EPALONS: SYNTHESIS OF NEW 16α-CARBOXYMETHYL DERIVATIVES+

Barbora SLAVÍKOVÁ[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] barbora@uochb.cas.cz, [2] kasal@uochb.cas.cz

Received June 25, 1999
Accepted August 18, 1999

The title compounds (16α-carboxymethyl-3α-hydroxy-5α-pregnan-20-one and its sodium and triethylammonium salts) were prepared from 20-oxopregna-5,16-dien-3β-yl acetate by conjugate addition of dimethyl malonate and decarboxylation. Inversion of configuration at carbon C-3 was carried out by Mitsunobu acylation with diethyl azodicarboxylate and formic acid in the presence of triphenylphosphine.

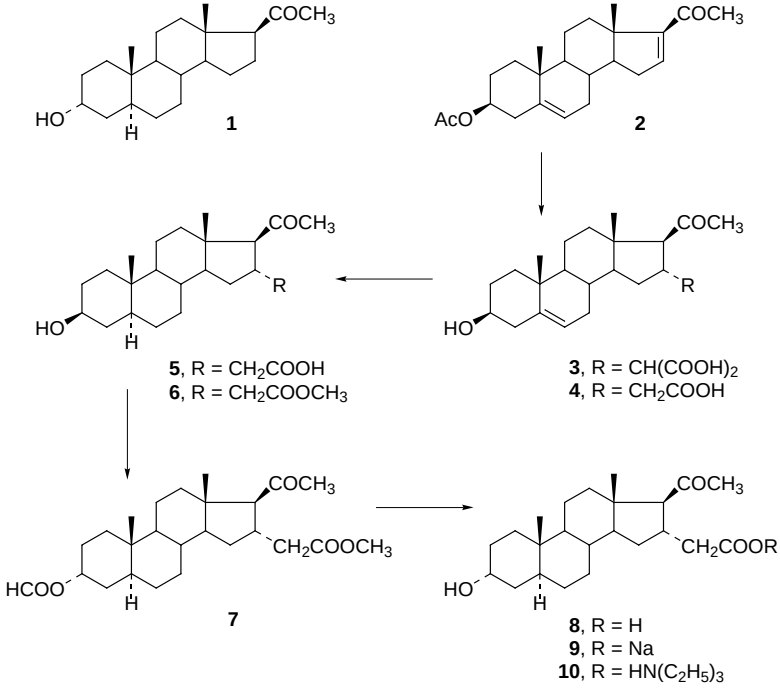
Key words: Steroids; Epalon; 3α,5α-Tetrahydroprogesterone; $GABA_A$-Receptor; Mitsunobu reaction; NMR spectroscopy, ^1H.

Endogenous anaesthetic neurosteroids (*e.g.* compound **1**, see Scheme 1; in medical literature called "allopregnanolone" or "3α,5α-tetrahydroprogesterone" or THP) function in the body by positive allosteric modulation of action of γ-aminobutyric acid ($GABA_A$) to its receptors in neuronal membranes. Thus, these compounds help to stop the transmission of excessive signals of pain and anxiety. They have been used clinically[2] as short-acting anaesthetics only because they are rapidly metabolized and removed from the tissue. Interest in neurosteroids as potential analgesics and tranquillizers recently revived in the hope of finding new compounds with slower metabolism and higher solubility in body fluids.

The conversion of 3α-alcohols into 3α-sulfates and phosphates does not fully answer the problem of stability because rapid equilibrium[3] between the conjugates and the corresponding free 3α-alcohol anyway opens the way to inactivation of such derivatives. The stability is rather achieved by the conversion of the secondary 3α-alcohols into tertiary[4] ones.

+ Part CDIV in the series On Steroids; Part CDIII see ref.[1]

Quite a different type of structure modification consists in the introduction of a solubilizing moiety into the molecule of the parent compound **1**. This approach turned out to be successful[5]: a quaternary amino group in the 2β-position made the compound soluble in water. A similar procedure is now described here: a carboxymethyl group was introduced into the molecule of "allopregnanolone" (**1**).



