ALLOPREGNANOLONE DERIVATIVES: SYNTHESIS OF 3α-HYDROXY-7a-HOMO-5α-PREGNAN-20-ONE+

Hana CHODOUNSKÁ[1,*] and Alexander KASAL[2]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic; e-mail: [1] hchod@uochb.cas.cz, [2] kasal@uochb.cas.cz

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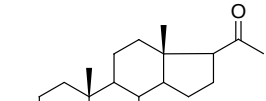
3α-Hydroxy-7a-homo-5α-pregnan-20-one and 3α-hydroxy-7a-homo-5α-pregnane-7,20-dione were prepared from (20*R*)-3α-(methoxymethoxy)-7-oxo-5α-pregnan-20-yl benzoate. Homologation of the B ring was carried out by the Demyanov rearrangement of the corresponding cyanohydrin. Alkaline hydrolysis of the protecting group and oxidation of the formed 20-hydroxy group and deprotection of the 3-hydroxy group were performed either with (20*R*)-3α-(methoxymethoxy)-7-oxo-7a-homo-5α-pregnan-20-ol derivative or with the product of the Huang Minlon deoxygenation at carbon 7.

Key words: Steroids; Epalons; Allopregnanolone; $GABA_A$-Modulators; 3α,5α-Tetrahydroprogesterone; Huang Minlon reduction.

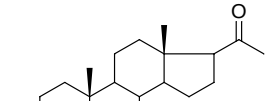
A link between mental health and the level of reproductive steroids has been long suspected; *e.g.* epileptic seizures were most frequent in preovulatory-phase women with the peak levels of circulating estrogens; the least frequency was found for the luteal phase characterized by a high level of progesterone[2] (**1**). Progesterone was experimentally shown to reduce the seizure frequency to a half, which, however, could be prevented by co-application of 5α-reductase inhibitors blocking its conversion into dihydro (**2**) and tetrahydro (**3**) derivatives[3]. One of the progesterone metabolites – 3α-hydroxy-5α-pregnan-20-one (**3**, allopregnanolone) was identified as an endogenous neurosteroid, which binds to the α_4 subunit of the $GABA_A$ receptor[4]. The neuroprotective properties of this compound are about 10 times higher than those of progesterone (ED_{50} 10–30 mg/kg *vs* 180 mg/kg) but about 10 times lower than those of diazepam. In spite of that,

+ Part CDVIII in the series On Steroids; Part CDVII see ref.[1]

interest in the new allopregnanolone derivatives is maintained because tolerance to activity of neuroactive steroids does not develop as it does with other $GABA_A$ potentiating drugs (*e.g.* benzodiazepines[5]). Since the forties until the eighties, alphaxolone (3α-hydroxy-5α-pregnane-11,20-dione, **4**) was used as an anaesthetic, however its use was discontinued owing to occasional allergy bouts[6]. The recently introduced allopregnanolone derivative – "ganaxolone" (3α-hydroxy-3-methyl-5α-pregnan-20-one[7], **5**) has an additional advantage: a higher stability in the body (the endogenous allopregnanolone derivative is metabolized very fast[5], $t_{1/2}$ = 16–19 min).



