

# Synthesis, Metabolism, and Pharmacological Activity of 3 $\alpha$ -Hydroxy Steroids Which Potentiate GABA-Receptor-Mediated Chloride Ion Uptake in Rat Cerebral Cortical Synaptoneurosomes<sup>1</sup>

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Certain 3 $\alpha$ -hydroxy steroids have recently been shown to bind to the  $\gamma$ -aminobutyric acid (GABA) receptor gated chloride ion channel with high affinity and to potentiate the inhibitory effects of GABA when measured both in vitro and in vivo. In the present study, a series of natural and synthetic 3 $\alpha$ -hydroxy steroids were tested for their ability to potentiate GABA-receptor-mediated chloride ion (Cl<sup>-</sup>) uptake into cerebral cortical synaptoneurosomes. The naturally occurring metabolites 3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one (allopregnanolone) and 3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one (allotetrahydroDOC) were found to be the most active in augmenting GABA<sub>A</sub>-receptor-mediated Cl<sup>-</sup> uptake. Pharmacological activity was reduced in the corresponding isomers with the 5 $\beta$ -pregnane configuration and by some, but not all, modifications of the side chain. The ability of these steroids to potentiate muscimol-stimulated Cl<sup>-</sup> uptake is lost by acetylation at C<sub>3</sub>, introduction of unsaturation at C<sub>9(11)</sub>, inversion to the 3 $\beta$ -hydroxy isomer, or inversion of configuration at C<sub>17</sub>. A facile procedure is reported for the synthesis of unlabeled and tritium-labeled allopregnanolone and allotetrahydroDOC. The 9 $\alpha$ ,11 $\alpha$ ,12 $\alpha$ -<sup>3</sup>H-labeled derivatives of allopregnanolone and allotetrahydroDOC were used to identify the distribution and metabolic products of these active steroids. Uptake of the more hydrophobic [<sup>3</sup>H]allopregnanolone into brain was significantly greater than that of [<sup>3</sup>H]allotetrahydroDOC. The principal <sup>3</sup>H-labeled metabolites recovered from brain were the 3-ketone derivatives of allopregnanolone and allotetrahydroDOC, which are both inactive on GABA-receptor-mediated Cl<sup>-</sup> flux. Molecular modeling of the active steroids based on quantitative structure-activity relationships provides evidence to support the stereospecificity of the binding interactions and suggests that there may be more than one type of steroid binding site associated with the GABA<sub>A</sub>-receptor-mediated chloride ionophore.

The  $\gamma$ -aminobutyric acid (GABA<sub>A</sub>)/benzodiazepine receptor-chloride channel complex has been shown to be modulated by several metabolites of the steroid hormones progesterone and deoxycorticosterone (DOC), most notably 3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one (2a, allopregnanolone) and 3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one (4a, allotetrahydroDOC).<sup>2</sup> These metabolites compete with [<sup>35</sup>S]-*tert*-butylbicyclophosphorothionate (TBPS) in binding to the GABA<sub>A</sub> receptor complex, with an affinity for this recognition site in the high nanomolar range.<sup>2</sup> In the presence of low concentrations of GABA, these metabolites have significantly higher affinity for [<sup>35</sup>S]TBPS binding,<sup>2c</sup> suggesting an allosteric interaction between the recognition site for GABA and the recognition site for these metabolites. In addition, allopregnanolone and allotetrahydroDOC enhance benzodiazepine receptor binding in vitro in a manner similar to that of barbiturates.<sup>2a</sup> Electrophysiological studies have shown that these metabolites can directly alter GABA-receptor-mediated chloride ion conductance in cultured rat hippocampal neurons.<sup>2a</sup> Moreover, we have recently demonstrated<sup>2e,3</sup> that these steroid hormone metabolites enhance GABA-receptor-mediated chloride uptake into rat cerebral cortical synaptoneurosomes at concentrations between 0.025 and 1  $\mu$ M. Thus, allopregnanolone and allotetrahydroDOC are extremely potent modulators of GABA-receptor-mediated inhibitory neurotransmission in the central nervous system (CNS), suggesting that they may serve as anxiolytic or hypnotic agents in animals and man.

Behavioral studies have indicated that one or both of these steroid hormone metabolites have anxiolytic, sedative, analgesic, and antiaggressive effects when administered to laboratory animals. AllotetrahydroDOC has been shown to have anticonflict effects in rats,<sup>4</sup> potent dose-

dependent sleep-inducing properties in rats,<sup>5</sup> and to reduce aggressive behavior in mice.<sup>6</sup> Allopregnanolone also produces analgesic effects in mice when administered in the cerebroventricular space.<sup>7</sup> These compounds are closely related structurally to anesthetic steroids such as alphaxalone, which have also been shown to have GABA<sub>A</sub>-receptor-mediated activity in the CNS.<sup>8</sup>

The objectives of the present study were (1) to develop a procedure for the facile synthesis of these steroid hor-

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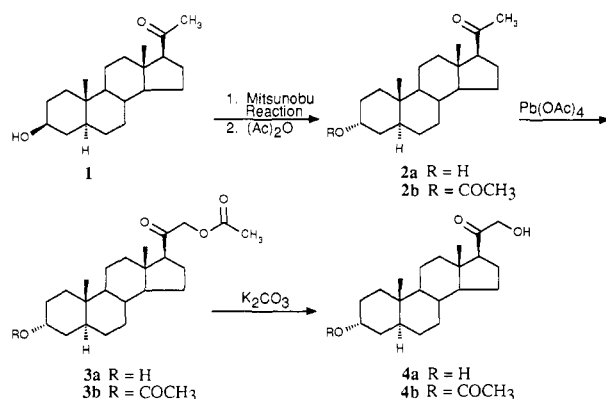
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## Scheme I



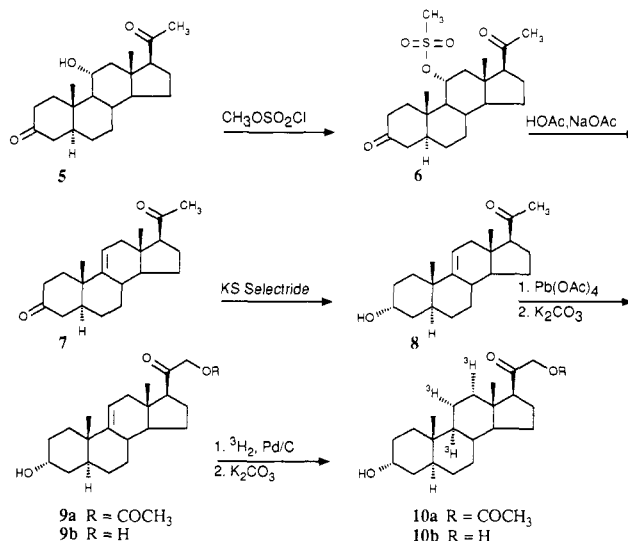
mone metabolites and other closely related compounds, (2) to prepare the tritiated forms of the natural steroids in order to investigate their metabolism and pharmacokinetics, and (3) to determine some of the structural requirements for biological activity by correlating the potentiation of GABA-receptor-mediated Cl<sup>-</sup> uptake in synaptoneurosomes with molecular graphic analyses of the steroid structures.

The ability of allopregnanolone, allotetrahydroDOC, and their derivatives to potentiate GABA-receptor-mediated stimulation of Cl<sup>-</sup> flux provides a sensitive assay for studying the effects of structural changes in these steroids. We have previously shown that the 3α-hydroxy metabolites in the 5α- and 5β-pregnane series have similar, but not identical, activity as modulators of GABA<sub>A</sub>-receptor-mediated Cl<sup>-</sup> flux.<sup>3b</sup> We now demonstrate the activity of the allylic 3α-hydroxypregn-4-en-20-one, another metabolite of progesterone.<sup>9</sup> We also investigated the effects of C<sub>3</sub> acetylation, unsaturation at C<sub>9(11)</sub>, C<sub>20</sub> ketone modification, C<sub>21</sub> modification, C<sub>11</sub> and C<sub>12</sub> hydroxylation, and several other structural modifications which alter pharmacological activity in vitro. This report provides a description of the structure-activity relationships for the interaction of the GABA<sub>A</sub> receptor with these hormonally inactive steroid metabolites and suggests the possibility of "partial agonist" activity by some of these compounds. In addition, we have identified the principal brain metabolites of allopregnanolone and allotetrahydroDOC in vivo and have demonstrated that they are inactive in potentiating GABA-receptor-mediated Cl<sup>-</sup> uptake. These studies also indicate that uptake of <sup>3</sup>H-labeled 2a into the brain is significantly greater than the uptake of <sup>3</sup>H-labeled 4a.