SCHEME 1

The carboxymethyl group has been previously used as a spacer for binding a steroid hapten to protein in the preparation of antigens. Conjugate addition of diethyl malonate to derivatives of androst-15-en-17-one[6,7] or pregn-16-en-20-one[8] produced corresponding haptens with the carboxymethyl group in positions 15 and 16. Since Atkinson's classical paper on neurosteroids[9] claimed that the methyl group in positions 16α and 16β did not substantially alter the binding of such products with $GABA_A$ receptors, 16α-carboxymethyl-3α-hydroxy-5α-pregnan-20-one (**8**) might be a suitable emulator competing with endogenous compound **1**.

20-Oxopregna-5,16-dien-3β-yl acetate (**2**) was treated with dimethyl malonate in the presence of sodium methoxide in methanol; the adduct

was immediately hydrolyzed to diacid **3**. Its decarboxylation was achieved by heating at 200 °C without solvent and the monoacid obtained (**4**) was hydrogenated to the compound **5** using palladium on calcium carbonate. Before the inversion of the configuration at carbon 3 by the Mitsunobu reaction[10], the carboxylic group in compound **5** was protected by esterification. On treatment of the 3β-hydroxy derivative **6** with diethyl azodicarboxylate, triphenylphosphine and formic acid, 3α-formate **7** was formed. Both ester groupings in compound **7** were hydrolyzed to yield the target compound **8**. For binding assays, sodium and triethylammonium salts **9** and **10** were also prepared.

The identity of each product was determined using IR and ^{1}H NMR spectra. The introduction of the 16α-substituent was clearly manifested by the presence of a signal of H-16a proton(s) at δ 3.20 (multiplet, compound **3**) or δ 2.13 to 2.30 (doublet, compounds **4**, **5**, **8** to **10**; two doublets of doublets in esters **6** and **7**). All the above 16-substituted derivatives had a broad multiplet at δ 2.78 to 3.20 due to the 16β-proton. Still another significant signal confirmed the presence of the 16α-substituent: a triplet of the H-17 proton, which appears at δ 2.50 (J = 9.0 Hz) in unsubstituted 20-oxopregnane derivatives (*e.g.* compound **1**), collapses into a doublet (J = 8.8 Hz) in compounds **3** to **10**.

Compounds **8**–**10** were subjected to biological screening using [^{3}H]-muscimol binding to $GABA_A$-receptors from synaptic membranes of the male rat brain, the results will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured in chloroform (unless stated otherwise), $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}, IR spectra of chloroform solutions (unless stated otherwise) were recorded on a Bruker IFS 88 spectrometer, wavenumbers are given in cm^{-1}. NMR spectra were measured on an FT NMR spectrometer Varian UNITY-200 (at 200 MHz) in $CDCl_3$ with tetramethylsilane as internal reference (unless stated otherwise). Chemical shifts are given in ppm (δ-scale), coupling constants (J) and multiplet half-width ($W_{1/2}$) in Hz. Unless otherwise stated, the data were interpreted as the first-order spectra. Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals). For column chromatography, silica gel 60–120 μm was used. Prior to evaporation on a rotary evaporator in vacuum, bath temperature 50 °C, solutions in organic solvents were dried over anhydrous sodium sulfate.

16α-(Dicarboxymethyl)-3β-hydroxypregn-5-en-20-one (**3**)

Dimethyl malonate (1.32 g, 10.0 mmol) and 20-oxopregna-5,16-dien-3β-yl acetate (**2**; 712 mg, 2.0 mmol) were added to a solution of sodium (200 mg, 8.7 mmol) in methanol

