_2	0 °C, 10 min	70[b]
2	Br_2Zn/THF	25 °C, 28 hs	ND[c]
3	$AlCl_3$/THF	25 °C, 3.5 hs	ND[c]
4	Br_2Zn/THF	MW 120 °C, 10 min	87
5	$AlCl_3$/THF	MW 65 °C, 10 min	80

ND: not determined. MW: microwave irradiation.
[a] After chromatographic purification.
[b] Data taken from ref. [8].
[c] Complex mixture.



Scheme 1. *Reagents and conditions*: (a) see Table 1. (b) i. $LiAlH_4$, THF ii. 1 N HCl.



Scheme 2. *Reagents and conditions*: (a) ketone **10** (30% mol), Oxone, tetrabutylammonium acetate, K_2CO_3(aq), CH_3CN/DME (1:2); (b) i. $LiAlH_4$, THF ii. 1 M HCl; (c) PCC, $BaCO_3$, MS 4Å, CH_2Cl_2; (d) 1 M K-Selectride, THF, −50 °C.