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Suppressing aggressive behavior with analogs of allopregnanolone (epalon)

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Abstract

3α-Hydroxy-20-oxo-5α-pregnan-21-yl hemisuccinate (**8**) was produced by partial acylation of 3α,21-dihydroxy-5α-pregnan-20-one (**14**). 3α-Fluoro-5α-pregnan-20-one (**9**) was prepared by treatment of 3β-hydroxy-5α-pregnan-20-one (**11**) with DAST and by solvolysis of tosylate **12** with tetrabutylammonium fluoride. A behavioral test on mice was performed using 3α-hydroxy-5α-pregnan-20-one (**1**) and compounds **8** and **9**. Compound **8** was found to be inactive, while the fluoro derivative **9** selectively reduced aggressive behavior in mice more than the corresponding 3α-hydroxy compound **1**; locomotion and other behavioral features were not affected.

Keywords: Steroid; Allopregnanolone; Behavior; $GABA_A$-receptor; DAST; ^1H NMR spectroscopy; Epalon

1. Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the brain that is involved in controlling many conditions ranging from anxiety to epilepsy [1–3]. The effects of GABA can be magnified by several types of compounds (e.g. benzodiazepines), which bind to $GABA_A$ receptors. Some metabolites of progesterone, e.g. 3α-hydroxy-5α-pregnan-20-one (**1**, 'allopregnanolone,' sometimes also 'epalon' see Fig. 1), also bind allosterically to the $GABA_A$ receptor and increase inhibitory effects of GABA by opening the membrane chloride ion channel with little tolerance [4]. The 3α-hydroxyl of allopregnanolone is considered essential for its anesthetic activity [5]; the hydroxy group acts within the binding site as a hydrogen-bond donor.

The practical use of allopregnanolone **1** (see Fig. 1) is restricted by its fast metabolism [6]: its half-life in serum is about 16 min. One solution to this problem was found by the introduction of a 3β-substituent (like **2** [7]): such a compound cannot be oxidized to an inactive 3-oxo derivative and thus, acts longer in the body.

The practical use of allopregnanolone **1** is also restricted by its low solubility in body liquids. This property allows the compound to pass the blood-brain barrier, but also affects methods of its application. In the search for new types of allopregnanolone, several water-soluble compounds like **3** and **4** were discovered [8–10].

The collection of steroid compounds, obtained over the past forty years by our Laboratory, was repeatedly searched for new types of potential lead structures: here, Hamilton and his colleagues [11] found good $GABA_A$ receptor ligands (e.g. **5** and **6**), which led them to a new product **7** with improved anesthetic properties. We picked other compounds for preliminary evaluation of their binding to the $GABA_A$ receptor using [^{3}H]muscimol tests; some of these compounds were developed into new products **8** and **9** whose synthesis and behavioral effects are reported in this paper. In vitro studies of the binding of our products to the $GABA_A$ receptor (Refs. [12–14]) will be reported elsewhere.

2. Methods

2.1. Synthesis of compounds tested

Commercial pregnenolone (**10**, 3β-hydroxypregn-5-en-20-one) was hydrogenated to the desired 5α dihydro product **11**

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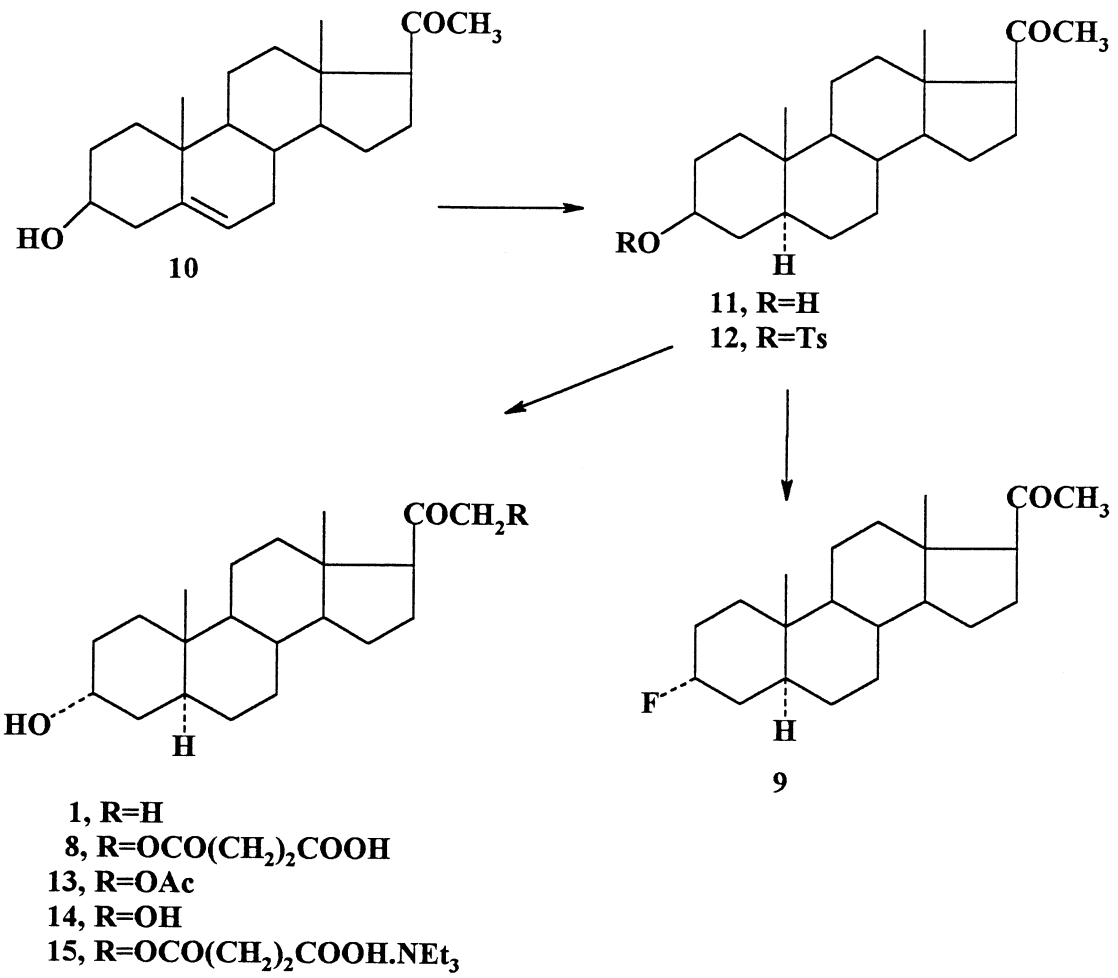