1



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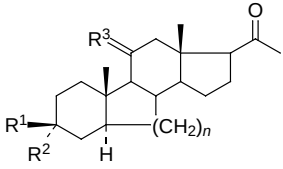
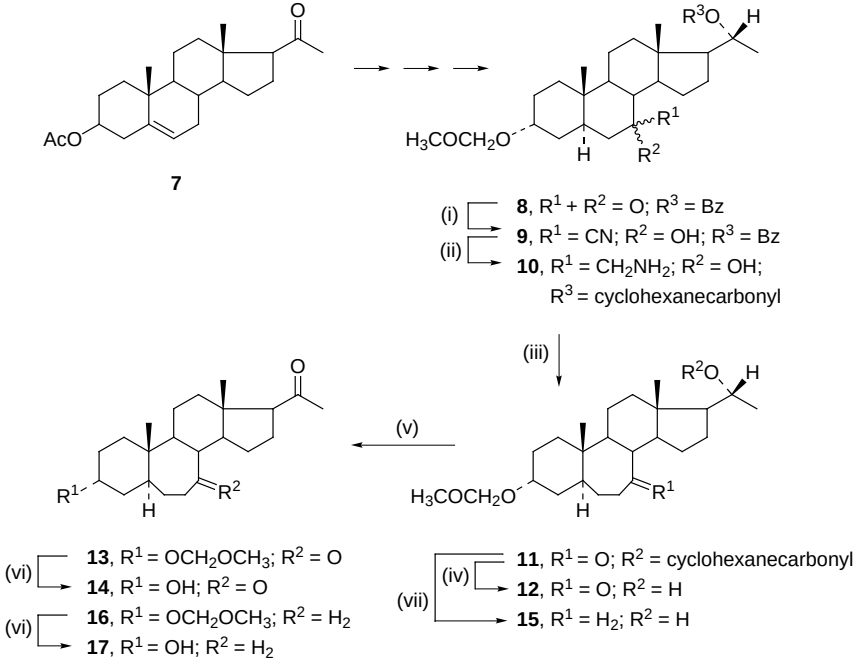


3, R^1 = H; R^2 = OH; R^3 = H_2; n = 2
4, R^1 = H; R^2 = OH; R^3 = O; n = 2
5, R^1 = CH_3; R^2 = OH; R^3 = H_2; n = 2
6, R^1 = H; R^2 = OH; R^3 = H_2; n = 1

Though a lot has been found out about the structure activity relationship[8,9], nothing is known about the neural activity of B-modified steroids apart from the information on the lower activity of 7-oxoallopregnanolone[10]. Recently we published a paper on the synthesis of B-norallopregnanolone[11] (3α-hydroxy-7-nor-5α-pregnan-20-one, **6**), one of whose derivatives binds the $GABA_A$ receptor[12]. The present paper describes the synthesis of 7a-homoallopregnanolone and its derivatives, the physiological activity of which could enrich our understanding of the allopregnanolone binding site.

The synthesis (see Scheme 1) started with 20-oxopregn-5-en-3β-yl acetate (**7**) which was converted into (20*R*)-3α-(methoxymethoxy)-7-oxo-5α-pregnan-20-yl benzoate (**8**) in 8 steps[13]. Ketone **8** was treated with hydrogen cyanide to yield cyanohydrin **9** which was hydrogenated to amino alcohol **10** using the Adams catalyst in acetic acid. On diazotation of compound **10**, ketone **11** was formed: its oxo group was flanked by two methylene groups, as suggested by the IR spectrum (see Experimental, CH_2 group band at 1 431 cm^{-1}). The final proof was brought by ^1H and ^{13}C NMR spectra. Starting from the unambiguously assigned signals in the 1D ^1H

spectrum it was possible to deduce proton–proton coupling patterns from $^1H,^1H$ 2D-COSY spectra and complete the structure assignment of all protons. The APT ^{13}C NMR spectra were used to determine chemical shifts and distinguish the multiplicity of individual carbon signals. The structure assignment of CH, CH_2, and CH_3 signals was derived from $^1H,^{13}C$ 2D-HMQC spectra by correlation with the previously assigned proton signals. The remaining quaternary carbons were assigned from the chemical shifts (see Experimental). Apparently, it was the more substituted carbon C-8 that migrated to bind the intermediate C-7a carbonium ion within the Demyanov rearrangement.