## Results and Discussion

**Chemistry.** The synthesis of the rare 3α-hydroxy-5α-pregnanes 2a, 3a, and 4a and their 3-acetates was accomplished via the route outlined in Scheme I. The relatively common 3β-hydroxy-5α-pregnan-20-one (1) was selected over 5α-pregnane-3,20-dione (10) as starting material for the preparation of 2a. Although stereospecific reduction of 10 to axial alcohol 2a was reported in about 35% yield after purification with either the Henbest reduction<sup>10</sup> or 1 equiv of potassium trisiamylborohydride,<sup>11</sup> we were unable to obtain 2a in pure form from 10 without extensive chromatographic purification by HPLC. We therefore used the Mitsunobu reaction<sup>12</sup> employing the very mild

## Scheme II



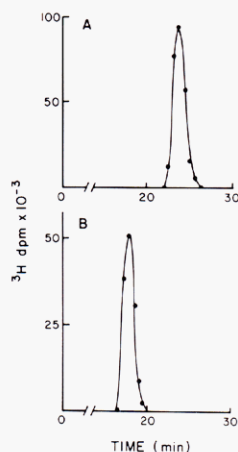
conditions of Varasi et al.<sup>13</sup> for accomplishing the inversion of the 3β-hydroxy group of 1 in 54% yield after final purification. Analogous to the preparation of 3β-hydroxy-5β-pregnan-20-one from 3α-hydroxy-5β-pregnan-20-one, where Varasi et al.<sup>13</sup> found no detectable starting material after crystallization, we were able to remove, by crystallization, the 5% of 1 found in the crude 2a. In this procedure the trifluoroacetate ester (not isolated) was hydrolyzed without the accompanying formation of 3α-hydroxy-5α,17α-pregnan-20-one. This 17α-isomer is difficult to separate from 2a and was formed when the more stable formate ester intermediate was prepared by using the general procedure of Bose et al.<sup>14</sup>

Modifying the conditions of Cocker et al.<sup>15</sup> for the 21-acetoxylation of 3β-acetoxy-5α-pregnane-11,20-dione by lead tetraacetate, we were able to obtain 3a in 35% yield from 2a. The yield was only 24% with the protected 3-acetate 2b for similar preparation of the 3,21-diacetate 3b. Furthermore, hydrolysis of the 3-acetyl group of 3b does not occur under the mild basic conditions used to remove the 21-acetyl group. Hydrolysis of 4b required acidic conditions due to the instability of the α-ketol group of 4b under the more basic conditions required for the hydrolysis of the 3-acetoxy group. Under these acidic conditions, an impurity, presumed to be the 17α-isomer of 4a, was formed which could be removed only by HPLC. When the *tert*-butyldimethylsilyl ether of 2a was reacted with lead tetraacetate in an attempt to increase the yield of 21-acetoxylation, this silyl ether did not survive in the presence of the boron trifluoride etherate catalyst.

The sequence of reactions shown in Scheme II was used to obtain the analogous 9(11)-pregnene isosteres. The mesyl group of 11α-hydroxy-5α-pregnane-3,20-dione (6) was easily eliminated to provide 5α-pregn-9(11)-ene-3,20-dione (7). Stereospecific reduction with trisiamylborohydride gave 8, followed by 21-acetoxylation to provide 9a. After separate 6-day catalytic reductions of 8 and 9a with tritium gas and palladium oxide in dioxane, purification by HPLC gave the desired 9α,11α,12α-<sup>3</sup>H-labeled products. The purity of <sup>3</sup>H-labeled 2a and 4a as demonstrated by HPLC is illustrated in Figure 1. No radioactive impurities

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 (10) Browne, P. A.; Kirk, D. N. *J. Chem. Soc. C* 1969, 1653.  
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**Figure 1.** Chromatogram of the separation of  $250 \times 10^3$  dpm [ $9\alpha,11\alpha,12\alpha$ - $^3\text{H}$ ]- $3\alpha$ -hydroxy- $5\alpha$ -pregnan-20-one (41 Ci/mmol, allopregnanolone) (A) and  $140 \times 10^3$  dpm [ $9\alpha,11\alpha,12\alpha$ - $^3\text{H}$ ]- $3\alpha,21$ -dihydroxy- $5\alpha$ -pregnan-20-one (61 Ci/mmol, allotetrahydroDOC) (B) by HPLC on two  $100 \times 9.4$  mm Partisil 5 ODS-3 RAC columns (Whatman, Inc.) in tandem using the solvent system 75% aqueous methanol with a flow rate of 3 mL/min in A and 2 mL/min in B. Fractions were collected at 0.5-min intervals and measured by liquid scintillation spectrometry for their content of radioactivity. The radioactive steroids eluted with the same retention times as unlabeled authentic standards measured by their absorption at 280 nm (not shown).

**Table I.** Molecular Modeling of Allopregnanolone:  $\text{C}_{16}\text{--C}_{17}\text{--C}_{20}\text{--O}_{20}$  Torsion Angles and Relative Energies

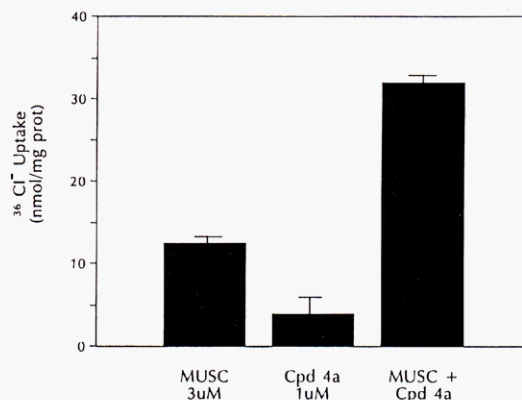
$\tau$ , deg	relative energy, kcal/mol
-8.5 (minimum)	0
-26.7 (saddle point)	0.177
-30.0 (minimum)	0.162
-140.7 (minimum)	0.284

were detected. A final specific activity of 41 and 63 Ci/mmol of  $^3\text{H}$ -labeled **2a** and **4a** respectively was achieved with a radiochemical purity of greater than 99%.

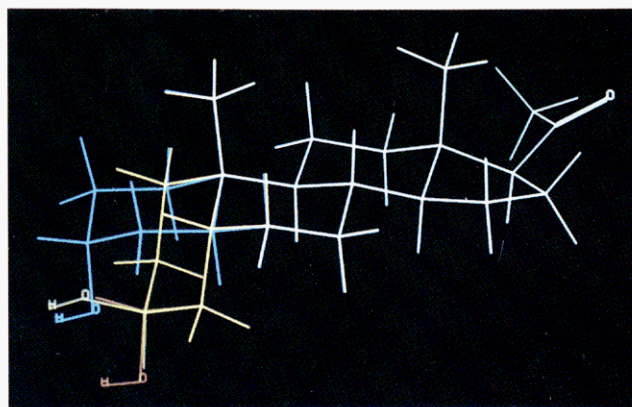
21-Mesylate **14** was prepared by the sequence of reactions shown in Scheme III. 21-Acetate **3a** was treated with *tert*-butyldimethylsilyl chloride to give **9c**. Hydrolysis of the 21-acetate group with  $\text{K}_2\text{CO}_3$  gave the intermediate **9d** which was then reacted with methanesulfonyl chloride in pyridine at  $0^\circ\text{C}$  to provide the protected 21-mesylate **9e**. The hydrolysis of the 3-ether group was achieved in tetrahydrofuran/acetic acid (1:3) at  $60^\circ\text{C}$  for 7 h to give the desired product **14**.

### Molecular Modeling

**Side-Chain Conformation.** The energetically preferred side-chain conformations of allopregnanolone (**2a**) were first investigated to determine a minimum conformation for use in subsequent comparisons with other steroid structures. The  $\text{C}_{17}\text{--C}_{20}$  torsion angle of **2a** was searched at a  $10^\circ$  resolution, to give three minima shown in Table I after minimization (confirmed by eigenvalue test). The minimum at  $\tau = -30^\circ$  is not physically meaningful since there is only a 0.015 kcal/mol barrier between it and the minimum at  $\tau = -8.5^\circ$ . The two minima together effectively constitute a single broad minimum with torsion angles between  $\tau = 0^\circ$  and  $-40^\circ$  readily accessible, which fits well with X-ray crystallographic data for a large number of steroids.<sup>16</sup> The minimum at  $\tau = -140.7^\circ$  is similar to one proposed on the basis of the  $^1\text{H}_{17\alpha}\text{--}^{13}\text{C}_{21}$



**Figure 2.** Potentiation of muscimol-stimulated  $^{36}\text{Cl}^-$  uptake by allotetrahydroDOC. Synaptoneurosomes were incubated for 5 s in the presence of muscimol (3  $\mu\text{M}$ ) alone, allotetrahydroDOC (1  $\mu\text{M}$ ) alone, or in combination. Muscimol produced a net increase in  $^{36}\text{Cl}^-$  uptake of  $12.8 \pm 0.8$  nmol/mg of protein. AllotetrahydroDOC had no significant effect on  $^{36}\text{Cl}^-$  uptake alone; however, it significantly enhanced muscimol stimulation;  $31.6 \pm 0.6$  nmol/mg of protein,  $p < 0.01$ . Data represent the mean  $\pm$  SE of five independent experiments, each conducted in quadruplicate.



**Figure 3.** Rigid BCD-ring overlay of allopregnanolone (**2a**, blue),  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one (red), and  $3\beta$ -hydroxy- $5\beta$ -pregnan-20-one (green).

coupling constant,<sup>17</sup> but this conformation is rarely if ever found in crystal structures of related steroids<sup>16</sup> and is somewhat higher in energy by molecular mechanics as well. Thus, the torsional minima corresponding to the  $\tau = -8.5^\circ$  minimum in allopregnanolone were used for modeling of these steroids.