Fig. 1.

(see Scheme 1), which was tosylated to yield tosylate **12.** Its treatment with sodium nitrite in hexamethylphosphoroamide afforded alcohol **1** in which the configuration of the 3-OH group was inverted. The change of the configuration was apparent by the narrow width of the H-3 signal in its ^{1}H NMR spectrum ($W_{1/2}$ = 7.7 Hz while 3β-alcohol **11** has $W_{1/2}$ = 24 Hz). Ketone **1** was oxidized with lead tetraacetate to yield the 21-acetoxy compound **13**. With respect to the susceptibility of this compound to oxidation in alkaline medium [13], we carried out the hydrolysis in acid medium and obtained diol **14**. Partial acylation of the diol **14** with succinic anhydride yielded the 21-hemisuccinate **8** sought. Its triethylammonium salt **15** was obtained in crystalline form by dissolving acid **8** in hot triethylamine.

Treatment of 3β-hydroxy-5α-pregnan-20-one **11** with (diethylamino)sulfur trifluoride (DAST [15]) led to the 3α-fluoro product **9** with the reversed C-3 configuration (see Experimental).

2.2. Behavioral testing

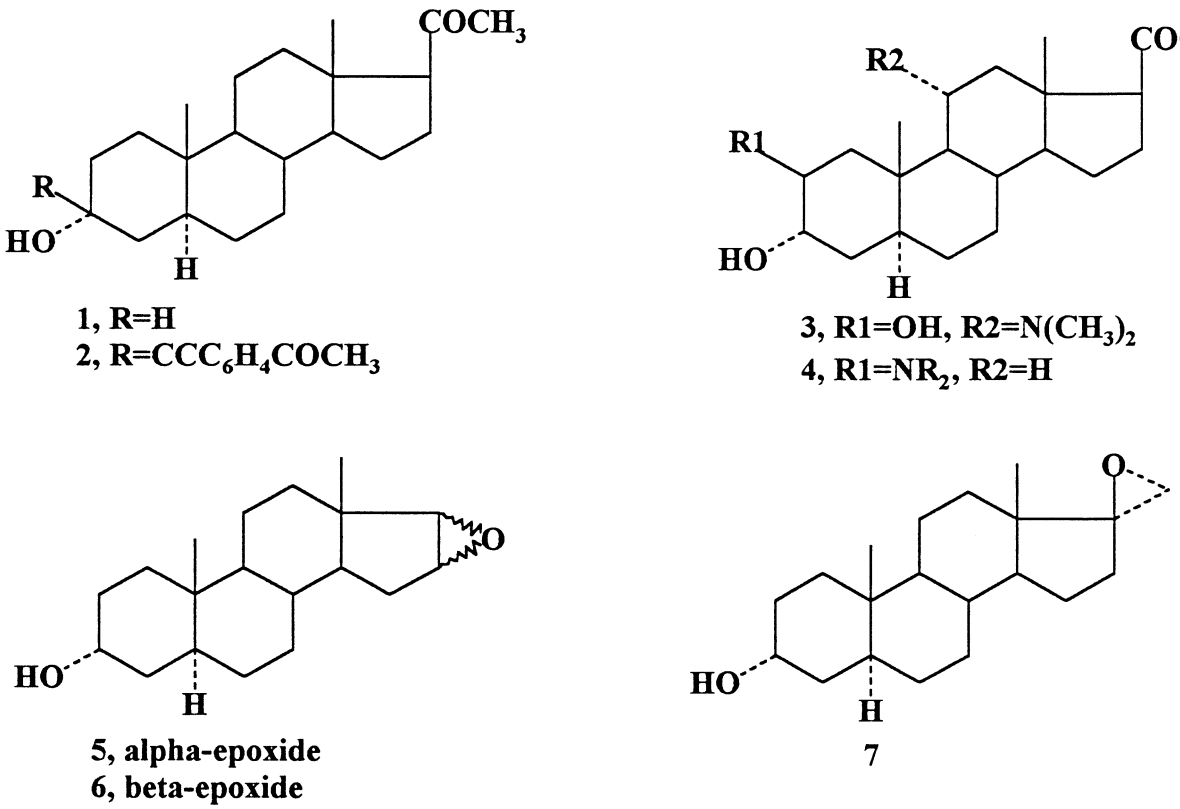
The behavioral effects of the drugs were tested in an experimental model of social conflict in mice. The model is based on interactions between an aggressive or timid male mouse housed individually and a non-aggressive group-housed male mouse in an observational cage. In this ethologically oriented laboratory model, analysis of aggressive, defensive-escape, sociable, and locomotor acts as well as postures occurring in the individually housed males enables the evaluation of potential antiaggressive and anxiolytic effects of the tested drugs as well as the degree of motor impairment and sedation they might produce [16,17].