(25 ml). After 3 h reflux a solution of potassium hydroxide (4.0 g, 71.3 mmol) in aqueous ethanol (50%, 20 ml) was added and the reaction mixture was refluxed for 5 min, then diluted with water and extracted with ethyl acetate. The organic layer was washed with water and dried; evaporation of the solvent gave the starting compound **2** (23 mg, 3%). The aqueous layer was acidified with dilute hydrochloric acid (10%) and the white precipitate was filtered off and dried in the vacuum desiccator at 60 °C. Yield: 752 mg (90%) of compound **3**; m.p. 249–252 °C (acetone–methanol) (ref.[11] gives 250–253 °C). ^{1}H NMR: 0.66 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 2.13 s, 3 H (3 × H-21); 2.76 d, 1 H, J = 8.8 (H-17); 3.20 m, 2 H, $W_{1/2}$ = 5 (H-16 and H-16a); 3.5 m, 1 H, $W_{1/2}$ = 22 (H-3); 5.34 m, 1 H, $W_{1/2}$ = 8 (H-6).

16α-Carboxymethyl-3β-hydroxypregn-5-en-20-one (**4**)

Dicarboxylic acid **3** (50 mg, 0.12 mmol) was heated for 1 h at 200 °C in vacuum (2.67 kPa) affording compound **4** (43 mg, 96%); m.p. 242–247 °C (aqueous methanol) (ref.[8] gives 254–258 °C, ref.[12] gives 211–213 °C). IR: 3 609, 3 511 (OH); 1 743, 1 703 (C=O); 1 045 (C–OH). ^{1}H NMR: 0.65 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.25 d, 2 H, J = 8.1 (2 × H-16a); 2.40 d, 1 H, J = 8.8 (H-17); 2.98 m, 1 H, $W_{1/2}$ = 22 (H-16); 3.50 m, 1 H, $W_{1/2}$ = 22 (H-3); 5.32 m, 1 H, $W_{1/2}$ = 7.2 (H-6).

16α-Carboxymethyl-3β-hydroxy-5α-pregnan-20-one (**5**)

A solution of olefin **4** (2.0 g, 5.3 mmol) in dichloromethane (20 ml) and methanol (120 ml) was stirred with a palladium catalyst (5% Pd/$CaCO_3$, 600 mg) in a hydrogen atmosphere at room temperature for 3 h. After filtration the solvent was evaporated in vacuum. The residue (2.0 g, 99%) was crystallized from ethanol–toluene; m.p. 232–235 °C, $[\alpha]_D$ +61 (*c* 1.4, chloroform–methanol 1 : 1). IR (KBr): 3 493 (OH); 1 738, 1 682 (C=O); 1 029 (C–OH). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.77 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.30 d, 2 H, J = 7.6 (2 × H-16a); 2.38 d, 1 H, J = 8.8 (H-17); 3.00 m, 1 H, $W_{1/2}$ = 22 (H-16); 3.6 m, 1 H, $W_{1/2}$ = 22 (H-3). For $C_{23}H_{36}O_4$ (376.5) calculated: 73.37% C, 9.64% H; found: 73.26% C, 9.51% H.

3β-Hydroxy-16α-[(methoxycarbonyl)methyl]-5α-pregnan-20-one (**6**)

A solution of carboxylic acid **5** (2.0 g, 5.3 mmol) in methanol (200 ml) was treated with hydrochloric acid (35%, 2.5 ml, 28.3 mmol) in methanol (15 ml). The reaction mixture was allowed to stand at room temperature for 24 h. Potassium hydrogencarbonate (2.83 g, 28.3 mmol) was added and the reaction mixture was concentrated to a quarter of its volume. The product was extracted with chloroform, washed with brine (saturated solution of sodium chloride in water) and dried. The solvent was evaporated and the residue crystallized from acetone–heptane to yield compound **6** (1.4 g, 68%); m.p. 130–134 °C, $[\alpha]_D$ +60 (*c* 1.25). IR: 3 610 (OH); 1 729, 1 700 (C=O); 1 438 (OCH_3); 1 232, 1 161 (C–O). ^{1}H NMR: 0.63 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.21 dd, 1 H, J = 7.4, J' = 14.2 (H-16a$_1$); 2.30 dd, 1 H, J = 7.4, J' = 14.2 (H-16a$_2$); 2.38 d, 1 H, J = 8.8 (H-17); 3.00 m, 1 H, $W_{1/2}$ = 22 (H-16); 3.59 m, 1 H, $W_{1/2}$ = 22 (H-3); 3.61 s, 3 H (OCH_3). For $C_{24}H_{38}O_4$ (390.6) calculated: 73.81% C, 9.81% H; found: 73.63% C, 9.76% H.

