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Chemical synthesis of 7α-hydroxypregnenolone, a neuroactive steroid that stimulates locomotor activity

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ABSTRACT

7α-Hydroxypregnenolone is an endogenous neuroactive steroid that stimulates locomotor activity. A synthesis of 7α-hydroxypregnenolone from pregnenolone, which takes advantage of an orthogonal protecting group strategy, is described. In detail, the C7-position was oxidized with CrO_3 and 3,5-dimethylpyrazole to yield a 7-keto steroid intermediate. The resulting 7-ketone was stereoselectively reduced to the 7α-hydroxy group with lithium tri-*sec*-butylborohydride. In contrast, reduction of the same 7-ketone intermediate with $NaBH_4$ resulted in primarily the 7β-hydroxy epimer. Furthermore, in an alternative route to the target compound, the 7α-hydroxy group was successfully incorporated by direct C–H allylic benzoyloxylation of pregnenolone-3-acetate with CuBr and *tert*-butyl peroxybenzoate followed by saponification. The disclosed syntheses to 7-oxygenated steroids are amenable to potentially obtain other biologically active sterols and steroids.

1. Introduction

7α-Hydroxypregnenolone (Fig. 1, **4**) is a neurosteroid that has been shown to stimulate locomotor activity in newts [1], juvenile birds (biosynthetically produced in the pineal gland) [2], and salmon (stimulating upstream migration) [3]. The biological target that stimulates locomotor activity is unknown, however, it has been suggested that the $GABA_A$ and N-methyl-d-aspartate receptor may be the targets of this neurosteroid since pregnenolone stimulates these receptors [4]. Injection of 7α-hydroxypregnenolone at 6.25 mg/kg body weight has been shown to increase the immune response in mice [5]. Additionally, administration of 7α-hydroxypregnenolone had enhanced spatial memory retention in cognitively impaired aged rats [6]. Moreover, 7α-hydroxypregnenolone has been shown to promote microtubule polymerization [7].

1.1. Biosynthesis of 7α-hydroxypregnenolone

Cytochrome P450 7B1 is the enzyme responsible for directly converting pregnenolone to 7α-hydroxypregnenolone (Fig. 1, **3** to **4**) [8,9]. P450 7B1 has a broad substrate scope and has been shown to 7α-hydroxylate 27-hydroxycholesterol and dehydroepiandrosterone [10]. Mutations in *CYP7B1*, the gene that encodes the P450 7B1 protein, results in motor neuron degeneration and hereditary spastic paraplegia type 5 [11–13].

The 7-oxygenated C19-androgens (i.e. 7α-hydroxy-, 7β-hydroxy-, and 7-oxo- dehydroepiandrosterone) have been shown to be interconverted by 11β-hydroxysteroid dehydrogenase type 1 [14].

Other possible biosynthetic pathways to 7α-hydroxypregnenolone may arise from the activity of the cholesterol side chain cleavage enzyme, P450 11A1 (Fig. 1), onto 7α-hydroxycholesterol, which is the enzymatic product of P450 7A1 acting on cholesterol [15].

Despite its physiological importance, a report on the synthesis of 7α-hydroxypregnenolone with spectral characterization has yet to be disclosed. A previous synthesis without NMR data was reported by treating 7α-bromopregnenolone-3-acetate with acetic acid/sodium acetate – resulting in mixtures of 7α- and 7β- acetoxy epimers, which were saponified in KOH/CH_3OH and purified by preparative silica gel thin-layer chromatography with ethyl acetate [16]. A more recent synthesis of 7α-hydroxypregnenolone has been reported through displacement of an allylic bromide intermediate with $CaCO_3$ in H_2O [7]. A convenient chemical synthesis of this neuroactive steroid may open the possibility of the synthesis of other neuroactive steroid analogs. Here, two complementary syntheses of 7α-hydroxypregnenolone (**4**) from pregnenolone (**3**) are reported.

1.2. Retrosynthetic analysis

The ideal synthesis of 7α-hydroxypregnenolone would come from a one-step biomimetic oxidation (Fig. 1, **3** to **4**), where a diastereo- and

Abbreviations: GABA, gamma-aminobutyric acid; NMR, nuclear magnetic resonance; CYP, cytochrome P450; TLC, thin-layer chromatography