2.2.1. Subject

Male, albino random-bred mice derived from ICR strain (Velaz, Prague), weighing 18–20 g at the beginning of the experimental housing, were used. All mice were housed under room lighting (with lights on from 6 a.m. to 6 p.m.) and with the temperature ranging from 22 to 24°C. Food and water were available ad libitum.

2.2.2. Procedure

After 4 weeks of isolation, mice were ready for testing. Drugs were tested during a series of interactions repeated at 1 week intervals. A particular dose of tested compound or



Scheme 1.

vehicle was given intraperitoneally (i.p.) to the singly housed males 30 min before interactions, the order of treatments was balanced according to a Latin square design.

Social interactions always involved one singly housed and one group-housed mouse, placed as a pair in an observational cage. Each isolate mouse was paired with the same group-housed partner throughout the experimental period. The isolated mice were allowed 30 min of adaptation in the observational cages before the group-housed partners were introduced; interactions ended after 4 min.

The observational cages were transparent boxes (20 × 30 × 20 cm) with wood shavings on the floor and covered with a transparent cover with apertures for air. Observations were made under moderate room lighting from 8 a.m. to 1 p.m. The behavior of animals during the interactions was recorded on videotape. The tapes were later analyzed by an observer blinded with respect to the drug treatment. Analysis was performed with a key-board connected to a standard PC and special software [18].

2.2.3. Measures

The frequency, total duration, and latency of a number of aggressive, defensive-escape (timid), social, and locomotor activities, derived from the ethogram of mice [19] and described in detail previously [20,21], were recorded. In short, the acts and postures evaluated in the present paper were defined as follows: *attack*—a fierce lunging at the partner often associated with biting, *threat*—a sideways or an upright stance with head and forebody movements toward the partner, and trying to bite the partner (also termed Offensive Sideways or Upright Posture), *tail rattle*—rapid vibrations of the tail, *walk*—any walking across the cage that is not apparently related to the partner, and *rear*—the mouse stands only on his hind legs and simultaneously sniffs the air or walls.

The interobserver reliability of the recorded items was satisfactory as determined by several observers independently judging a videotaped record of behavior of 70 mice in interactions lasting 4 min each. The correlations ranged from $r = 0.83$ to 0.97.

2.2.4. Drugs

Compound **1**, **8**, and **9** were dissolved in saline with two drops of Tween 80 and injected i.p. in a volume 0.1 ml/10 g of body weight.

2.2.5. Data analysis

The isolate mice that exhibited attacks in the control interaction (aggressive isolates) were selected for data analysis in the present paper. The differences between the control and experimental values were evaluated by the Paired *t* test or the Wilcoxon Signed Rank test (nonparametric tests were used when conditions for parametric tests were not satisfied).

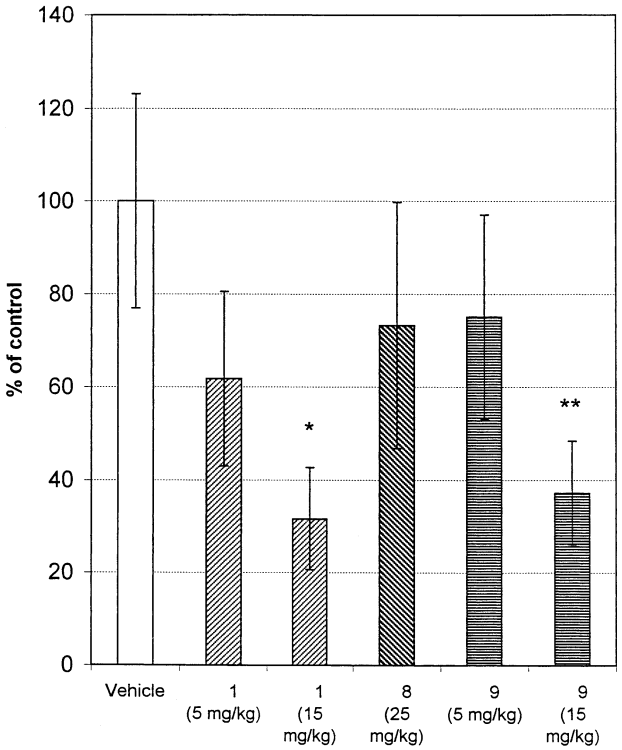


Fig. 2. Effects of tested compounds (**1**, **8** and **9**) on duration of threat postures illustrated as a percentage of control behavior (duration of threat postures after vehicle administration [12.1 ± 2.8 s] = 100 ± 23.1%). Effects of each dose represent mean results (± SEM) from 15 to 23 mice. * $P < 0.05$, ** $P < 0.01$.