(i) HCN, AcOH; (ii) Adams' catalyst, H_2, AcOH; (iii) $NaNO_2$, H^+, AcOH–H_2O; (iv) OH^-, MeOH; (v) Jones reagent, $(CH_3)_2CO$; (vi) H^+, MeOH; (vii) $N_2H_4 \cdot 2H_2O$, OH^-, diethylene glycol

SCHEME 1

The hydrolysis of cyclohexanecarboxylate **11**, oxidation of alcohol **12** and deprotection of the methoxymethoxy ether **13** led to 7-oxo-7a-homoallopregnanolone **14**. Its 7-deoxy analogue **17** was prepared from the above-mentioned intermediate **11**: its Huang Minlon reduction afforded

compound **15** which was analogously oxidized and deprotected to 7a-homoallopregnanolone **17**.

IR and ^{1}H NMR spectra of all intermediates agreed with the proposed structures, molecular rotation differences of the intermediates and 7-homo-5α-cholestane standards[14] were compatible with the structures. Binding of compounds **14** and **17** to $GABA_A$ receptor will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Circular dichroism (Mark V apparatus) was measured in methanol. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer in chloroform. ^{1}H NMR and ^{13}C NMR spectra were measured on a Varian UNITY-200 FT NMR spectrometer (^{1}H at 200.04 MHz and 500 MHz and ^{13}C at 125.7 MHz) in $CDCl_3$ or $CDCl_3$–C_6D_6 (1 : 2) with TMS as internal reference. The absolute value ^{1}H,^{1}H 2D-COSY spectra were obtained with spectral width 5 kHz, pulse width 13 μs (90°), relaxation delay 1 s, acquisition time 0.17 s. Four free induction decays (FIDs) were acquired for each of 416 time increments and the data matrix was zero filled to 2 048 a 2 048 data points. A sine bell weighing function was used before Fourier transformation in both dimensions. Chemical shifts in ppm (δ-scale) were referenced to internal tetramethylsilane. Coupling constants (*J*) and width of multiplets (*W*) are given in Hz. Thin-layer chromatography (ICN Silica G, TLC-60 A) was used for checking the purity of individual intermediates. Column chromatography was carried out on silica gel (Kieselgel 60, Merck, 0.04–0.063 mm) using a slight overpressure. The concentration of the used aqueous solutions of hydrochloric acid, potassium hydrogencarbonate and potassium carbonate was 5%. Solutions in organic solvents were dried with magnesium sulfate, the solvents were removed using a rotatory vacuum evaporator at 50 °C.

(20*R*)-7-Cyano-7-hydroxy-3α-(methoxymethoxy)-5α-pregnan-20-yl Benzoate (**9**)

Ketone[13] **8** (670 mg, 1.39 mmol) was dissolved in dioxane (6.7 ml) and ethanol (6.7 ml) and cooled to 0 °C. The stirred solution was treated with powdered potassium cyanide (969 mg, 14.9 mmol) and then acetic acid (0.95 ml, 16.6 mmol) diluted with dioxane (2 ml) was added dropwise during 30 min under stirring. The mixture was stirred at 0 °C for 1 h and at room temperature for additional 2 h. It was then poured into a mixture of ice and water. After 1 h, the precipitate was separated by suction. The product was dissolved in ether, the solution washed with a sodium hydrogencarbonate solution and water. Two-dimensional TLC showed that cyanohydrin **9** decomposed on contact with silica gel yielding starting ketone **8**. The solid residue **9** (700 mg; 99%) crystallized from ethyl acetate, m.p. 103–106 °C, $[\alpha]_D$ –10 (*c* 1.2). IR: 3 588 (O–H); 2 233 (C≡N); 1 707 (C=O); 1 147, 1 039, 1 096, 912 (C–O–C–O–C). ^{1}H NMR: 8.05 m, 2 H (H-2 and H-6, benzoate); 7.48 m, 3 H (H-3, H-4 and H-5, benzoate); 5.14 m, 1 H (H-20); 4.67 s, 2 H (OCH_2O); 3.82 quintet, 1 H, *J* = 2.5 (H-3); 3.36 s, 3 H (CH_3-O); 1.28 d, 3 H, *J* = 6 (3 × H-21); 0.77 s, 3 H (3 × H-18); 0.69 s, 3 H (3 × H-19). For

$C_{31}H_{43}NO_5$ (509.7) calculated: 73.05% C, 8.50% H, 2.75% N; found: 72.78% C, 8.60% H, 2.37% N.

