Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 343–346

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and $GABA_A$ Receptor Activity of a 6,19-Oxido Analogue of Pregnanolone

Adriana S. Veleiro,[a] Ruth E. Rosenstein,[b] Carolina O. Jaliffa,[b] María L. Grilli,[b] Florencia Speroni[a] and Gerardo Burton[a,*]

[a]*Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, C1428EHA Buenos Aires, Argentina*

[b]*Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Argentina*

Received 24 September 2002; revised 18 November 2002; accepted 22 November 2002

Abstract—3α-Hydroxy-6,19-oxidopregn-4-ene-20-one (**4**) was prepared in seven steps from pregnanolone acetate. At 0.1 μM concentration **4** significantly increased GABA induced $^{36}Cl^-$ influx in hamster cerebral cortex synaptoneurosomes while at 20 mg/kg it decreased the percentage of hamsters showing seizures induced by 3-mercaptopropionic acid.

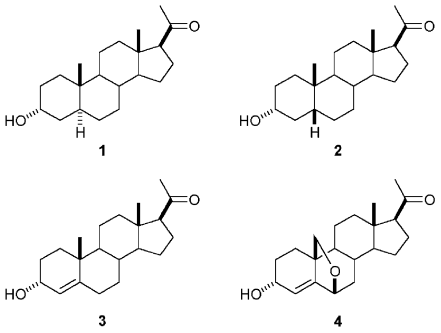
γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). Modulation of GABAergic levels and metabolism induce profound physiological and behavioral changes, in particular the inhibition of its synthesis or its binding to specific receptors may evoke seizures in a variety of animal models. The $GABA_A$ receptor is also the site for allosteric modulation by several compounds such as benzodiazepines, barbiturates and neurosteroids.[1] Both neurosteroids (which are synthesized by neural tissues) and neuroactive steroids (either synthetic or produced elsewhere, but acting on neurons or glial cells) have been shown to affect GABAergic neurotransmission in the brain.[2–5] The major site of neuronal activity appears to be a specific steroid sensitive site on the $GABA_A$ receptor/chloride ionophore complex.[6] Neuroactive steroids can either antagonize Cl^- conductance, e.g., the sulfate ester of pregnenolone, or potentiate Cl^- influx by increasing the probability that the Cl^- channel enters in an open state of long duration as well as by increasing the frequency of channel opening.[7] These GABAergic positive modulators form an interesting class of compounds with potential clinical use as anesthetics, anticonvulsants, anxiolytics, hypnotics and analgesics. Those with an ability to potentiate the $GABA_A$ receptor reponse also show a potent anticonvulsant effect in pentylenetetrazol (PTZ), bicuculline and picrotoxin seizure tests in mice.[8] 3-Mercaptopropionic acid (3-MPA) has also been used to induce seizures, since its inhibition of glutamate decarboxylase activity has been thoroughly studied in several species including the golden hamster.[9] It has been recently suggested that neurosteroid-related anticonvulsants may offer a potentially new nonhormonal approach for the treatment of infantile spasms and other developmental epilepsies.[10]

Among the naturally endogenous neurosteroids are 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, **1**) and its 5β-isomer (pregnanolone, **2**). Several synthetic analogues of these compounds with improved activities and bioavailability have been developed.[6,11–14] In the search for conformationally restricted analogues that could imitate the molecular shape of neurosteroids **1** or **2** we turned our attention to 6,19-oxido bridges that, when incorporated into Δ^4 steroids, bend the molecule at the A/B ring junction mimicking an A/B *cis* fused steroid.[15] The gonadal steroid 3α-hydroxy-4-pregnen-20-one (**3**), an unsaturated analogue of allopregnanolone (**1**) with similar overall shape and conformation, exhibits a potency and efficacy similar to this neurosteroid.[7] The introduction of an oxido bridge between C-6 and C-19 of the former compound, gives the highly bent 6,19-oxidopregnene **4**, with a torsioned steroid nucleus at the A/B-ring junction. The overall conformation of **4** as predicted by semiempirical AM1

*Corresponding author. Tel.: +54-11-4576-3385; fax: +54-11-4576-3385; e-mail: burton@qo.fcen.uba.ar

0960-894X/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)01019-3

calculations is very similar to that of pregnanolone (**2**) (Fig. 1) with the side chain at C-17 and the 3α-OH occupying almost identical relative positions (less than 0.05 Å displacement).[16] Thus **4** would be the equivalent of **3** in the 5β series, however at variance with the latter steroid, the 6,19-oxido bridge gives rise to a highly rigid framework.