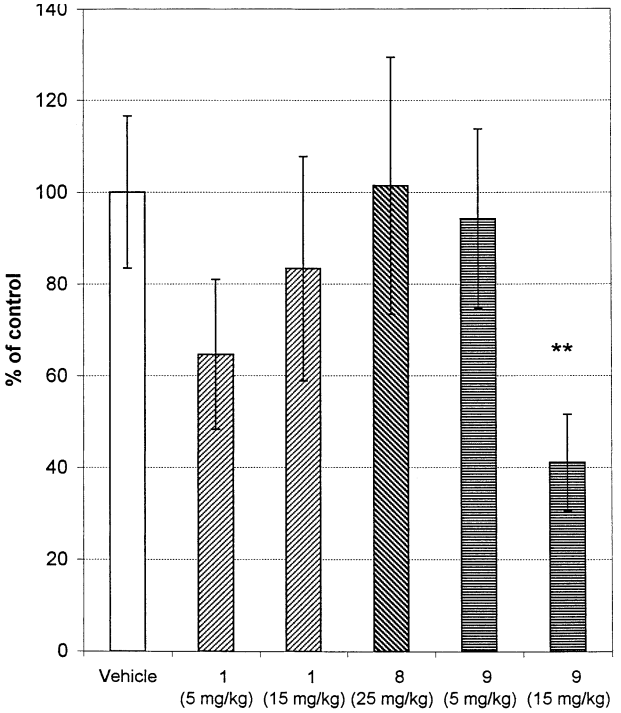


Fig. 3. Effects of tested compounds (**1**, **8** and **9**) on the frequency of attacks illustrated as a percentage of control behavior (frequency of attacks after vehicle administration [16.9 ± 2.8] = 100 ± 16.6%). Effects of each dose represent mean results (± SEM) from 15 to 23 mice. ** $P < 0.01$.

3. Experimental

Melting points were determined on a Boetius micro melting point apparatus (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50°C/100 Pa. Optical rotations were measured in chloroform ($[\alpha]_D$ values are given in 10^{-1}.deg.cm^2.g^{-1}), and IR spectra of chloroform solutions 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 the internal reference. Chemical shifts are given in ppm (δ-scale); coupling constants and the widths of multiplets are given 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). Preparative TLC (PLC) was carried out on 200 × 200 mm plates coated with a 0.7 mm thick layer of the same material. For column chromatography, silica gel (60–120 μm) was used. Whenever aqueous solutions of hydrochloric acid, potassium hydrogen carbonate, and potassium carbonate were used, their concentration was always 5%. Before evaporation on a rotary vacuum evaporator in (bath temperature 50°C), solutions in organic solvents were dried over anhydrous sodium sulfate.

*3.1. 3α-Hydroxy-5α-pregnan-20-one (**1**)*

Tosylate **12** (1.5 g, 3.17 mmol) was solvolyzed with sodium nitrite (4.5 g, 65.22 mmol) in hexamethylphosphoramide (20 ml) at 90°C for 2 h under a nitrogen atmosphere. The mixture was poured into water, and the product was extracted with chloroform. The extract was washed sequentially with water, dilute hydrochloric acid, water, the solution of potassium hydrogen carbonate, and water. After evaporation of the solvent, the product was purified by chromatography. The silica gel column was eluted with light petroleum:ether, 8:2. Crystallization of the major fraction afforded 514 mg (51%) of compound **1**. M.p. 167–169°C (toluene), Ref. [22] gives 168–169°C. ^{1}H NMR spectrum: 0.60 s, 3H (H-18); 0.78 s, 3H (H-19); 2.11 s, 3H (H-21); 2.53 t, 1H (J = 8.8, H-17); 4.05 m, 1H ($W_{1/2}$ = 7.7, H-3).