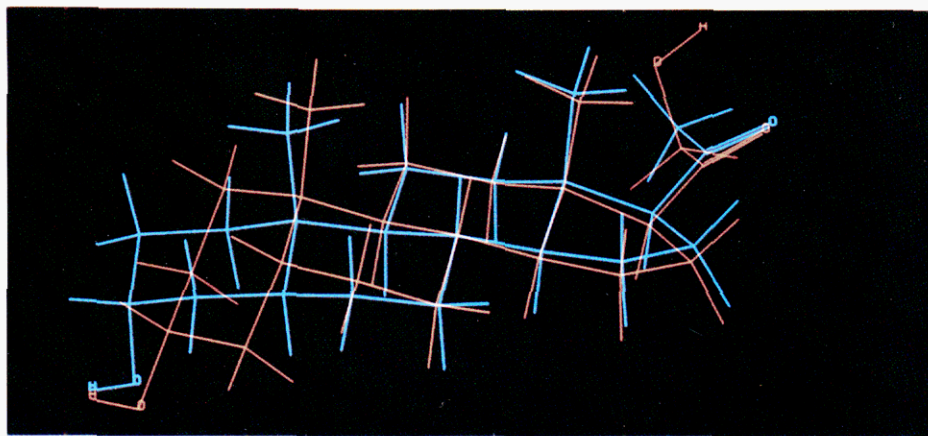
**Role of A Ring Conformation: Chair vs Boat.** The biologically active A ring conformation of analgesic steroids has been proposed by Phillips<sup>18</sup> to be a so-called " $\alpha$ -boat" structure. To test this hypothesis, the A ring of alphaxalone was searched at a  $30^\circ$  resolution with the MULTIC submode, giving structures which included the chair, twist boat, and the putative  $\alpha$ - and  $\beta$ -boats. Energy minimization of the structures showed that all boat variants minimized to the expected twist-boat conformation; the  $\alpha$ - and  $\beta$ -boats are not minima. The twist-boat conformation was 3.97 kcal/mol higher in energy than the chair conformation. Conformational searching of several of the compounds (**2a**, **3a**, **4a**, **11b**) showed the same behavior for the A rings, with the twist boat at least 4 kcal/mol higher in energy than the chair conformation in each case. These data demonstrate that the normal chair conformation is

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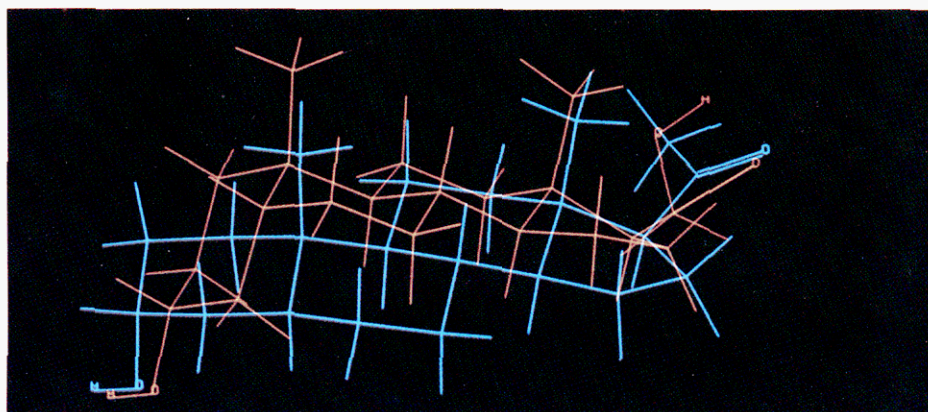
(17) Fesik, S. W.; Makriyannis, A. *Mol. Pharmacol.* **1985**, *27*, 624.

(18) Phillips, G. H. *J. Steroid Biochem.* **1975**, *6*, 607.



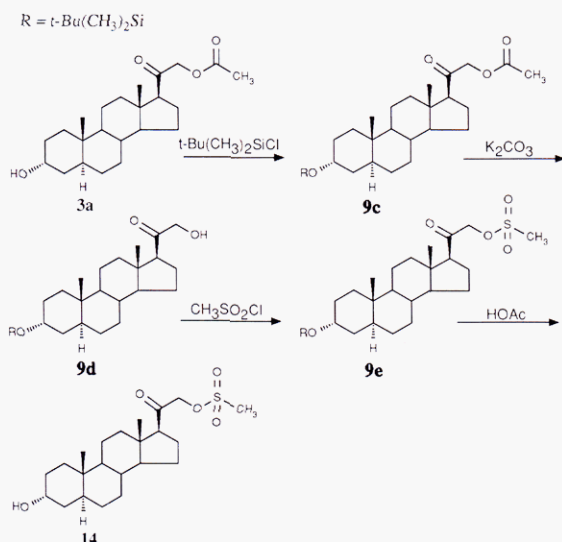


**Figure 4.** Template forcing of 3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one (**11b**, red) onto allopregnanolone (**2a**, blue) matching atoms O<sub>3</sub>, C<sub>6</sub>, C<sub>8</sub>, C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>17</sub> and O<sub>20</sub> with an added 2.0 kcal/mol per Å potential.



**Figure 5.** Rigid overlay of allopregnanolone (**2a**, blue) and 3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one (**11b**, red) matching atoms O<sub>3</sub>, C<sub>11</sub> and O<sub>20</sub>. Corresponding carbonyl and hydroxyl groups are closer than in the case of template forcing (Figure 4).

### Scheme III



clearly preferred in comparison to the “ $\alpha$ -boat” structure proposed by Phillipps.<sup>18</sup>

**Role of AB Ring Fusion: Cis vs Trans.** An essential feature in the pharmacologically active 5 $\alpha$ - and 5 $\beta$ -pregnane structures is the presence of a 3 $\alpha$ -hydroxyl group.<sup>2</sup> This is exemplified in the comparison of allopregnanolone (**2a**) and 3 $\alpha$ -hydroxy-5 $\beta$ -pregnan-20-one versus the inactive 3 $\beta$ -hydroxy-5 $\beta$ -pregnan-20-one. A BCD ring rigid overlay of the three structures in Figure 3 shows that the O<sub>3</sub> of the 3 $\beta$ ,5 $\beta$ -structure lies within 0.28 Å of the corresponding O<sub>3</sub> of allopregnanolone, whereas the corresponding distance

between the O<sub>3</sub>'s of allopregnanolone and the 3 $\alpha$ ,5 $\beta$ -structures is 1.96 Å. Nevertheless, it is allopregnanolone and the 3 $\alpha$ ,5 $\beta$ -compound which are active.

The preceding overlay implicitly assumes that the BCD ring location of the 5 $\beta$ -steroids, when bound to the GABA receptor, is similar to that of the 5 $\alpha$ -steroids. To test this assumption, template forcing<sup>19</sup> was used to force the chair and boat forms of 3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one (**11b**) to conform to the minimized chair form of allopregnanolone, matching eight sets of atoms (O<sub>3</sub>, C<sub>6</sub>, C<sub>8</sub>, C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>17</sub>, O<sub>20</sub>) with an added 2.0 kcal/mol per Å potential. After template forcing, the distance between O<sub>3</sub>'s for the twist-boat conformation of **11b** and allopregnanolone had been reduced to 0.45 Å at a cost of 0.32 kcal/mol in addition to the 4.3 kcal/mol penalty for being in the twist-boat conformation rather than the chair form. The corresponding chair conformation of **11b**, after template forcing to the structure of allopregnanolone, has a distance between the O<sub>3</sub>'s of 0.60 Å at a cost of 0.59 kcal/mol (Figure 4). Thus neither the chair nor the twist-boat forms of **11b** are able to deform sufficiently to achieve concurrent good overlap of both the oxygens and the steroid skeleton. This is consistent with the reduction of pharmacological activity of **11b** compared to **4a** (Table II).

It was not possible to achieve good overlap of the BCD rings of the 5 $\alpha$ - and 5 $\beta$ -structures concurrently with overlap of their 3 $\alpha$ -hydroxyl and C<sub>20</sub> carbonyl groups, so

(19) Duchamp, D. J. In *Computer-Assisted Drug Design*; Olson, E. C., Christoffersen, R. E., Ed.; ACS Symposium Series 112; American Chemical Society: Washington, DC, 1979; p 79.

the overlay requirement was eased to match only O<sub>3</sub>, C<sub>11</sub>, and O<sub>20</sub>. Rigid overlay of the minimum energy structures (Figure 5) now places the corresponding oxygens within 0.3 Å of each other without needing to deform the 5 $\beta$ -structure. In contrast, the A ring boat conformation proposed by Phillips<sup>18</sup> requires that the 5 $\beta$ -structure have both the A and B rings in the boat conformation in order to place the corresponding oxygen atoms in reasonable proximity. This would be extremely unfavorable energetically, and it is unnecessary if the rings are left in the chair conformation.