The 6,19-oxidosteroid **5** was used as starting material for the preparation of compound **4**, (Scheme 1); the former compound was obtained from commercially available pregnenolone acetate following essentially the procedure described previously by de Armas et al.[17] The first step of the synthetic sequence was the protection of the C-20 carbonyl as the ethylene ketal with ethyl

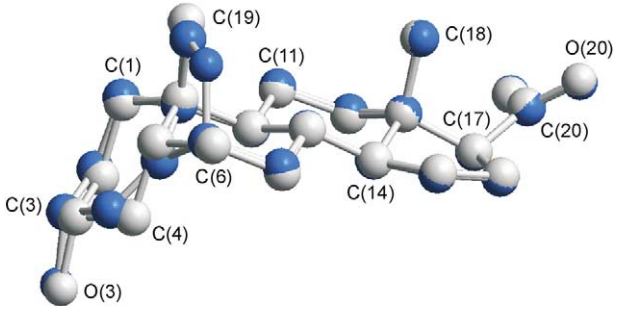
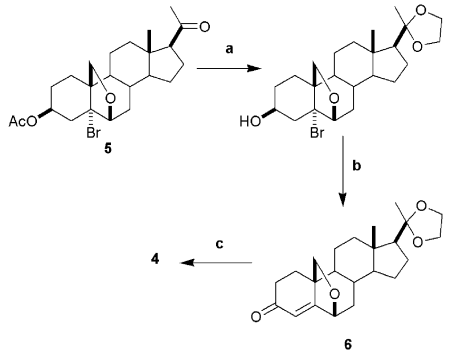


Figure 1. Superposition of the AM1 calculated structures of pregnanolone (**2**, white) and 3α-hydroxy-6,19-oxidopregn-4-en-20-one (**4**, blue). The overall error for the RMS fit of both structures was 0.139 Å (all heavy atoms considered except C-19 and the bridge oxygen).[16] Hydrogens are not shown for clarity.



Scheme 1. Reagents and conditions: (a) (i) Ethyleneglycol, *p*-TsOH, $HC(OEt)_3$; (ii) K_2CO_3, MeOH; (b) (i) PCC, $BaCO_3$, Cl_2CH_2; (ii) filtration through Al_2O_3; (c) (i) $LiAlH(t\text{-}BuO)_3$, THF; (ii) Acetone, *p*-TsOH, H_2O, Cl_2CH_2.

orthoformate and ethyleneglycol in the presence of *p*-toluenesulfonic acid. Hydrolysis of the acetate at position 3β with potassium carbonate in methanol followed by oxidation with pyridinium chlorochromate (PCC) in the presence of barium carbonate gave the 3-ketone **6**, dehydrohalogenation occurring spontaneously upon filtration through alumina. Stereoselective reduction with lithium tri(*t*-butoxy) aluminum hydride in THF,[18] followed by deprotection of the C-20 carbonyl with *p*-toluenesulfonic acid in acetone gave **4** in 41% yield from **5**.

The structure of **4** was established on the basis of spectroscopic evidence.[19] Confirmation of the stereochemistry at C-3 was carried out by the combined analysis of the NOESY spectrum and molecular modeling of compound **4** and its 3β-stereoisomer. The correlations observed in the NOESY spectrum for the pairs H-1β (δ 1.34)/H-19b (δ 3.31) and H-1β (δ 1.34)/H-3β (δ 4.38) were in agreement with the distances between those hydrogens predicted by AM1 calculations (2.27 and 2.73 Å, respectively).[16] On the other hand for the 3β-hydroxysteroid, calculations indicated that no hydrogen would simultaneously correlate with H-3α and H-19b. Application of the Altona equation[20] to the minimum energy conformation of compound **4**, gave couplings of 9.8, 6.6 and 3.0 Hz for the pairs H-3β/H-2β, H-3β/H2α, and H-3β/H-4 respectively, in good agreement with the observed data (8.4, 5.7, and 2.5 Hz, respectively). The same calculations performed on the isomeric 3β-stereoisomer predicted couplings of 1.51, 5.43 and 4.11 Hz for the pairs H-3α/H-2β, H-3α/H2α, and H-3α/H-4 respectively.