16α-[(Methoxycarbonyl)methyl]-20-oxo-5α-pregnan-3β-yl Formate (**7**)

A stirred solution of 3β-hydroxy derivate **6** (770 mg, 2.0 mmol) and triphenylphosphine (1.037 g, 4.0 mmol) in toluene (40 ml) was cooled to 0 °C and diethyl azodicarboxylate (697 mg, 4.0 mmol) was added. After 20 min, formic acid (98%, 0.15 ml, 4.0 mmol) was added and the reaction mixture was stirred for 4 h at room temperature. After an additional 20 h, the solvent was evaporated and the residue was chromatographed on a column of silica gel (40 g). A mixture of petrolether–ether (8 : 2), yielded compound **7** (556 mg, 66%); m.p. 134–136 °C (acetone–heptane), $[\alpha]_D$ +69 (*c* 1.05). IR: 1 740, 1 716, 1 703 (C=O); 1 230, 1 198, 1 160 (C–O); 1 438 (OCH_3). ^{1}H NMR: 0.63 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.21 dd, 1 H, J = 7.4, J' = 14.2 (H-16a$_1$); 2.30 dd, 1 H, J = 7.4, J' = 14.2 (H-16a$_2$); 2.39 d, 1 H, J = 8.8 (H-17); 3.00 m, 1 H, $W_{1/2}$ = 22 (H-16); 3.61 s, 3 H (OCH_3); 5.16 m, 1 H, $W_{1/2}$ = 7 (H-3); 8.06 s, 1 H (formate). For $C_{25}H_{38}O_5$ (418.6) calculated: 71.74% C, 9.15% H; found: 71.70% C, 9.01% H.

16α-Carboxymethyl-3α-hydroxy-5α-pregnan-20-one (**8**)

A solution of formate **7** (300 mg, 0.72 mmol) in acetone (20 ml) was treated with hydrochloric acid (17%, 4 ml) and left standing at room temperature for 48 h. The reaction mixture was concentrated to a quarter of its volume, dichloromethane (20 ml) was added and the solution was washed with brine. Evaporation of the solvent yielded acid **8** (139 mg, 53%); m.p. 215–217 °C (acetone–heptane), $[\alpha]_D$ +109 (*c* 1.47, chloroform–methanol 1 : 1). IR: 3 617, 3 513 (OH); 1 740, 1 704 (C=O); 1 000 (C–OH). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.28 d, 2 H, J = 7.3 (2 × H-16a); 2.38 d, 1 H, J = 8.2 (H-17); 2.98 m, 1 H, $W_{1/2}$ = 22 (H-16); 4.05 m, 1 H, $W_{1/2}$ = 8 (H-3). For $C_{23}H_{36}O_4$ (376.5) calculated: 73.37% C, 9.64% H; found: 73.19% C, 9.54% H.

16α-Carboxymethyl-3α-hydroxy-5α-pregnan-20-one Sodium Salt (**9**)

A solution of carboxylic acid **8** (50 mg, 0.13 mmol) in methanol (1 ml) was mixed with 0.87 M methanolic solution of sodium methoxide (0.15 ml, 0.13 mmol), after 30 min, the solvent was evaporated in vacuum and the residue was triturated with ether. The residue (48 mg, 90%) was compound **9**; m.p. >360 °C. IR (KBr): 3 350, 3 268 (OH); 1 703, 1 694, 1 680 (C=O); 1 570, 1 404, 701 (COO^-); 1 003 (C–OH). ^{1}H NMR (D_2O): 0.64 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.13 d, 2 H, J = 7.9 (2 × H-16a); 2.20 s, 3 H (3 × H-21); 2.56 d, 1 H, J = 8.8 (H-17); 2.78 m, 1 H, $W_{1/2}$ = 22 (H-16); 4.04 m, 1 H, $W_{1/2}$ = 8 (H-3). For $C_{23}H_{35}NaO_4 \cdot H_2O$ (416.5) calculated: 66.32% C, 8.95% H; found: 65.80% C, 8.47% H.