*3.2. 3α-Hydroxy-20-oxo-5α-pregnan-21-yl hemisuccinate (**8**)*

Succinic anhydride (245 mg, 2.45 mmol) was added to a solution of diol **14** (280 mg, 0.84 mmol) in pyridine (2.8 ml) at 0°C. After 24 h, the mixture was poured into a mixture of ethyl acetate (50 ml), hydrochloric acid (4 ml), and ice. The solution was washed with water, and then repeatedly washed with the solution of potassium hydrogen carbonate. The organic phase was separated, and the solvent was evap-

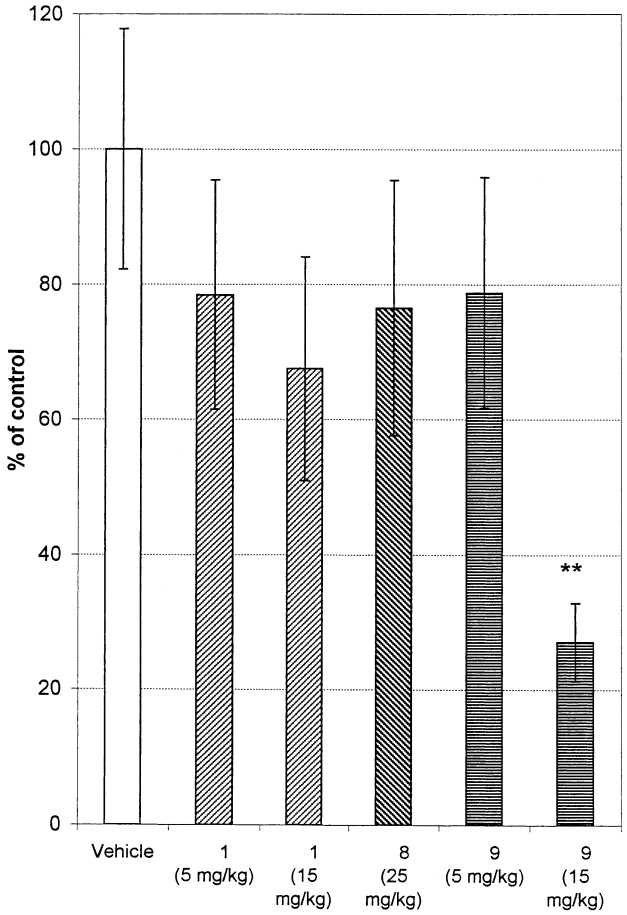


Fig. 4. Effects of tested compounds (**1**, **8** and **9**) on the duration of tail rattling illustrated as a percentage of control behavior (duration of tail rattling after vehicle administration [12.9 ± 2.3 s] = 100 ± 17.8%). Effects of each dose represent mean results (± SEM) from 15 to 23 mice. ** $P < 0.01$.

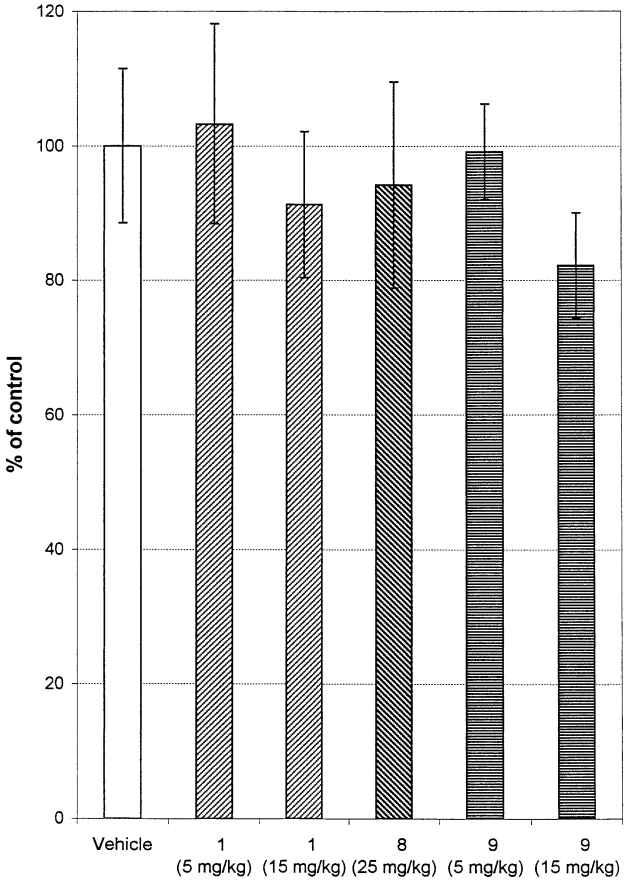


Fig. 5. Effects of tested compounds (**1**, **8** and **9**) on the frequency of walks illustrated as a percentage of control behavior (frequency of walks after vehicle administration [32.1 ± 3.7] = 100 ± 11.5%). Effects of each dose represent mean results (± SEM) from 15 to 23 mice.

orated to yield the starting compound (163 mg, 66%). The combined aqueous layers were acidified with dilute hydrochloric acid, the precipitate formed was filtered off, washed with water, and dried. Yield of compound **8** 107 mg (70% with allowance for the starting material recovered). M.p. 203–206°C (acetone-toluene), $[\alpha]_D$ +90 (c 0.99). IR spectrum: 3619, 3512 (OH), 1747, 1718 (C = O), 1166 (C-O). ^{1}H NMR spectrum: 0.64 s, 3H (H-18); 0.78 s, 3H (H-19); 2.76 dt, 4H (J = 4, J' = 1.2, $COCH_2CH_2CO$); 4.05 m, 1H ($W_{1/2}$ = 7, H-3); 4.55 d and 4.77 d, 2H (J = 17, H-21). Anal. calc. for $C_{24}H_{38}O_6$: C, 69.10; H, 8.81. Found: C, 68.93; H, 8.99.