### Pharmacology

The potentiation of muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake in cerebral cortical synaptoneurosomes by steroid hormone metabolites provides an accurate and sensitive in vitro assay for the functional activity of these metabolites. The potency and efficacy of allopregnanolone and allotetrahydroDOC for the enhancement of muscimol-stimulated Cl<sup>-</sup> flux are much greater than the direct effects of these steroids on Cl<sup>-</sup> flux, suggesting that potentiation of GABA receptor-mediated Cl<sup>-</sup> conductance may be the physiologically relevant mechanism of action of these steroids.<sup>2a,3</sup> In an initial study the net uptake of <sup>36</sup>Cl<sup>-</sup> into synaptoneurosomes in the presence of muscimol (3  $\mu$ M) or allotetrahydroDOC (1  $\mu$ M) alone and in combination is presented in Figure 2. Although allotetrahydroDOC had no significant direct effect on <sup>36</sup>Cl<sup>-</sup> uptake at this concentration, it enhanced the effect of muscimol. The ability of the steroid hormone derivatives prepared in this study to potentiate muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake in subsequent experiments is shown in Table II. AllotetrahydroDOC produced the maximal potentiation, increasing muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake by approximately 120%. The 21-acetate derivative of allotetrahydroDOC (3a) or the isomeric 3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one 21-acetate (tetrahydroDOC 21-acetate, 11a) were both very effective in potentiating Cl<sup>-</sup> flux. The relatively high potency of the 21-acetate derivative of allotetrahydroDOC has not been observed previously in vitro. Nevertheless, the anesthetic activities of the 21-acetate derivatives in mice are very similar to those of the corresponding alcohols. However, the effect of the 21-acetate group may have only pharmacological significance, since 3a was not found in the brain as a metabolite of <sup>3</sup>H-labeled 4a when the latter compound was administered to rats (see below). 3,21-Diacetate 3b, 3-monoacetates 2b and 4b, and 3-oxime 21 were essentially inactive as potentiators of muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake. This further supports the conclusion that a free 3 $\alpha$ -hydroxyl group is essential for activity, both in vitro and in vivo.<sup>2,18</sup> 3-Ketone metabolites 20 and 22 either had very low activity or were devoid of activity, consistent with their lack of anesthetic effect in vivo.<sup>18</sup> Progesterone (23) is also inactive at 1  $\mu$ M in vitro (Table II).

Interestingly, the 5 $\beta$ -isomer (tetrahydroDOC, 11b), as well as the 21-mesylate of allotetrahydroDOC (14), were less active than allotetrahydroDOC itself at concentrations of 1  $\mu$ M. The reduced activity of tetrahydroDOC (11b) compared to allotetrahydroDOC in this functional assay is consistent with the comparative anesthetic potency of these steroids in rats,<sup>20</sup> but contrasts with the equivalent effects of these two isomers in comparison with [<sup>35</sup>S]TBPS binding.<sup>2b</sup> This reduction of activity in the 5 $\beta$ -isomer on GABA-receptor-mediated chloride flux, but not on the affinity for [<sup>35</sup>S]TBPS binding, suggests that this com-

**Table II.** Effect of Structural Modification on the Potentiation of Muscimol-Stimulated <sup>36</sup>Cl<sup>-</sup> Uptake in Rat Brain Synaptoneurosomes by Steroids

no.	steroid <sup>a</sup> (1 $\mu$ M)	potentiation, <sup>b</sup> nmol/mg of protein (mean $\pm$ se)
<b>Active Compounds</b>		
4a	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one (allotetrahydroDOC)	13.1 $\pm$ 1.1
16	3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17 $\beta$ -carbo- nitrile	13.0 $\pm$ 2.6
3a	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one 21-acetate	12.2 $\pm$ 1.1
2a	3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one (allopregnanolone)	10.9 $\pm$ 2.2
12	3 $\alpha$ -hydroxypregn-4-en-20-one	10.4 $\pm$ 1.0
11a	3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one 21-acetate	10.3 $\pm$ 0.6
14	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one 21-mesylate	7.9 $\pm$ 0.7
11b	3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one (tetrahydroDOC)	7.7 $\pm$ 0.2
15	3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one	7.0 $\pm$ 2.3
18	3 $\alpha$ -hydroxy-5 $\beta$ -androstane-17-one	4.9 $\pm$ 0.7
19	3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17 $\beta$ - carboxylic acid methyl ester	3.6 $\pm$ 0.6
<b>Inactive Compounds</b>		
<b>A Ring Modification</b>		
2b	3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one acetate	2.57 $\pm$ 1.65
3b	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one diacetate	1.37 $\pm$ 1.10
4b	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one 3-acetate	0.48 $\pm$ 0.32
13	3 $\beta$ -hydroxypregn-4-en-20-one	1.43 $\pm$ 1.20
20	5 $\alpha$ -pregnane-3,20-dione	2.63 $\pm$ 1.30
21	5 $\alpha$ -pregnane-3,20-dione 3-oxime	-3.26 $\pm$ 1.80
22	21-hydroxy-5 $\alpha$ -pregnane-3,20-dione	-1.95 $\pm$ 0.30
23	progesterone	-1.80 $\pm$ 1.20
<b>C Ring Modification</b>		
8	3 $\alpha$ -hydroxy-5 $\alpha$ -pregn-9(11)-en-20-one	1.23 $\pm$ 1.20
9b	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregn-9(11)-en-20- one	1.40 $\pm$ 0.69
9a	3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregn-9(11)-en-20- one 21-acetate	0.34 $\pm$ 0.32
24	3 $\alpha$ ,11 $\alpha$ -dihydroxy-5 $\alpha$ -pregnan-20-one	0.70 $\pm$ 0.85
25	3 $\alpha$ ,11 $\alpha$ -dihydroxy-5 $\beta$ -pregnan-20-one	0.98 $\pm$ 0.44
26	3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -pregnan-20-one	-2.58 $\pm$ 1.49
<b>Side-Chain Modification</b>		
27	3 $\alpha$ -hydroxy-5 $\alpha$ ,17 $\alpha$ -pregnan-20-one	1.12 $\pm$ 0.33
17	3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17 $\alpha$ -carbo- nitrile	-2.10 $\pm$ 0.98
28	3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one oxime	-1.91 $\pm$ 0.81
29	20-ethylenedioxy-3 $\alpha$ -hydroxy-5 $\alpha$ -preg- nane	0.16 $\pm$ 0.90
30	20-ethylenedioxy-3 $\alpha$ -hydroxy-5 $\beta$ -preg- nane	-0.96 $\pm$ 0.86
31	5 $\alpha$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol	1.97 $\pm$ 0.50

<sup>a</sup> Steroid (1  $\mu$ M) and muscimol (3  $\mu$ M) were added simultaneously. <sup>b</sup> Potentiation represents the relative rate of <sup>36</sup>Cl<sup>-</sup> uptake in the presence of steroid and muscimol, compared to that produced by muscimol alone. Uptake of <sup>36</sup>Cl<sup>-</sup> was measured for 5 s. Data are the mean  $\pm$  SE of five to 10 separate measurements.

pound has reduced efficacy compared with that of allotetrahydroDOC.

The ability of 3 $\alpha$ -hydroxypregn-4-en-20-one (12) to potentiate muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake contrasts with its 3 $\beta$ -hydroxy isomer (13), which is inactive. This stereospecificity is consistent with radioligand binding and behavioral studies where 3 $\beta$ -hydroxy-5 $\alpha$ -pregnan-20-one was shown to be inactive in competing with [<sup>35</sup>S]TBPS binding<sup>2</sup> or in producing analgesia.<sup>7</sup> The bioreduction of progesterone by 3 $\alpha$ -hydroxysteroid dehydrogenase to form allylic alcohol 12 results in a unique ring A metabolite

(20) Norberg, L.; Wahlström, G.; Bäckström, T. *Pharmacol. Toxicol.* 1987, 61, 42.

which has been reported in brain,<sup>11</sup> and found to suppress follicle stimulating hormone secretion in the pituitary without also inhibiting luteinizing hormone secretion.<sup>9</sup> The potentiation of muscimol-stimulated <sup>36</sup>Cl<sup>-</sup> uptake in rat cerebral cortical synaptoneurosome by 12 suggests that this metabolite may also have anxiolytic or sedative properties. With use of molecular graphics, the structure of 12 can be rigidly fitted to allopregnanolone (matching O<sub>3</sub>, C<sub>11</sub>, C<sub>17</sub>, O<sub>20</sub>) with a root mean square (RMS) difference of 0.20 Å. Template forcing (*k* = 2.0 kcal/mol per Å) reduces the RMS difference to only 0.11 Å at a cost of 0.07 kcal/mol (Figure 6).

In contrast to the 3 $\alpha$ -hydroxy metabolites with 5 $\alpha$ -pregnane or 5 $\beta$ -pregnane steroid skeletons, the 3 $\alpha$ -hydroxy derivatives in the 5 $\alpha$ -pregn-9(11)-ene series (8, 9a, and 9b) were essentially inactive as potentiators of muscimol-stimulated Cl<sup>-</sup> flux (Table II). These results demonstrate that the interaction of the steroids with this chloride ion channel complex is markedly altered when the 9(11)-double bond is introduced in ring C, making this ring more planar. Comparing structure 8 by molecular graphics (built from the alphaxone crystal structure and minimized as usual) with allopregnanolone shows that C<sub>11</sub> and C<sub>12</sub> of 8 protrude well into the  $\alpha$ -face of the steroid, relative to the active structure (Figure 7). There are no other significant differences between these two structures.