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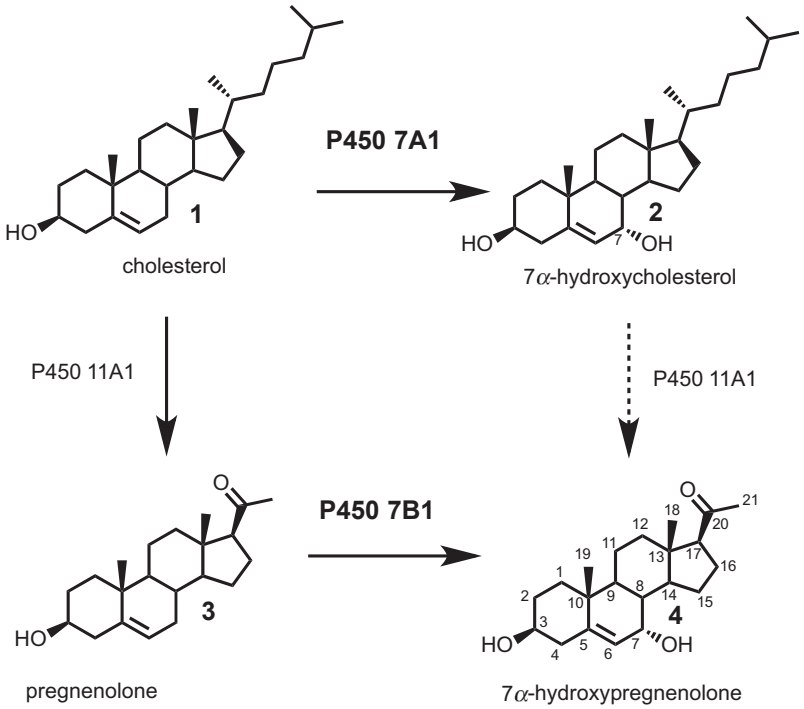

Fig. 1. Biosynthesis of 7α-hydroxypregnenolone (**4**).

regio- selective monohydroxylation would occur at the C7-position of pregnenolone to directly furnish 7α-hydroxypregnenolone. In this situation, there are two allylic positions (C4- and C7-) and during the allylic oxidation conditions, the 3β-hydroxy group will not be oxidized. Realistically, the 3-hydroxy group should be protected during the C7-H oxidation process to avoid oxidation to the 3-keto moiety.

Because of the potential oxidation of any free hydroxy during the proposed allylic oxidation process (*vide supra*, **3** to **4**), the chemical synthesis required the protection of the 3-hydroxy group of pregnenolone as the acetate (Fig. 2, Schemes 1 and 3, **9**). The available method to introduce a 7α-hydroxy substituent in the steroid backbone involved the regioselective oxidation of the C7-position to the 7-keto group using CrO_3 and 3,5-dimethylpyrazole, followed by diastereoselective reduction with L-selectride. This strategy was previously employed in the synthesis of 7α-hydroxycholestenone from cholesterol [17]. Other methods of C7-oxidation to the ketone exist, which involves a dirhodium-caprolactamate complex in the presence of *tert*-butyl hydroperoxide [18]. In order to follow the oxidation and subsequent diastereoselective reduction strategy, the C20-keto group of pregnenolone would have to be masked. The C20-ketone was therefore reduced to the alcohol, which was orthogonally protected as the silyl ether (Fig. 2, **8**), allowing the 3- and 7- hydroxy groups to be protected as acetates (**6**).

2. Results and discussion

To begin, pregnenolone was protected as the acetate to furnish pregnenolone-acetate (Scheme 1, **3** to **9**). The resulting C20-keto group was reduced with $NaBH_4$ to afford the C20-alcohol (**10**). The *R*-stereochemistry at the C20 position of alcohol **10** was determined by the chemical shift of the C18-methyl (δ 0.75 ppm) in comparison to the known literature value (δ 0.78 ppm for the C20-*R* stereochemistry compared with δ 0.68 ppm for the C20-*S* stereochemistry) [19]. In addition, the reduction of pregnenolone-3-acetate (**9**) has been performed with $NaBH_4$ [20] or L-selectride [21] to afford the alcohol (**10**) in 99% or 96% reported yields prior to purification. Furthermore, the

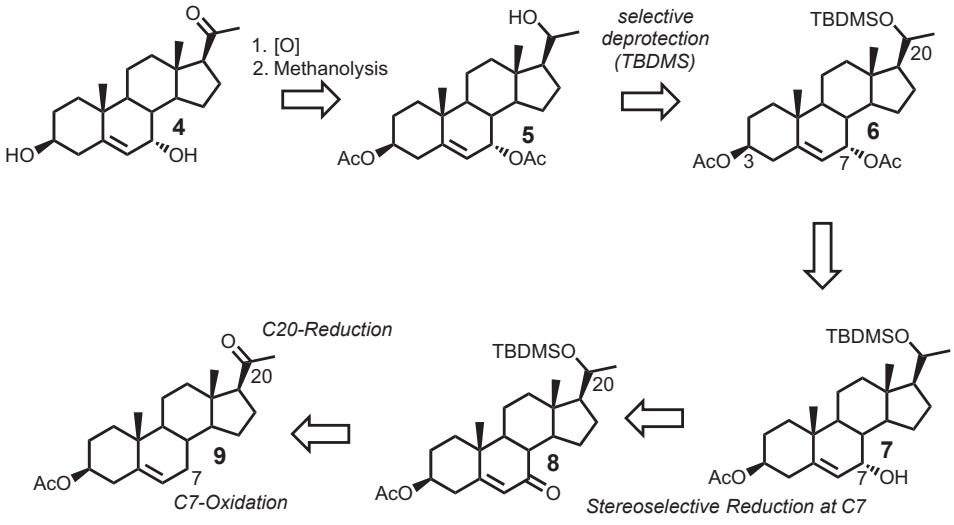

Fig. 2. Retrosynthetic analysis of 7α-hydroxypregnenolone using an orthogonal protecting group strategy at C3 and C7 vs. C20 (**6**).

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