$GABA_A$ receptor activity was evaluated by assessing $^{36}Cl^-$ influx in hamster cerebral cortex synaptoneurosomes.[21] Figure 2 shows the effect of **1**, **2**, and **4** on GABA-induced $^{36}Cl^-$ uptake. Allopregnanolone (**1**) significantly increased this parameter at a range of concentrations of 1–50 μM, being ineffective at 0.1 and 100 μM. A higher sensitivity was observed for the 6,19-oxido

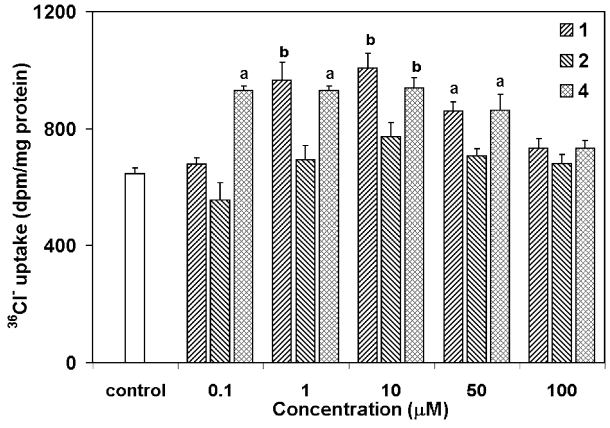


Figure 2. Effect of compounds **1**, **2**, and **4** on GABA-induced $^{36}Cl^-$ influx. Hamster cerebral cortex synaptoneurosomes were incubated with 10 μM GABA, in the presence or absence of these compounds (0.1–100 μM).[21] Data are mean ± SEM ($n = 15$/group). GABA alone significantly increased $^{36}Cl^-$ uptake (white bar on the left), being basal levels of this parameter (in the absence of GABA) 452 ± 35 dpm/mg protein; $^a p < 0.05$, $^b p < 0.01$, by Dunnett's *t* test.

analogue **4** since it was effective in the increase of GABA-induced Cl^- influx even at 0.1 μM. The β analogue **2** did not differ significantly from controls at all the concentrations tested.

Naturally occurring or synthetic neuroactive steroids such as androsterone and alphaxalone showed anticonvulsant activity, presumably by acting at the GABA receptor complex.[22,23] The highly effective modulation of GABA-induced $^{36}Cl^-$ uptake exhibited by analogue **4**, indicates a positive modulation of GABA activity. As mentioned above, 3-mercaptopropionic acid (3-MPA) is a highly specific inhibitor of glutamate decarboxylase, its administration resulting in seizures due to a significant decrease in brain GABA levels. Positive allosteric modulators of the $GABA_A$ receptor should counteract the proconvulsant effect of 3-MPA by potentiating GABAergic response. Present results confirm this presumption, since compound **4** was also shown to behave as an effective anticonvulsant.[21] Figure 3 shows the percentage of animals reaching tonic and tonic-clonic seizures when injected with 3-MPA alone or 3-MPA and the 6,19-oxido analogue **4**. At 10 mg/kg, **4** was ineffective, whereas at 20 and 50 mg/kg it significantly decreased the percentage of hamsters showing seizures. These data are in line with the reported effectiveness of several neurosteroids that positively modulate $GABA_A$ receptors (at similar doses to those used in the present report) in inhibiting the seizures induced by $GABA_A$ antagonists.[8,24] It should be noted that at variance with 3-MPA both bicuculine and pentylenetetrazol induce seizures by inhibiting GABA response, thus their use may mask the putative anticonvulsant effect of a positive modulator of the $GABA_A$ receptor.

Interestingly, although polar groups at position 6, for example, the replacement of the 6-methylene by oxygen, adversely affect the $GABA_A$ receptor activity of neurosteroid analogues,[18] this is not the case with the oxygen bridge in compound **4**. Although several pieces of information are still lacking (e.g. side effects, pharmacodynamics, etc.) this novel compound appears as a new tool with potential application for the treatment of epilepsy and other behavioral alterations associated to a GABAergic system disfunction. Furthermore, the oxidation product of **4**, 6,19-oxidoprogesterone, which is a plausible metabolite in vivo, has been shown to be completely devoid of hormonal activities.[15,25]

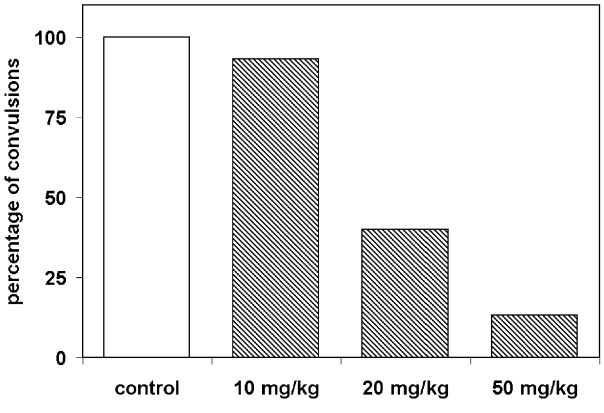


Figure 3. Effect of compound **4** on 3-mercaptopropionic acid induced seizures in hamsters. Compound **4** or its vehicle (white bar on the left) was injected ip 10 min before 3-MPA (40 mg/kg).[21] Shown are the percentage of animals reaching tonic and tonic-clonic seizures. ($n=15$ animals/group). Animals injected with vehicle alone (no 3-MPA) did not show any pro- or anticonvulsant effects.