In previous studies it has been shown that a steroidal C<sub>20</sub> ketone group is not absolutely required for significant interaction of a steroid with the GABA-receptor complex. Thus 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one (15) was shown to effectively enhance [<sup>3</sup>H]muscimol binding to rat brain membranes,<sup>2h</sup> and 3 $\alpha$ ,17 $\beta$ -dihydroxy-5 $\alpha$ -androstane weakly displaced [<sup>35</sup>S]TBPS binding to these membrane preparations.<sup>2g</sup> For natural C<sub>21</sub> steroid metabolites, reduction of the C<sub>20</sub> ketone group led to a decrease in binding potency, but did not abolish [<sup>35</sup>S]TBPS binding activity in the 3 $\alpha$ -hydroxy-5 $\alpha$ -pregnane series.<sup>2g</sup> We found that the 17-oxime (28) of 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one (15) was inactive in the potentiation of Cl<sup>-</sup> flux in vitro (Table II). Similarly, the synthetic 20-ethylenedioxy derivatives (29, 30) of allopregnanolone and pregnenolone were also inactive, as was 5 $\alpha$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol (31), in contrast to other results<sup>21</sup> for this metabolite of progesterone. This supports the importance of a C<sub>17</sub> or C<sub>20</sub> ketone group as a presumed hydrogen-bond acceptor in natural steroid metabolites. The synthetic 17 $\beta$ -carboxylic acid methyl ester 19 contains the ester carbonyl group, which can also serve this function; molecular modeling of the ester shows the same two minima for the C<sub>17</sub>-C<sub>20</sub> torsion angle as for structure 2a, and rigid overlay of 19 and 2a matching the ABCD ring atoms places the carbonyl oxygens within 0.11 Å of each other. However, this methyl ester (19) has much less pharmacological activity than 2a.

Since the 17 $\beta$ -carbonitrile of 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-11-one was reported to be active as an anesthetic in rats,<sup>18</sup> we prepared the 17 $\alpha$ - and 17 $\beta$ -carbonitrile derivatives of 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane. As expected from the importance of the side-chain configuration, 17 $\alpha$ -carbonitrile 17 was inactive, analogous to the inactive 17 $\alpha$ -epimer 3 $\alpha$ -hydroxy-5 $\alpha$ ,17 $\alpha$ -pregnan-20-one (25). However, the synthetic 17 $\beta$ -carbonitrile 16 was as active at 1  $\mu$ M as allotetrahydroDOC in the potentiation of Cl<sup>-</sup> flux, and 17-ketone 15 was less active. The ABC ring overlay of compounds 2a, 15, and 16 in Figure 8 shows that these compounds differ primarily in the location of the side-chain heteroatom (O<sub>20</sub> for 2a, O<sub>17</sub> for 15, nitrile N for 16).

**Table III.** Recovery of Radioactivity 20 min after Administration of <sup>3</sup>H-Labeled Steroids to Rats<sup>a</sup>

plasma dpm/ mL $\times 10^3$	brain dpm/ g $\times 10^3$	ratio brain/ plasma <sup>b</sup>	% of total tritium in extracts of brain	
Treatment: 100 $\mu$ Ci				
[9 $\alpha$ ,11 $\alpha$ ,12 $\alpha$ - <sup>3</sup> H]-3 $\alpha$ ,21-Dihydroxy-5 $\alpha$ -pregnan-20-one (4a)				
			4a	22
316 $\pm$ 14	212 $\pm$ 13	0.68 $\pm$ 0.05	48.1 $\pm$ 3.6	46.1 $\pm$ 2.3
Treatment: 100 $\mu$ Ci				
[9 $\alpha$ ,11 $\alpha$ ,12 $\alpha$ - <sup>3</sup> H]-3 $\alpha$ -Hydroxy-5 $\alpha$ -pregnan-20-one (2a)				
			2a	20
176 $\pm$ 1	342 $\pm$ 11	1.95 $\pm$ 0.05	50.4 $\pm$ 3.9	40.6 $\pm$ 1.1

<sup>a</sup> Adult male Sprague-Dawley rats (240–260 g) administered <sup>3</sup>H-labeled steroid by tail vein injection. <sup>b</sup> Column 3 divided by column 2. <sup>c</sup> All values are means  $\pm$  SE.

Structure 2a (O<sub>3</sub>-O<sub>20</sub> distance = 11.15 Å) can be reconciled with 15 (O<sub>3</sub>-N<sub>20</sub> distance = 11.09 Å) by a slight reorientation to align the side-chain heteroatoms. Since both 15 (O<sub>3</sub>-O<sub>17</sub> distance = 9.66 Å) and 16 are rigid, the 1.43 Å difference in O<sub>3</sub>-heteroatom distance suggests that these compounds may interact with different binding sites on the GABA<sub>A</sub> receptor complex, although this possibility or the existence of multiple conformational states of a single binding site or the interaction of these ligands with multiple GABA<sub>A</sub> isoreceptors cannot be distinguished by molecular modeling of the ligands. The presence of different 3 $\alpha$ -hydroxysteroid binding sites is strongly implied from recent neurophysiological evidence demonstrating differences in the effects of these steroids on membrane current recorded from cells with recombinantly expressed human GABA<sub>A</sub> isoreceptors (S. Vincini et al., unpublished results).

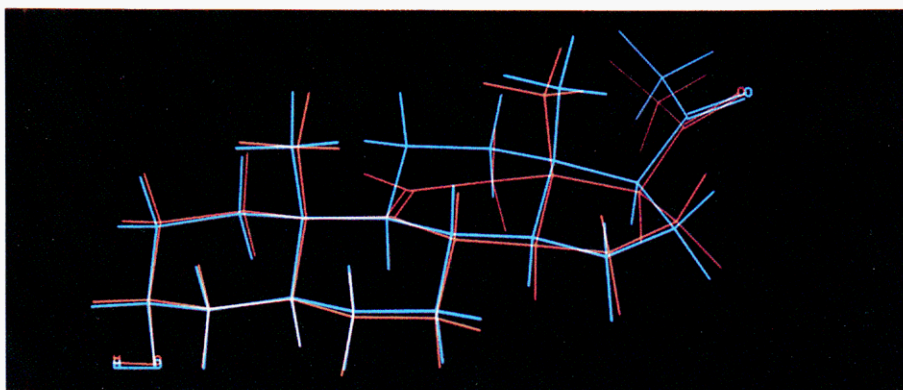
The formation of progesterone metabolites (2a and 12) and DOC metabolites (4a and 11b) in vivo may be an important mechanism in the regulation of GABA-mediated inhibition in the central nervous system. The comparative hypnotic effects of 2a and 4a were studied by Mendelson et al.<sup>5</sup> in the rat at doses of 5 and 10 mg/kg, where 4a but not 2a had potent sleep-inducing properties and increased non-REM sleep. Similarly, 4a but not 2a was shown by Crawley et al.<sup>4a</sup> to have anxiolytic activity in rodents in the range of 5–15 mg/kg. These comparative results in vivo might be explained by a more rapid metabolism of 2a compared to that of 4a. These possibilities were explored in a preliminary experiment in rats following the administration by tail vein of 100  $\mu$ Ci of <sup>3</sup>H-labeled 2a or 4a. From the data in Table III, the concentration of radioactivity in plasma obtained 20 min after the labeled steroid was injected was 80% higher for allotetrahydroDOC (4a) than the level obtained from rats given allopregnanolone (2a). It is therefore possible that an increased metabolic clearance rate of 2a compared to that of 4a might account for the greater anxiolytic and sedative effects of 4a in vivo.<sup>4,5</sup> However, in brain tissue obtained 20 min after the radioactive compound was administered, there was a 60% higher level of total radioactivity/g of brain in the group receiving 2a compared to the group treated with 4a (Table III). After purification by solvent extraction and HPLC, there was an equal amount (50.4%  $\pm$  3.9%) of 2a and (48.1%  $\pm$  3.6%) of 4a as the mean percent of total radioactivity in brain tissue in each group. A more extensive pharmacokinetic study is required to resolve this dilemma.

The principal metabolites of the <sup>3</sup>H-labeled 3 $\alpha$ -hydroxysteroids in brain were the 3-ketones, with 5 $\alpha$ -pregnane-3,20-dione (20) as 40.6%  $\pm$  1.1% of total radioactivity in brain tissue from 2a and 21-hydroxy-5 $\alpha$ -preg-

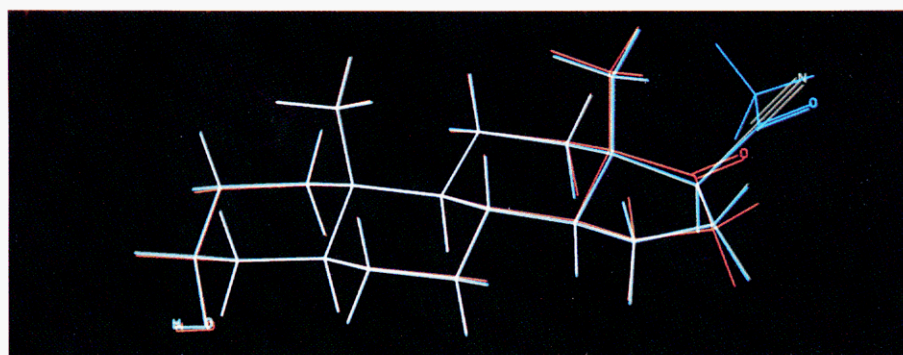




**Figure 6.** Template forcing of 3 $\alpha$ -hydroxypregn-4-en-20-one (12, red) onto allopregnanolone (2a, blue) matching atoms O<sub>3</sub>, C<sub>11</sub>, C<sub>17</sub>, O<sub>20</sub> with an added 2.0 kcal/mol per Å potential.