(20*R*)-3α-(Methoxymethoxy)-7-oxo-7a-homo-5α-pregnan-20-yl Cyclohexanecarboxylate (**11**)

The cyanohydrin **9** (300 mg, 0.59 mmol) was dissolved in acetic acid (6 ml), Adams' catalyst was added (40 mg) and mixture hydrogenated for 16 h. The catalyst was filtered off and washed with chloroform and acetone. Most solvents was evaporated on a vacuum evaporator yielding crude (20*R*)-7-(aminomethyl)-7-hydroxy-3α-(methoxymethoxy)-5α-pregnan-20-yl cyclohexanecarboxylate (**10**) in acetic acid. A solution of sodium nitrite (1.0 g, 14.5 mmol) in water (3 ml) was then added dropwise to the solution under stirring at 0 °C. The mixture was stirred at room temperature for additional 2 h, diluted with water (10 ml) and the precipitate formed was extracted with ether. The extract was washed with a sodium hydrogencarbonate solution and water. The residue after removal of the solvent was chromatographed on a silica gel column in benzene–ether (49 : 1). The major product crystallized from methanol yielding 7a-homoketone **11** (136 mg; 49%), m.p. 123–126 °C, $[\alpha]_D$ –10 (*c* 0.8). CD: $\Delta\varepsilon_{286}$ –2.12. IR: 1 713, 1 698 (C=O); 1 431 (H–C–H, CH_2CO); 1 248 (C–O); 1 146, 1 040, 1 096, 914 (C–O–C–O–C). ^{1}H NMR: 4.84 m, 1 H (H-20); 4.66 s, 2 H (OCH_2O); 3.83 quintet, 1 H, *J* = 2.5 (H-3); 3.38 s, 3 H (CH_3O); 2.59–2.00 m, 4 H (2 × H-7 and 2 × H-8); 1.11 d, 3 H, *J* = 6 (3 × H-21); 0.79 s, 3 H (3 × H-19); 0.64 s, 3 H (3 × H-18). MS, *m*/*z* (%): 374 (100, $M^+ - C_6H_{11}COOH - C_2H_4O$). For $C_{31}H_{50}O_5$ (504.7) calculated: 74.06% C, 10.02% H; found: 73.95% C, 10.11% H.

(20*R*)-20-Hydroxy-3α-(methoxymethoxy)-7a-homo-5α-pregnan-7-one (**12**)

A solution of compound **11** (100 mg, 0.20 mmol) and sodium hydroxide (0.4 g, 15 mmol) in methanol (10 ml) was heated on a water bath for 4 h. After dilution with water, compound 12 (75 mg, 95%) was isolated by filtration. M.p. 79–82 °C (acetone), $[\alpha]_D$ –53 (*c* 0.6). IR: 3 610 (O–H); 1 694 (C=O); 1 147, 1 041, 1 099, 913 (C–O–C–O–C). ^{1}H NMR: 4.66 s, 2 H (OCH_2O); 3.83 quintet, 1 H, *J* = 2.5 (H-3); 3.73 m, 1 H (H-20); 3.38 s, 3 H (OCH_3); 2.59–2.00 m, 4 H (2 × H-7 and 2 × H-8); 1.13 d, 3 H, *J* = 6 (3 × H-21); 0.80 s, 3 H (3 × H-18); 0.76 s, 3 H (3 × H-19). For $C_{23}H_{38}O_4$ (392.6) calculated: 73.43% C, 10.27% H; found: 73.75% C, 10.31% H.