Acknowledgements

This work was supported by grants from Universidad de Buenos Aires, ANPCYT and CONICET (Argentina).

References and Notes

1. Sieghart, W. *Pharmacol. Rev.* **1995**, *47*, 182.
2. Baulieu, E. E. *Psychoneuroendocrinol.* **1998**, *23*, 963.
3. Baulieu, E. E. *Recent Prog. Horm. Res.* **1997**, *52*, 1.
4. Compagnone, N. A.; Mellon, S. H. *Front. Neuroendocrinol.* **2000**, *21*, 1.
5. Tsutsui, K.; Ukena, K.; Usui, M.; Sakamoto, H.; Takase, M. *Neurosci. Res.* **2000**, *36*, 261.
6. Gasior, M.; Carter, R. B.; Witkin, J. M. *Trends Pharmacol. Sci.* **1999**, *20*, 107.
7. Lambert, J. J.; Belelli, D.; Hill-Venning, C.; Peters, J. A. *Trends Pharmacol. Sci.* **1995**, *16*, 295.
8. Belelli, D.; Lan, N. C.; Gee, K. W. *Neurosci. Biobehav. Rev.* **1990**, *14*, 315.
9. Golombek, D. A.; Fernández, D. D.; De Brito Sánchez, M. G.; Burin, L.; Cardinali, D. P. *Eur. J. Pharmacol.* **1991**, *210*, 253.
10. Rogawski, M. A.; Reddy, D. S. *Int. Rev. Neurobiol.* **2002**, *49*, 199.
11. Beekman, M.; Ungard, J. T.; Gasior, M.; Carter, R. B.; Dijkstra, D.; Golberg, S. R.; Witkin, J. M. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 868.
12. Upasani, R. B.; Yang, K. C.; Acosta-Burruel, M.; Konkoy, C. S.; McLellan, J. A.; Woodward, R. M.; Lan, N. C.; Carter, R. B.; Hawkinson, J. E. *J. Med. Chem.* **1997**, *40*, 73.
13. Anderson, A.; Boyd, A. C.; Byford, A.; Campbell, A. C.; Gemmell, D. K.; Hamilton, N. M.; 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.
14. Anderson, A.; Boyd, A. C.; Clark, J. K.; Fielding, L.; Gemmell, D. K.; Hamilton, N. M.; Maidment, M. S.; May, V.; McGuire, R.; McPhail, P.; Sansbury, F. H.; Sundaram, H.; Taylor, R. *J. Med. Chem.* **2000**, *43*, 4118.
15. Burton, G.; Galigniana, M. D.; de Lavallaz, S.; Brachet-Cota, A. L.; Sproviero, E. M.; Ghini, A. A.; Lantos, C. P.; Damasco, M. C. *Mol. Pharmacol.* **1995**, *47*, 535.
16. Semiempirical AM1 calculations and RMS fitting of structures were performed with Hyperchem 7.0 (Hypercube Inc., USA).
17. de Armas, P.; Concepción, J. I.; Francisco, C. G.; Hernández, R.; Salazar, J. A.; Suárez, E. *J. Chem. Soc., Perkin Trans. 1* **1989**, 405.
18. Nicoletti, D.; Ghini, A. A.; Furtmuller, R.; Sieghart, W.; Dodd, R. H.; Burton, G. *Steroids* **2000**, *65*, 349.
19. **3α-Hydroxy-6,19-oxidopregn-4-ene-20-one** (**4**): mp 166 °C (methanol); IR (KBr) 3408, 2938, 1712, 1448, 1362, 1035, 1016 cm^{-1}; UV (methanol) λ_{max} 210, 236 nm; ^1H NMR (500.13 MHz, $CDCl_3$) δ 5.48 (1H, d, $J=2.5$ Hz, H-4) 4.44 (1H, d, $J=4.8$ Hz, H-6) 4.38 (1H, ddd, $J=2.5$, 5.7 and 8.4 Hz, H-3) 4.04 (1H, d, $J=7.8$ Hz, H-19a), 3.31 (1H, d, $J=7.8$ Hz, H-19b) 2.50 (1H, t, $J=8.9$ Hz, H-17) 2.15 (1H, m, H-16) 2.11 (3H, s, H-21) 2.07 (1H, m, H-12β) 1.99 (1H, m, H-1α) 1.94