**Figure 7.** Rigid overlay of allopregnanolone (2a, blue) and 3 $\alpha$ -hydroxy-5 $\alpha$ -pregn-9(11)-en-20-one (8, red) showing the added steric hindrance on the  $\alpha$ -face at C<sub>11</sub> and C<sub>12</sub> of structure 8.



**Figure 8.** Rigid overlay of the skeletons of allopregnanolone (2a, blue), 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17 $\beta$ -carbonitrile (16, green), and 3 $\alpha$ -hydroxy-5 $\alpha$ -androstan-17-one (15, red).

nane-3,20-dione (22) as  $46.1 \pm 2.3\%$  of total radioactivity in the brain after administration of 4a. After acetylation, the retention time of radioactive 20 remained unchanged and radioactive 22 was converted to a derivative with the retention time of 21-hydroxy-5 $\alpha$ -pregnane-3,20-dione acetate. This provides additional confirmation of the identification of 20 and 22 as metabolites. No evidence was found for the presence of the 21-acetate of 4a as a metabolite of 4a in the brain extracts.

The availability of the tritium-labeled compounds will be of assistance in measuring physiological concentrations of 2a and 4a in blood and brain tissue by radioimmunoassay. In fact, we have recently demonstrated the presence of 2a in both blood and brain tissue of rats at concentrations capable of potentiating muscimol-stimulated Cl<sup>-</sup> flux.<sup>22</sup> The formation of these metabolites in

vivo may therefore, be a significant mechanism in regulating GABA<sub>A</sub> receptor-mediated inhibition in the central nervous system. The synthesis of a bioavailable analogue of 2a or 4a may be a useful starting point for the development of novel anxiolytic/hypnotic compounds.

### Experimental Section

**Steroid Synthesis.** Melting points were determined with a Thomas-Hoover Model 6406-H apparatus. IR spectra were recorded in KBr with a Perkin-Elmer 1600 FTIR spectrometer. <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> solutions with a Varian EM-390 90-MHz spectrometer and are reported in ppm downfield from Me<sub>4</sub>Si as the internal reference. Elemental microanalyses

(22) Purdy, R. H.; Moore, P. H., Jr.; Rao, P. N.; Hagino, N.; Yamaguchi, T.; Schmidt, P.; Rubinow, D. R.; Morrow, A. L.; Paul, S. M. *Steroids*, in press.

were performed by Midwest Microlab, Ltd., Indianapolis, IN, and are within  $\pm 0.3\%$  of the theoretical values. Emission spectroscopy was carried out by Huffman Laboratories, Inc., Golden, CO. Chromatographic purification of products was performed by using the technique of Still et al.<sup>23</sup> with Merck silica gel (grade 60, 230–400 mesh, Aldrich Chemical Co.). High-performance liquid chromatography (HPLC) was performed with a Waters Associates Model 6000A pump, two Partisil 5 ODS-3 RAC columns ( $9.4 \times 100$  mm, Whatman, Inc.) in tandem using 75–85% aqueous methanol as solvent system at a flow rate of 1–3 mL/min. A Milton Roy SpectroMonitor 3100 detector was used to monitor the chromatographic effluent at 205 and 280 nm. A SuperRac Model 2211 LKB fraction collector was used to collect the effluent for determination of radioactivity after the addition of 8 mL ScintiVerse LC scintillation fluid (Fisher Scientific Co.). Radioactivity was measured with a Beckman Model LS 7500 liquid-scintillation spectrometer.

The preparations of 3 $\alpha$ -hydroxypregn-4-en-20-one (12) and 3 $\beta$ -hydroxypregn-4-en-20-one (13) were carried out by the method of Wiebe et al.<sup>11</sup> The 3-oxime of 5 $\alpha$ -pregnane-3,20-dione was synthesized by the procedure of Nace and Watterson.<sup>24</sup> 3 $\alpha$ -Hydroxy-5 $\alpha$ ,17 $\alpha$ -pregnan-20-one (27) was synthesized by isomerization of allopregnanolone (2a).<sup>25</sup> The 17-oxime of 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one (28) and the 20-ethylenedioxy derivatives (29, 30) were prepared in the usual manner. The starting material 3 $\beta$ -hydroxy-5 $\alpha$ -pregnan-20-one (1) was a gift from Dr. John S. Baran, G. D. Searle Co., and 11 $\alpha$ -hydroxy-5 $\alpha$ -pregnane-3,20-dione (5)<sup>26</sup> was purchased from Steraloids, Inc.

**3 $\alpha$ -Hydroxy-5 $\alpha$ -pregnan-20-one (2a).** The reaction of 3 $\beta$ -hydroxy-5 $\alpha$ -pregnan-20-one (1) with trifluoroacetic acid in the presence of triphenylphosphine and diethyl azodicarboxylate without isolation of the intermediate trifluoroacetate<sup>13</sup> yielded 2a in 74% yield after the usual workup and crystallization from ethyl acetate and aqueous ethanol free of detectable 1 by HPLC, mp 168–170 °C (lit.<sup>10</sup> mp 170–173 °C). The mother liquor from the first crystallization contained 5% of total steroids as 1 by HPLC analysis. The final product was purified to homogeneity with dry column chromatography on silica gel with 2% acetone in  $\text{CH}_2\text{Cl}_2$  and crystallized from aqueous ethanol; yield 54%, mp 174–176 °C.

**3 $\alpha$ -Acetoxy-5 $\alpha$ -pregnan-20-one (2b).** Treatment of 2a in the usual manner gave 2b, mp 135–138 °C (lit.<sup>27</sup> mp 139–140 °C).

**21-Acetoxy-3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one (3a).** The reaction of 5.23 g of 2a in 300 mL of benzene, 16 mL of methanol, and 29 mL of boron trifluoride etherate with 10.9 g of lead tetraacetate was carried out under argon for 3 h at 0 °C. The reaction mixture was diluted with 300 mL of saturated aqueous sodium bicarbonate and extracted twice with 300 mL of ether at 0 °C. The combined ether extracts were washed with 300 mL of water, dried over  $\text{Na}_2\text{SO}_4$ , and crystallized from ethyl acetate to afford 2.07 g (35%) of 3a, mp 210–216 °C (lit.<sup>28</sup> mp 216–218 °C). Recrystallization gave product, mp 215–219 °C, which was free of detectable impurities by HPLC.

**3 $\alpha$ ,21-Diacetoxy-5 $\alpha$ -pregnan-20-one (3b).** In a similar manner 1.71 g of 2b was converted to 0.46 g (24%) of 3b after crystallization from ether/petroleum ether, mp 162–163 °C (lit.<sup>29</sup> mp 164–165 °C).

**3 $\alpha$ ,21-Dihydroxy-5 $\alpha$ -pregnan-20-one (4a).** A suspension of 0.68 g of 3a in 21 mL of methanol was treated with 1.80 mL of aqueous 10%  $\text{K}_2\text{CO}_3$  under argon, stirred at room temperature for 30 min, cooled to 0 °C, and acidified (pH 5) with 0.7 mL of 2 M acetic acid. Methanol was removed in vacuo until crystallization occurred, giving 0.58 g (96%) of 4a, mp 162–164 °C (lit.<sup>28</sup> mp 163–166 °C). To remove all traces of lead, 3a was purified by dry column chromatography on silica gel using  $\text{CH}_2\text{Cl}_2$ /ethyl acetate (9:1) prior to hydrolysis. This provided 4a with less than

10 ppm lead by emission spectroscopy.

**3 $\alpha$ -Acetoxy-21-hydroxy-5 $\alpha$ -pregnan-20-one (4b).** A methanolic suspension of 3b was hydrolyzed by the procedure described for compound 4a to provide 125 mg (93%) of 4b. An analytical sample was crystallized from aqueous methanol: mp 168–172 °C; IR (KBr) 3501 (OH), 1721 (CO), 1703 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.62 (s, 3 H, 18- $\text{CH}_3$ ), 0.77 (s, 3 H, 19- $\text{CH}_3$ ), 2.05 (s, 3 H,  $\text{COCH}_3$ ), 4.20 (d, 2 H, 21- $\text{CH}_2$ ), 5.05 (b s, 1 H, 3 $\beta$ -H). Anal. ( $\text{C}_{23}\text{H}_{36}\text{O}_4$ ) C, H.

**11 $\alpha$ -[(Methylsulfonyl)oxy]-5 $\alpha$ -pregnan-20-one (6).** A solution of 1.02 g of 11 $\alpha$ -hydroxy-5 $\alpha$ -pregnane-3,20-dione (5)<sup>26</sup> in 5 mL of dry pyridine at 0 °C was treated with 0.4 mL of methanesulfonyl chloride for 2 h. The mixture was poured on ice, and the precipitate was collected and washed with cold 1 N HCl to afford 1.22 g (97%) of crude 6, mp 145–146 °C dec. An analytical sample was crystallized from acetone/hexane: mp 146–148 °C dec; IR (KBr) 1165 (SO), 1321 (SO), 1700 (CO), 1715 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.70 (s, 3 H, 18- $\text{CH}_3$ ), 1.19 (s, 3 H, 19- $\text{CH}_3$ ), 2.13 (s, 3 H, 21- $\text{CH}_2$ ), 3.00 (s, 3 H,  $\text{CH}_3\text{SO}_2$ ), 5.13 (m, 1 H, 11 $\beta$ -H). Anal. ( $\text{C}_{22}\text{H}_{34}\text{O}_5\text{S}$ ) C, H, S.