16α-Carboxymethyl-3α-hydroxy-5α-pregnan-20-one Triethylammonium Salt (**10**)

A solution of carboxylic acid **8** (50 mg, 0.13 mmol) in triethylamine (4.0 ml, 28.7 mmol) was refluxed for 20 min. The product **10** crystallized from the reaction mixture after cooling. Yield 52 mg (82%); m.p. 213–214 °C, $[\alpha]_D$ +57.7 (*c* 1.5). IR: 3 615 (OH); 2 470 (Et_3HN^+); 1 700 (C=O); 1 602 (COO^-); 1 387 (CH_3); 1 001 (C–OH). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.77 s, 3 H (3 × H-19); 1.17 t, 9 H, J = 7.2 (3 × C**H**$_3$-CH_2); 2.12 s, 3 H (3 × H-21); 2.19 d, 2 H, J = 7.3 (2 × H-16a); 2.43 d, 1 H, J = 8.8 (H-17); 2.86 q, 6 H, J = 7.2 (3 × CH_2-N); 2.96 m, 1 H, $W_{1/2}$ = 22 (H-16); 4.04 m, 1 H, $W_{1/2}$ = 8 (H-3). For $C_{29}H_{51}NO_4$ (477.73) calculated: 72.91% C, 10.76% H, 2.93% N; found: 72.67% C, 10.82% H, 2.93% N.

The authors are indebted to Dr P. Fiedler for interpretation of IR spectra measured by Ms K. Matoušková. ^{1}H NMR spectra were measured by Ms M. Snopková. Elemental analyses were carried out in the Analytical Laboratory (Dr V. Pechanec, Head). This work was supported by the Ministry of Health of the Czech Republic (grant No. 4668-3).

REFERENCES

1. Kasal A.: *Collect. Czech. Chem. Commun.* **1999**, *64*, 1471.
2. Robertson J. D. in: *Recent Advances in Anaesthetics and Analgesia* (C. Langton Hewer, Ed.), 9th ed., p. 30. J. and A. Churchill, London 1963.
3. Mensah-Nyagan A. G., Do-Rego J. L., Beaujean D., Luu-The V., Pelletier G., Vaudry H.: *Pharmacol. Rev.* **1999**, *51*, 63.
4. Hawkinson J. E., Acosta-Burruel M., Yang K. C., Hogenkamp D. J., Chen J. S., Lan N. C., Drewe J. A., Whittemore E. R., Woodward R. M., Carter R. B., Upasani R. B.: *J. Pharmacol. Exp. Ther.* **1998**, *287*, 198.
5. Anderson A., Boyd A. C., Byford A., Campbell A. C., Gemmel D. K., Hamilton N., Hill D. R., Hill-Venning C., Lambert J. J., Maidment M. S., May V., Marshall R. J., Peters J. A., Rees D. C., Stevenson D., Sundaram H.: *J. Med. Chem.* **1997**, *40*, 1668.
6. Miyake Y., Kubo Y., Iwabuchi S., Kojima M.: *Steroids* **1982**, *40*, 245.
7. Miyake Y., Shigetoshi M., Kojima M.: *Chem. Pharm. Bull.* **1990**, *38*, 150.
8. Igarashi K., Mori Y., Takeda K.: *Steroids* **1969**, *13*, 627.
9. Atkinson R. M., Davis B., Praft M. A., Shape H. M., Tomich E. G.: *J. Med. Chem.* **1964**, *8*, 426.
10. Morzycki J. W.: *Can. J. Chem.* **1986**, *64*, 1536.
11. Bladon P.: *J. Chem. Soc.* **1958**, 3723.
12. Perez G., Halpern O., Iriarte J., Crabbe P.: *Bull. Soc. Chim. Fr.* **1968**, 4063.