3α-(Methoxymethoxy)-7a-homo-5α-pregnane-7,20-dione (**13**)

A solution of hydroxy derivative **12** (60 mg, 0.14 mmol) in benzene (5 ml) was stirred with pyridinium chlorochromate on aluminium oxide (200 mg, 0.2 mmol) at room temperature. After 8 h, inorganic solids were filtered off and the filtrate was evaporated. Crystallization from ether afforded 57 mg (95%) of compound **13**, m.p. 69–72 °C (ether–ligroin), $[\alpha]_D$ –5 (*c* 0.3). IR: 1 698 (C=O); 1 147, 1 095, 1 041, 914 (C–O–C–O–C). ^{1}H NMR: 4.66 s, 2 H (OCH_2O); 3.83 quintet, 1 H, *J* = 2.5 (H-3); 3.38 s, 3 H (CH_3O); 2.11 s, 3 H (3 × H-21); 0.79 s, 3 H (3 × H-19); 0.62 s, 3 H (3 × H-18). For $C_{24}H_{38}O_4$ (390.6) calculated: 73.81% C, 9.81% H; found: 73.84% C, 9.92% H.

3α-Hydroxy-7a-homo-5α-pregnane-7,20-dione (**14**)

Alcohol **13** (50 mg, 0.12 mmol) in a mixture of benzene (10 ml) and methanol (10 ml) was treated with 2 M hydrochloric acid (0.8 ml) at 40 °C. After 6 h, ether (50 ml) was added and

the solution was washed with a potassium hydrogencarbonate solution and water. The dried extract was evaporated and purified by chromatography on a thin layer of silica gel (ligroin–ether 4 : 1). Compound **14** (32 mg; 66%) has a m.p. 148–150 °C (ether), $[\alpha]_D$ +33 (*c* 0.8). CD: $\Delta\varepsilon_{295}$ +9.0. IR: 3 616, 3 490 (O–H); 1 698 (C=O); 1 016 (C–OH). MS, *m*/*z* (%): 346 (48, M^+); 328 (88, $M^+ - 18$). ^{1}H NMR: 4.05 quintet, 1 H, *J* = 2.5 (H-3); 2.12 s, 3 H (3 × H-21); 0.79 s, 3 H (3 × H-19); 0.62 s, 3 H (3 × H-18). ^{13}C NMR ($CDCl_3$–C_6D_6 1 : 2, 2D-HMQC, ^{1}H, ^{13}C): 213.26 (C-7); 209.02 (C-20); 65.43 (C-3); 63.79 (C-17); 58.34 (C-9); 56.19 (C-14); 48.50 (C-6); 47.77 (7a); 43.66 (C-13); 39.47 (C-10); 38.94 (C-12); 38.32 (C-4); 35.99 (C-8); 34.84 (C-5); 32.89 (C-1); 31.37 (C-21); 28.73 (C-2); 25.99 (C-15); 22.82 (C-11); 22.29 (C-16); 13.03 (C-18); 12.37 (C-19). ^{1}H NMR ($CDCl_3$–C_6D_6 1 : 2, 2D-COSY, ^{1}H, ^{1}H): 3.64 (H-3β); 2.27 (H-7ax); 2.22 (H-7ay); 2.18 (H-6x); 2.16 (H-17); 2.10 (H-16x); 2.05 (H-5); 1.89 (6-Hx); 1.84 (H-21); 1.75 (H-12x); 1.66 (H-11x); 1.58 (H-15x); 1.45 (H-16x); 1.40 (H-1x); 1.38 (H-1x); 1.35; (H-2x); 1.32 (H-8); 1.22 (H-4x); 1.19 (H-2x); 1.12 (H-15x); 1.10 (H-4x); 1.09 (H-12x); 1.08 (H-11x); 0.95 (H-14); 0.89 (H-9); 0.53 (H-19); 0.48 (H-18). For $C_{22}H_{34}O_3$ (334.5) calculated: 76.26% C, 9.89% H; found: 75.94% C, 10.02% H.