*3.3. 3α-Fluoro-5α-pregnan-20-one (**9**)*

a) DAST (0.366 g, 2.27 mmol) was added to a solution of alcohol **11** (0.5 g, 1.57 mmol) in dichloromethane (10 ml) at room temperature. After 30 min, the reaction mixture was poured into the potassium hydrogen carbonate solution, and the precipitate formed was extracted with dichloromethane. After evaporation of the solvent, the residue was separated by flash chromatography on a column of silica gel (50 g); light petroleum:ether (93:7) eluted compound **9** (153 mg, 30.4%), which crystallized from acetone-heptane, m.p. 143.5–145.5°C, $[\alpha]_D$ +93 (c 0.88). IR spectrum: 1706 (C = O), 1155 (C-F). ^{1}H NMR spectrum: 0.60 s, 3H (H-18); 0.78 s, 3H (H-19); 2.11 s, 3H (H-21); 4.81 dm, 1H (J = 48, H-3). Analysis calculated for $C_{21}H_{33}FO$: C, 78.70; H, 10.38. Found: C, 78.56; H, 10.27.

b) Tosylate **12** ([24], 100 mg, 0.21 mmol) was treated with a solution of tetrabutylammonium fluoride in tetrahydrofuran (1M, 1.3 ml) at 50°C for 4 days. The reaction was quenched with water, and the product was extracted with ether, washed with water, and dried. After evaporation, the mixture was separated by PLC (4 plates, light petroleum: ether, 9:1). The major product **9** (28 mg, 41%) was found to be identical to the above sample.

*3.4. 3α-Hydroxy-20-oxo-5α-pregnan-21-yl acetate (**13**)*

Compound **1** (0.4 g, 1.57 mmol) in benzene (16 ml), methanol (0.8 ml), and boron trifluoride etherate (2 g, 14.1 mmol) was stirred with lead tetraacetate (0.620 g, 1.4 mmol) for 3 h at room temperature. The mixture was diluted with

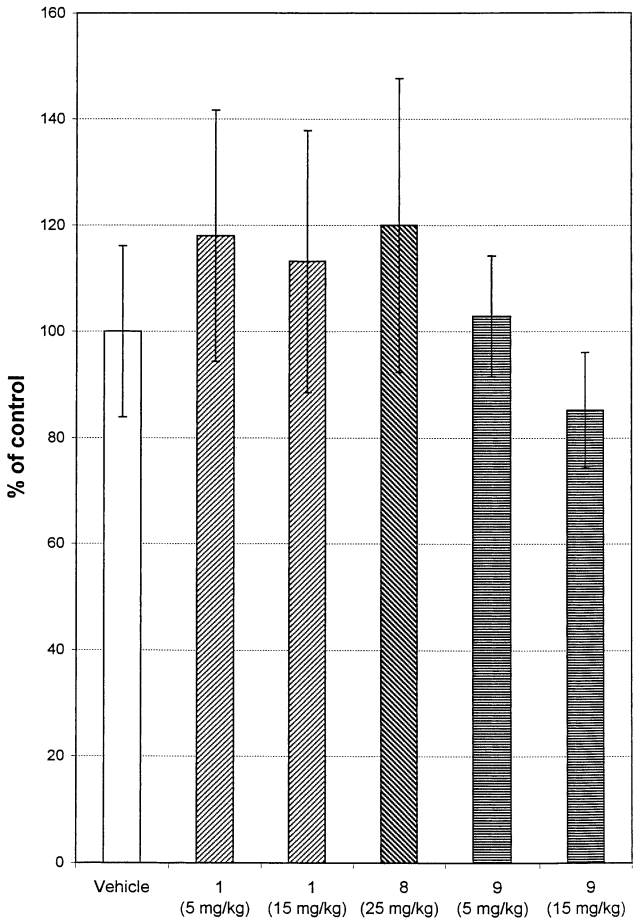


Fig. 6. Effects of tested compounds (**1**, **8** and **9**) on the frequency of rears illustrated as a percentage of control behavior (frequency of rears after vehicle administration [16.8 ± 2.7] = 100 ± 16.1%). Effects of each dose represent mean results (± SEM) from 15 to 23 mice.

water, and the product was extracted with ether and washed successively with the solution of potassium hydrogen carbonate, and water. Evaporation of the solvent afforded 0.310 mg (66%) of **13**. M.p. 215–216°C (ethanol), Ref. [23] gives 216–218°C. IR spectrum: 3615 (OH), 1747, 1722 (C = O), 1002 (C-OH). ^{1}H NMR spectrum: 0.64 s, 3H (H-18), 0.78 s, 3H (H-19), 2.17 s, 3H (CH_3CO), 4.05 m, 1H ($W_{1/2}$ = 7.2, H-3), 4.52 d and 4.71 d, 2H (J = 17, H-21).