**5 $\alpha$ -Pregn-9(11)-ene-3,20-dione (7).** A solution of 1.22 g of 6 and 1.7 g of anhydrous sodium acetate in 15 mL of glacial acetic acid was refluxed for 30 min, cooled to 0 °C, and diluted with 15 mL of  $\text{H}_2\text{O}$  to give 0.86 g (92%) of 7: mp 203–204 °C; IR (KBr) 1695 (CO), 1715 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.59 (s, 3 H, 18- $\text{CH}_3$ ), 1.15 (s, 3 H, 19- $\text{CH}_3$ ), 2.12 (s, 3 H, 21- $\text{CH}_2$ ), 5.43 (b t, 1 H, 11-H). Anal. ( $\text{C}_{22}\text{H}_{30}\text{O}_2$ ) C, H.

**3 $\alpha$ -Hydroxy-5 $\alpha$ -pregn-9(11)-en-20-one (8).** To a stirred solution of 0.86 g of 7 in 60 mL of dry tetrahydrofuran under argon at –78 °C was added 3.0 mL of 1 M K-Selectride (Aldrich Chemical Co.). After 1 h the solution was warmed to 0 °C and added with stirring to 55 mL of 0.5 M Tris HCl buffer (pH 7). After 45 min of stirring at 0 °C, the mixture was extracted twice with 100 mL of ethyl acetate and dried in vacuo to give 0.97 g of crude 8. The product was purified by HPLC on a Magnum 20 Partisil 10 ODS-3 column ( $2.25 \times 50$  cm, Whatman, Inc.) with a  $0.94 \times 15$  cm ODS-3 guard column using 75% aqueous methanol at a flow rate of 9.9 mL/min, eluting between 56 and 70 min. Crystallization from aqueous methanol provided 0.22 g (25%) of 8: mp 172–173 °C (lit.<sup>30</sup> mp 169–170 °C); IR (KBr) 3277 (OH), 1706 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.57 (s, 3 H, 18- $\text{CH}_3$ ), 0.90 (s, 3 H, 19- $\text{CH}_3$ ), 2.12 (s, 3 H, 21- $\text{CH}_2$ ), 4.10 (b s, 1 H, 3 $\beta$ -H), 5.38 (b t, 1 H, 11-H). Anal. ( $\text{C}_{21}\text{H}_{32}\text{O}_2$ ) C, H.

**21-Acetoxy-3 $\alpha$ -hydroxy-5 $\alpha$ -pregn-9(11)-en-20-one (9a).** By the procedure described for 3a, 0.158 g of 8 in 9.5 mL of benzene, 0.5 mL of methanol, and 0.9 mL of boron trifluoride etherate was reacted with 0.334 g of lead tetraacetate to give 98 mg (52%) of 9a after crystallization from ethyl acetate: mp 236–238 °C; IR (KBr) 3558 (OH), 1753 (CO), 1715 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3/\text{CD}_3\text{OD}$ , 1:1)  $\delta$  0.57 (s, 3 H, 18- $\text{CH}_3$ ), 0.90 (s, 3 H, 19- $\text{CH}_3$ ), 2.13 (s, 3 H,  $\text{COCH}_3$ ), 3.98 (b s, 1 H, 3 $\beta$ -H), 4.66 (dd, 2 H, 21- $\text{CH}_2$ ), 5.30 (b t, 1 H, 11-H). Anal. ( $\text{C}_{23}\text{H}_{34}\text{O}_4$ ) C, H.

**3 $\alpha$ ,21-Dihydroxy-5 $\alpha$ -pregn-9(11)-en-20-one (9b).** Under the conditions of hydrolysis described for 4a, 66 mg of 9a suspended in 4 mL of methanol and treated with 0.33 mL of aqueous 10%  $\text{K}_2\text{CO}_3$  gave 50 mg of crude 9b, which was purified by HPLC on a Magnum 9 Partisil ODS-3-column ( $2.25 \times 25$  cm, Whatman, Inc.) using 75% aqueous methanol at a flow rate of 5 mL/min, eluting between 13 and 16 min. Crystallization from aqueous methanol gave 31 mg (53%) of 9b: mp 152–154, 183–185 °C; IR (KBr) 3281 (OH), 1717 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3/\text{CD}_3\text{OD}$ , 1:1)  $\delta$  0.56 (s, 3 H, 18- $\text{CH}_3$ ), 0.91 (s, 3 H, 19- $\text{CH}_3$ ), 4.06 (b s, 1 H, 3 $\beta$ -H), 4.20 (b m, 2 H, 21- $\text{CH}_2$ ), 5.29 (b t, 1 H, 11-H). Anal. ( $\text{C}_{21}\text{H}_{32}\text{O}_3$ ) C, H.

**21-Acetoxy-3 $\alpha$ -[(*tert*-butyldimethylsilyl)oxy]-5 $\alpha$ -pregnan-20-one (9c).** A solution of 1.55 g of 9a and 0.73 g of imidazole in 12 mL of dry dimethylformamide was treated with 0.81 g of *tert*-butyldimethylsilyl chloride overnight at 24 °C.<sup>31</sup> After extraction with ether the crude product was purified by dry column chromatography on silica gel using petroleum ether/ether (5:1) to give 1.05 g (51%) of 9c. An analytical sample was

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crystallized from petroleum ether: mp 110–112 °C; IR (KBr) 1748 (CO), 1730 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.62 (s, 3 H, 18- $\text{CH}_3$ ), 0.73 (s, 3 H, 19- $\text{CH}_3$ ), 0.88 [s, 6 H,  $\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$ ], 2.13 (s, 3 H,  $\text{COCH}_3$ ), 3.96 (b s, 1 H, 3 $\beta$ -H), 4.55 (dd, 2 H, 21- $\text{CH}_2$ ). Anal. ( $\text{C}_{29}\text{H}_{50}\text{O}_5\text{Si}$ ) C, H.

**3 $\alpha$ -[*(tert*-Butyldimethylsilyl)oxy]-21-hydroxy-5 $\alpha$ -pregnan-20-one (9d).** A suspension of 1.0 g of 9c in 75 mL of methanol was hydrolyzed with 1.9 mL of aqueous 10%  $\text{K}_2\text{CO}_3$  as described for 4a to give 0.88 g of crude 9d. The product was purified by dry column chromatography on silica gel with petroleum ether/ether (5:1) to give 0.71 g (78%) of 9d, which crystallized from ether: mp 106–108 °C; IR (KBr) 3490 (OH), 1711 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.60 (s, 3 H, 18- $\text{CH}_3$ ), 0.72 (s, 3 H, 19- $\text{CH}_3$ ), 0.87 [s, 6 H,  $\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$ ], 3.97 (b s, 1 H, 3 $\beta$ -H), 4.17 (d, 2 H, 21- $\text{CH}_2$ ). Anal. ( $\text{C}_{27}\text{H}_{48}\text{O}_5\text{Si}$ ) C, H.

**3 $\alpha$ -[*(tert*-Butyldimethylsilyl)oxy]-21-[(methylsulfonyl)oxy]-5 $\alpha$ -pregnan-20-one (9e).** A solution of 0.71 g of 9d in 3.5 mL of pyridine was treated with 0.35 mL of methanesulfonyl chloride as described for the preparation of 6 to give 0.84 g of crude 9e. The product was purified by dry column chromatography on silica gel using petroleum ether/ether (2:1) to yield 0.70 g (82%) of 9e as a white gum which failed to crystallize: NMR ( $\text{CDCl}_3$ )  $\delta$  0.64 (s, 3 H, 18- $\text{CH}_3$ ), 0.74 (s, 3 H, 19- $\text{CH}_3$ ), 0.88 [s, 6 H,  $\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$ ], 3.22 (s, 3 H,  $\text{SO}_2\text{CH}_3$ ), 3.98 (b s, 1 H, 3 $\beta$ -H), 4.80 (dd, 2 H, 21- $\text{CH}_2$ ).

**3 $\alpha$ -Hydroxy-21-[(methylsulfonyl)oxy]-5 $\alpha$ -pregnan-20-one (14).** A solution of 0.60 g of 9e in 15 mL of a mixture of tetrahydrofuran/acetic acid/water (1:3:1) under argon was heated at 60 °C for 7 h. After evaporation of the solvent in vacuo, the crude product was purified by dry column chromatography on silica gel using 5% acetone in  $\text{CH}_2\text{Cl}_2$  and crystallized from acetone/heptane to give 0.39 g (82%) of 14: mp 171–172 °C; IR (KBr) 3558 (OH), 1723 (CO)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.67 (s, 3 H, 18- $\text{CH}_3$ ), 0.78 (s, 3 H, 19- $\text{CH}_3$ ), 3.23 (s, 3 H,  $\text{SO}_2\text{CH}_3$ ), 4.08 (b s, 1 H, 3 $\beta$ -H), 4.80 (dd, 2 H, 21- $\text{CH}_2$ ). Anal. ( $\text{C}_{22}\text{H}_{36}\text{O}_5\text{S}$ ) C, H, S.