(20*R*)-3α-(Methoxymethoxy)-7a-homo-5α-pregnan-20-ol (**15**)

Compound **11** (120 mg, 0.24 mmol) was dissolved in diethylene glycol (10 ml). After addition of 100% hydrazine hydrate (2 ml, 41 mmol), the mixture was refluxed for 2 h. Then it was cooled and potassium hydroxide (400 mg, 7.1 mmol) dissolved in minimum volume of water was added. The solvents boiling below 180 °C were distilled off and the solution was refluxed for another 2 h. On cooling, the mixture was poured into water and acidified with hydrochloric acid. The precipitate was extracted with ether and the extract was washed with a saturated sodium hydrogencarbonate solution and water. Evaporation of the solvents afforded 90 mg of a mixture. Chromatography on a column of silica gel (5 g) gave 30 mg (33%) of compound **15**, m.p. 105–107 °C (methanol), $[\alpha]_D$ –31 (*c* 0.6). IR: 3 610 (O–H); 1 147, 1 095, 1 041, 914 (C–O–C–O–C). ^{1}H NMR: 4.64 s, 2 H (OCH_2O); 3.76 quintet, 1 H, *J* = 2.5 (H-3); 3.38 s, 3 H (CH_3O); 1.11 d, 3 H, *J* = 6 (3 × H-21); 0.79 s, 3 H (3 × H-19); 0.74 s, 3 H (3 × H-18). For $C_{24}H_{40}O_3$ (378.6) calculated: 76.14% C, 11.18% H; found: 76.29% C, 11.26% H.

3α-(Methoxymethoxy)-7a-homo-5α-pregnan-20-one (**16**)

To a solution of hydroxy derivative **15** (30 mg, 0.08 mmol) in acetone (4 ml) was added dropwise the Jones' reagent until the yellow colour of the mixture persisted for 5 min. Then the excess of reagent was destroyed with methanol. The mixture was poured into water, the precipitate was extracted with ethyl acetate, the extract was washed with dilute hydrochloric acid, water, a sodium hydrogencarbonate solution and water. Evaporation of the solvent afforded 30 mg (99%) of the product **15** which was used directly in the next step. $[\alpha]_D$ +14 (*c* 0.8). IR: 1 699 (C=O); 1 147, 1 095, 1 041, 914 (C–O–C–O–C). ^{1}H NMR: 4.64 s, 2 H (OCH_2O); 3.76 quintet, 1 H, *J* = 2.5 (H-3); 3.38 s, 3 H (CH_3O); 2.09 s, 3 H (3 × H-21); 0.79 s, 3 H (3 × H-19); 0.63 s, 3 H (3 × H-18).

3α-Hydroxy-7a-homo-5α-pregnan-20-one (**17**)

Hydrochloric acid (0.2 ml; 37%) was added to a solution of ketone **16** (30 mg, 0.08 mmol) in methanol (5 ml) and the mixture was refluxed for 3.5 h. The precipitate formed on dilution with water was extracted with ethyl acetate, washed with water, a sodium hydrogen-

carbonate solution, and water. The residue after evaporation of solvents was chromatographed on a thin layer of silica gel (benzene–ether 4 : 1). Yield of compound **17** was 19 mg (63%), m.p. 67–72 °C (ether), $[\alpha]_D$ +40 (*c* 1.0). IR: 3 400 (O–H); 1 698 (C=O). ^{1}H NMR: 3.99 quintet, 1 H, *J* = 2.5 (H-3); 2.54 t, 1 H, *J* = 8.7 (H-17); 2.11 s, 3 H (3 × H-21); 0.80 s, 3 H (3 × H-19); 0.62 s, 3 H (3 × H-18). MS, *m*/*z* (%): 332 (49, M^+); 314 (82, M – 18); 149 (88). For $C_{22}H_{38}O_4$ (332.5) calculated: 79.46% C, 10.91% H; found: 78.84% C, 10.99% H.

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