*3.5. 3α,21-Dihydroxy-5α-pregnan-20-one (**14**)*

A solution of acetoxy compound **13** (300 mg, 0.80 mmol) in chloroform (3 ml) was treated with a mixture of methanol (9 ml), water (1 ml), and hydrochloric acid (0.6 ml). After 18 h, the mixture was concentrated to a quarter of its volume and poured into water. The resulting product was extracted with chloroform, washed sequentially with water, the solution of potassium hydrogen carbonate, and water and then dried. After evaporation of the solvent, the residue crystallized from acetone-heptane. Yield: 234 mg (88%). M.p. 162–163°C, Ref. [23] gives 163–166°C. IR spectrum: 3615, 3481 (OH), 1705 (C = O), 1077, 1022 (C-OH). ^{1}H NMR spectrum: 0.64 s, 3H (H-18), 0.78 s, 3H (H-19), 4.05 m, 1H ($W_{1/2}$ = 7.2, H-3), 4.13 d and 4.23 d, 2H (J = 18, H-21).

*3.6. 3α-Hydroxy-20-oxo-5α-pregnan-21-yl hemisuccinate, triethylammonium salt (**15**)*

A solution of compound **8** (12 mg, 0.03 mmol) in triethylamine (1.0 ml, 7.2 mmol) was refluxed for 20 min. Evaporation of the base and crystallization afforded salt **15** (8 mg, 54%). M.p. 194–196°C (acetone-heptane). ^{1}H NMR spectrum: 0.63 s, 3H (H-18); 0.77 s, 3H (H-19); 1.25 t, 9H (J = 7, 3 x CH_3-CH_2); 2.70 m, 4H ($W_{1/2}$ = 24, $COCH_2CH_2CO$); 3.04 q, 6 H (J = 7.3, 3× CH_2-N); 4.04 m, 1 H ($W_{1/2}$ = 7.6, H-3); 4.52 d and 4.73 d, 2H (J = 17, H-21).

4. Results

Allopregnanolone **1** appeared to be only moderately active in affecting the behavior of aggressive mice; this compound produced some reduction of aggressive activities (*Attacks*, *Threats*, and *Tail Rattles*, Figs. 2–4), but only the reduction of *Threats* was significant at the higher dose of compound **1** (15 mg/kg, P = 0.0479, Fig. 2). Its fluoro analog (compound **9**) was more active in reducing aggressive behavior; the higher dose (15 mg/kg) more markedly and significantly reduced all aggressive activities (*Attacks*, P = 0.0017; Fig. 3, *Threats*, P = 0.0067, Fig 2; *Tail Rattles*, P = 0.0065, Fig. 4). This reduction did not seem to be due to motor impairment or general sedation because walking and rearing were not affected significantly by these compounds (Figs. 5 and 6). Allopregnanolone **1** and its fluoro analog **9** did not affect other behavioral activities (social investigation, defensive-escape behavior) in aggressive mice (data not shown). Compound **8** was quite inactive; it did not produce any significant behavioral changes in aggressive mice, even at a relatively high dose (25 mg/kg, Figs. 1–6).

5. Discussion

The effects of compound **9** (the 3α-fluoro analog of allopregnanolone) on aggressive mice in the present study resembled those of diazepam and other benzodiazepines [17,20,21]. The present results suggest that the fluoro analog **9** may possess stronger tranquilizing activity than allopregnanolone **1,** which is probably due to the greater stability of its C-F bond at position 3.

The low activity or inactivity of the 3α-hydroxy compounds **1** and **8** in the present studies may be due to the rather long interval (30 min) between the injection of these compounds and the measurement of behavior (the half-life

of the compound **1** is only several minutes). When compound **1** was administered 10 min before measurement, it reportedly showed significant anxiolytic effects in a widely used plus-maze test in mice [25]. The interval (30 min) for adaptation of animals to the observational cages was derived from experiments with nonsteroidal modulators of the $GABA_A$ receptor, such as diazepam, which are not metabolized as quickly as the compounds used in the present studies.

Compound **9** reduced aggressive behavior of aggressive mice without causing overall sedation or motor impairment, which is a common effect of higher doses of benzodiazepines and other anxiolytics [20,21]. Social contacts (sniffing, climbing and following partners) and locomotion (walking across cage, rearing) were not affected by compound **9**.

The present results show that the 3α-hydroxy group of allopregnanolone like that of fluoro glucose can be replaced by fluorine which elicits strong inductive effects but does not yield a proton for a hydrogen bond. These results contrast the generally accepted belief that the presence of a 3α-hydroxy group is essential for GABA-like activity. Apparently, the fluorine atom, which also can participate in a hydrogen bond though in a reversed manner, can substitute for the hydroxyl. The slightly reduced $GABA_A$-receptor binding ability of the fluoro analog, when compared with the 3α-hydroxy compound, is more than compensated for by its metabolic stability [26,27].