**3 $\alpha$ -Hydroxy-5 $\alpha$ -androstane-17 $\beta$ -carbonitrile (16).** An approximate 2:1 mixture of the 17-epimers 16 and 17, respectively, was prepared from 3 g of 3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one (15) with tosylmethyl isocyanide.<sup>32</sup> The epimeric mixture was separated by dry-column chromatography on silica gel using hexane/ethyl acetate (2:1), to afford 0.73 g (24%) of 16 as the more polar epimer after crystallization from ethyl acetate: mp 156–158 °C; IR (KBr) 3550 (OH), 2237 (CN)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.79 (s, 3 H, 18- $\text{CH}_3$ ), 0.90 (s, 3 H, 19- $\text{CH}_3$ ), 4.05 (m, 1 H, 3 $\beta$ -H). Anal. ( $\text{C}_{20}\text{H}_{31}\text{NO}$ ) C, H, N.

**3 $\alpha$ -Hydroxy-5 $\alpha$ -androstane-17 $\alpha$ -carbonitrile (17).** The less polar epimer separated chromatographically in the preparation of 16 above was crystallized from ethyl acetate/hexane to give an analytical sample: mp 183–185 °C; IR (KBr) 3550 (OH), 2237 (CN)  $\text{cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  0.78 (s, 3 H, 18- $\text{CH}_3$ ), 0.80 (s, 3 H, 19- $\text{CH}_3$ ), 4.05 (m, 1 H, 3 $\beta$ -H). Anal. ( $\text{C}_{20}\text{H}_{31}\text{NO}$ ) C, H, N.

**Steroid Modeling.** Interactive molecular modeling was performed with the Mosaic molecular modeling program running on a VAX 8800 and an Evans and Sutherland PS300 or IBM PC-AT display terminal. Mosaic is derived from the MacroModel program.<sup>33</sup> Energy calculations used the MM2 force field of MacroModel 2.1 in either Mosaic (interactive) or BatchMin (batch) with a distant dependent dielectric ( $\epsilon = R$ ) and default nonbonded cutoffs. The Mosaic/MacroModel/BatchMin implementation of the MM2 force field differs from authentic MM2<sup>34</sup> primarily in the treatment of electrostatics; the Mosaic/MacroModel implementation uses a point-charge model whereas the MM2 program uses a dipole–dipole model. Energy minimizations were generally performed with the block diagonal Newton Raphson minimizer; the full matrix Newton Raphson minimizer was used to minimize to saddle point structures. The crystal structure of alphaxalone<sup>35</sup>

was used as a starting point for building models of the other steroid structures. Conformational searching was performed with MULTIC submode,<sup>36</sup> using 1.0–2.0 Å ring closures when searching rings, and 60° torsional resolution unless otherwise stated.

**Steroid Potentiation of Chloride Flux.** Synaptoneurosomes were prepared from fresh cerebral cortices of adult male Sprague–Dawley rats (180–200 g) as previously described.<sup>2a</sup> Briefly, cerebral cortices were homogenized in ice-cold (4 °C) Tris–Hepes buffer (pH 7.4) containing 118 mM NaCl, 4.7 mM KCl, 1.18 mM  $\text{MgSO}_4$ , 2.5 mM  $\text{CaCl}_2$ , and 10 mM D-glucose. The homogenate was filtered through three layers of nylon mesh (160  $\mu\text{m}$ ) and then through a 10- $\mu\text{m}$  Millipore filter. The filtrate was washed twice by centrifugation at 1000g for 15 min and the final pellet was resuspended in buffer at a concentration of 10 mg of protein per mL. In the present experiments the effect of the various steroids in potentiating muscimol-stimulated  $^{36}\text{Cl}^-$  uptake of synaptoneurosomes was measured as this has been previously shown to be a sensitive method to detect activity at the GABA<sub>A</sub> receptor coupled  $\text{Cl}^-$  ion channel.<sup>2a,e</sup> A muscimol concentration of 3  $\mu\text{M}$  to approximate the  $\text{ED}_{50}$  was used in all experiments. Chloride-36 uptake was measured in quadruplicate by the simultaneous addition of 0.5  $\mu\text{Ci}$   $^{36}\text{Cl}^-$  (Du Pont New England Nuclear) to the synaptoneurosomes (1 mg of protein per tube) which had been preincubated at 30 °C for 10 min. All drugs or steroids were added for the preincubation time of 10 min. Uptake was terminated after 5 s by dilution in 5 mL of ice-cold buffer containing 100  $\mu\text{M}$  picrotoxin, followed by rapid filtration over glass-fiber filters (Schleicher and Schuell No. 30) under vacuum. The filters were washed twice with 5 mL of buffer and the radioactivity was determined by liquid-scintillation spectroscopy in 5 mL of RediSolve (Beckman Instruments). Net uptake was calculated by subtracting  $^{36}\text{Cl}^-$  uptake observed in the absence of steroid (basal level) from that observed in the presence of steroid. Potentiation represents the increase in  $^{36}\text{Cl}^-$  uptake by steroids compared to the muscimol-stimulated uptake alone. Statistical comparisons are made with Student's *t* test. Protein determinations were performed by the method of Lowry et al.<sup>37</sup>

**Steroid Metabolism.** Groups of four adult male Sprague–Dawley rats (240–260 g) were administered 100  $\mu\text{Ci}$  of  $^3\text{H}$ -labeled allopregnanolone or allotetrahydroDOC in (70  $\mu\text{L}$ ) ethanol–(130  $\mu\text{L}$ ) saline solution by tail vein injection. After 20 min the animals were killed by decapitation, and blood and brain tissue were collected and immediately cooled on ice. After aliquots were taken for determination of radioactivity, the tissue was frozen at –40 °C. Prior to extraction, the brain tissue (1.7–1.9 g) was homogenized in 10 mL/g of chloroform/methanol (2:1, v/v) at 0–4 °C with a Polytron (Brinkman Instruments) at setting 2 for five 5-s intervals. Internal standards of 100  $\mu\text{g}$  of deoxycorticosterone were added for calculation of recoveries and the samples were centrifuged at 3000g for 15 min. The pellet was washed with an additional 10 mL/g ethanol at 0 °C and centrifuged, and the combined supernatant fractions were evaporated in vacuo. The residues were partitioned between hexane and 90% aqueous methanol, 100 mL of each phase, using a three transfer counter-current distribution with stripping. The 90% methanol phases containing over 95% of the radioactivity were combined and evaporated in vacuo. The residues were similarly partitioned between ethyl acetate and water, 100 mL of each phase. The combined ethyl acetate phases, containing over 95% of the radioactivity, were evaporated in vacuo. The resulting samples were purified by HPLC using the solvent system 75% aqueous methanol at 2 mL/min on two Whatman ODS-3 RAC columns in tandem. Fractions were collected every 0.4 min and 10% aliquots were used for the measurement of radioactivity. Internal standards of allopregnanolone (2a) or allotetrahydroDOC (4a), 3-ketone derivatives 20 or 22, and other metabolites were injected with the radioactive samples and their elution was monitored by ultraviolet absorption at 280 nm. The recovery standards of deoxycorticosterone were measured by absorption at 254 nm. For further identification, half of the fractions containing the purified radioactivated compounds were evaporated in vacuo and acety-

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lated with acetic anhydride (0.1 mL) in pyridine (0.1 mL) overnight at room temperature. After evaporation of the solvents under a stream of nitrogen, the acetylated residues were purified by HPLC as described above using the solvent system 85% aqueous methanol. Internal standards of the authentic acetates were located in the eluates by ultraviolet absorption at 280 nm.

**Acknowledgment.** This work was supported in part by a grant from the Upjohn Co. We are indebted to Perry H. Moore, Jr. for his excellent technical assistance in the synthetic work and in the metabolism study, to John R. Pace for some of the chloride-uptake determinations, and to Dr. Donald E. Ayer and Dr. Douglas C. Rohrer for assistance and helpful discussions. The formation of the  $^3\text{H}$ -labeled compounds was performed at Du Pont NEN

Products, Boston, MA. We are grateful to Dr. Mayo Cabell, Du Pont NEN for providing the  $^3\text{H}$  NMR and specific activity data of these products.

**Registry No.** 1, 516-55-2; 2a, 516-54-1; 2b, 6003-24-3; 3a, 96611-83-5; 3b, 96038-59-4; 4a, 567-02-2; 4b, 126451-95-4; 5, 565-96-8; 6, 126296-19-3; 7, 111464-63-2; 8, 38393-03-2; 9a, 126296-20-6; 9b, 102917-20-4; 11, 126296-21-7; 12, 126296-22-8; 13, 126296-23-9; 14, 126296-24-0; 15, 53-41-8; 16, 51872-53-8; 17, 126296-25-1; 18, 53-42-9; 19, 85611-15-0; 20, 566-65-4; 21, 6750-73-8; 22, 298-36-2; 23, 57-83-0; 24, 38398-44-6; 25, 600-52-2; 26, 6951-91-3; 27, 21788-58-9; 28, 5953-71-9; 29, 126451-96-5; 30, 18000-89-0; 31, 566-58-5; Cl<sup>-</sup>, 16887-00-6; 3 $\alpha$ -hydroxypregn-4-en-20-one, 25680-68-6; 3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one 21-acetate, 2402-24-6; 3 $\alpha$ ,21-dihydroxy-5 $\beta$ -pregnan-20-one, 567-03-3; 3 $\beta$ -hydroxypregn-4-en-20-one, 566-66-5.