(1H, m, H-2β) 1.92 (1H, m, H-7β), 1.81 (1H, m, H-8) 1.67 (1H, m, H-16) 1.67 (1H, m, H-11α) 1.61 (1H, m, H-15), 1.56 (1H, m, H-2α) 1.55 (1H, m, H-9), 1.45 (1H, m, H-12α) 1.34 (1H, m, H-1β), 1.31 (1H, m, H-11β) 1.30 (1H, m, H-14), 1.26 (1H, m, H-15) 1.23 (1H, m, H-7α), 0.69 (3H, s, H-18); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 209.3 (C-20), 149.4 (C-5), 115.7 (C-4), 77.0 (C-6), 75.3 (C-19), 67.5 (C-3), 63.5 (C-17), 55.3 (C-14), 50.2 (C-9), 44.8 (C-10), 44.7 (C-13), 39.5 (C-7), 38.8 (C-12), 34.4 (C-8), 31.3 (C-21), 28.9 (C-2), 25.1 (C-1), 23.9 (C-15), 23.0 (C-16), 22.9 (C-11), 13.7 (C-18); EIMS, *m*/*z* 330 (M^+, 4), 312 (1), 271 (1), 43 (100); Found C, 76.1; H, 9.5 ($C_{21}H_{30}O_3$ requires C, 76.3; H, 9.2%).

20. Haasnoot, C. A. G.; de Leew, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.

21. *Animals and drugs*. Male adult Syrian hamsters (*Mesocricetus auratus*, 80–140 g) were raised in our colony, under a 14 h light—10 h dark photoperiod, with lights on at 6:00 h. Hamsters were given access to food and water *ad libitum*. All drugs were from Sigma Chemical Co. (St. Louis, MO).

In vitro *experiments*. Chloride influx was assessed by a modification of the procedure described by Harris and Allan.[26] Hamsters were killed by decapitation, the brains were quickly removed and the brain cortex were dissected on an ice-cooled Petri dish. Brain cortex were homogenized by hand in ice cold HEPES buffer containing 140 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, (adjusted to pH 7.4 with Tris base) with a glass Teflon homogenizer. The homogenate was centrifuged at 900×*g* for 15 min at 4 °C, the supernatant was decanted and the pellet was suspended in buffer to yield 2–5 mg of protein per mL. A 200 μL aliquot of the suspension was incubated for 10 min at 30 °C. After incubation, chloride influx was initiated by adding 200 μL of a solution containing $^{36}Cl^-$ (final concentration: 0.5 μCi/mL). Five s after the addition of $^{36}Cl^-$ with or without GABA, influx was halted by adding 4 mL of buffer. The mixture was immediately poured onto Whatman GF/B filters under vacuum. The filters were washed twice with 4 mL-aliquots of ice-cold buffer and the radioactivity on the filters was counted in a liquid scintillation counter. The amounts of $^{36}Cl^-$ bound to the filters in the absence of membranes (no-tissue blank) was substracted from all values. GABA (10 μM) was added with $^{36}Cl^-$, while steroids were added 5 min before $^{36}Cl^-$ at variable concentrations (0.1–100 μM).

In vivo experimental procedure. Drugs were administered ip; 3-mercaptopropionic acid (3-MPA) was dissolved in saline, compound **4** was administered as a suspension in saline/DMSO (1:1, v/v) prepared just prior to the injections. Animals receiving 3-MPA were placed in plastic boxes and their behavior was recorded during 15-min trials. Compound **4** (10, 20 and 50 mg/kg) or its vehicle was administered 10 min before 3-MPA (40 mg/kg).

22. Guinjoan, S. M.; Golombek, D. A.; Cardinali, D. P. *Life Sci*. **1992**, *51*, 2089.
23. Guinjoan, S. M.; Golombek, D. A.; Kanterewicz, B. I.; Rosenstein, R. E.; Cardinali, D. P. *Neuroendocrinol. Lett*. **1992**, *14*, 329.
24. Kokate, T. G.; Svensson, B. E.; Rogawski, M. A. *J. Pharmacol. Exp. Ther*. **1994**, *270*, 1223.
25. Vicent, G. P.; Monteserín, M. C.; Veleiro, A. S.; Burton, G.; Lantos, C. P.; Galigniana, M. D. *Mol. Pharmacol*. **1997**, *52*, 749.
26. Harris, R. A.; Allan, A. M. *Science* **1985**, *228*, 1108.