Acknowledgments

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References

[1] Baulieu EE, Robel P. Neurosteroids. A new brain function? J Steroid Biochem Mol Biol 1990;37:395–403.
[2] Robel P, Baulieu EE. Neurosteroids. Biosynthesis and function. Trends Endocrinol Metab 1994;5:1–8.
[3] Rupprecht R, Holsboer F. Neuropsychopharmacological properties of neuro-active steroids. Steroids 1999;64:83–91.
[4] Marshall FH, Stratton SC, Mullings J, Ford E, Worton SP, Oakley NR, Hagan RM. Development of tolerance in mice to the sedative effects of the neuroactive steroid minaxolone following chronic exposure. Pharmacol Biochem Behav 1997;58:1–8.
[5] Covey DF, Evers AS, Zorumski CF. Neurosteroids: medicinal chemistry of steroids affecting GABA receptor function. Chem listy (Symposia) 1999;93:3–4.
[6] Kokate TG, Yamaguchi S-I, Pannell LK, Rajamani U, Carroll DM, Grossman AB, Rogawski MA. Lack of anticonvulsant tolerance to the neuroactive steroid pregnanolone in mice. J Pharmacol Exp Ther 1998;287:553–8.
[7] Hawkinson JE, Acosta-Burruel M, Yang KC, Hogenkamp DJ, Chen JS, Lan NC, Drewe JA, Whittemore ER, Woodward RM, Carter RB, Upasani RB. Substituted 3β-phenylethynyl derivatives of 3α-hydroxy-5α-pregnan-20-one: remarkably potent neuroactive steroid modulators of γ-aminobutyric $acid_A$ receptors. J Pharmacol Exp Ther 1998;287:198–207.
[8] Clark RSJ, Dundee JW, Garrett RT, McArdle GK, Sutton JA. Adverse reactions to intravenous anesthetics. Br J Anaesth 1976;47:575–85.
[9] Hope CE. Clinical trial. The minaxolone story. Can Anesth Soc J 1981;28:1–5.
[10] Anderson A, Boyd AC, Byford A, Campbell AC, Gemmell DK, Hamilton NM, Hill DR, Hill-Venning C, Lambert JJ, Maidment MSM, May V, Marshall RJ, Peters JA, Rees C, Stevenson D, Sundaram H. Anesthetic activity of novel water-soluble 2β-morpholinyl steroids and their modulatory effects at $GABA_A$ receptors. J Med Chem 1997;40:1668–81.
[11] Anderson A, Byford AJ, Campbell AC, Gemmell DK, Hamilton NM, Maidment MS, McGuire R, Rees DC, Sansbury FH, Sundaram H, Taylor R. Conformationally constrained anesthetic steroids that modulate $GABA_A$ receptors. Gordon Research Conference on Medicinal Chemistry. New London, New Hampshire, USA. August 1–6, 1999.
[12] Chodounská H, Kasal A. Epalons: synthesis of 3α,7α-dihydroxy-5α-pregnan-20-one. Coll Czech Chem Commun 1998;63:1543–8.
[13] Slavíková B, Kasal A, Buděšínský M. Autoxidation vs. hydrolysis in 16α-acyloxy steroids. Coll Czech Chem Commun 1999;64:1125–34.
[14] Kasal A. Epalons. Synthesis of 7-norallopregnanolone. Coll Czech Chem Commun 1999;64:1471–8.
[15] Bird TGC, Fredericks PM, Jones ERH, Meakins GD. Convenient general preparation of oxygenated monofluoro- and *gem*-difluoro-5α androstanes using diethylaminosulphur trifluoride. J Chem Soc Chem Commun 1979:65–9.
[16] Kršiak M. Timid singly-housed mice: their value in prediction of psychotropic activity of drugs. Br J Pharmac 1975;55:141–50.
[17] Kršiak M, Šulcová A, Donát P, Tomašíková N, Kosař E, Mašek K. Can social and agonistic interactions be used to detect anxiolytic activity of drugs? In: Miczek KA, Kruk MR, Olivier B, editors. Ethopharmacological aggression research. New York: Liss, 1984. pp. 93–114.
[18] Donát P. Measuring behaviour: the tools and the strategies. Neurosci Biobehav Rev 1991;15:447–54.
[19] Grant EC, Mackintosh JH. A comparison of the social postures of some common laboratory rodents. Behaviour 1963;21:246–59.
[20] Kršiak M. Effects of drugs on behaviour of aggressive mice. Br J Pharmacol 1979;65:525–33.
[21] Kršiak M, Šulcová A. Differential effects of six structurally related benzodiazepines on some ethological measures of timidity, aggression and locomotion in mice. Psychopharmacology 1990;101:396–402.
[22] Kasal A, Pásztorová S. Synthesis of RIA haptens. 3α,11α-Dihydroxy-5α-pregnan-20-one 11-hemisuccinate. Collect Czech Chem Commun 1993;58:619–28.
[23] Harnik M. Studies in syntheses of steroid metabolites. Steroids 1964; 3:359–79.
[24] Ruff A, Reichstein T. Monoketone der Pregnan- und Allopregnan-Reihe. Helv Chim Acta 1951;34:70–81.
[25] Rodgers RJ, Johnson NJT. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol Biochem Behav 1998;59:221–32.
[26] Wester HJ, Herz M, Weber W, Heiss P, Senekowitsch-Schmidtke R, Schwaiger M, Stocklin G. Synthesis and radiopharmacology of O-(2-[^{18}F]fluoroethyl)-L-tyrosine for tumor imaging. J Nucl Med 1999;40: 205–12.
[27] Holmgren SK, Bretscher LE, Taylor KM, Raines RT. A hyperstable collagen mimic. Chem Biol 1999;6:63–70.