Neurosteroid Analogues. 3. The Synthesis and Electrophysiological Evaluation of Benz[e]indene Congeners of Neuroactive Steroids Having the 5β-Configuration

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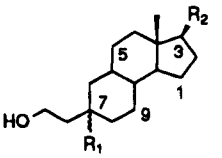
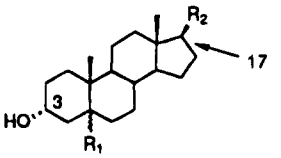
A series of 7-(2-hydroxyethyl)benz[e]indene analogues of 3α-hydroxy-5β-pregnan-20-one (**7**), a neuroactive steroid known to be a positive allosteric modulator of $GABA_A$ receptor function, was prepared. Electrophysiological measurements carried out on cultured rat hippocampal neurons were used to evaluate the modulatory effects of the analogues on $GABA_A$ receptor function. Analogues were tested for their ability to potentiate 1 μM GABA-mediated chloride currents and for their ability to directly gate chloride currents at this ligand-gated ion channel. Active analogues typically enhanced GABA-mediated currents at concentrations below those required to directly gate chloride currents. The dose–response relationships for potentiation of 1 μM GABA-mediated chloride currents were studied for [3*S*-(3α,3aα,5aβ,7β,9aα,9bβ)]-1-[dodecahydro-7-(2-hydroxyethyl)-3a-methyl-1*H*-benz[e]inden-3-yl]ethanone (**3**), steroid **7**, 3α-hydroxy-5α-pregnan-20-one (**5**), and the analogous 7α-(2-hydroxyethyl)benz[e]indene analogue of steroid **5** (compound **1**). Compound **3** was the most active potentiator (EC_{50} = 0.017 μM) of GABA-mediated current. The direct gating actions of compound **3** were not observed at a concentration of 1 μM, but were observed at a concentration of 10 μM.

Steroids of the 5α- or 5β-pregnane or androstane series containing a 3α-hydroxy group and a hydrogen bond acceptor group attached with the β-configuration at C-17 are known to have anesthetic activity.[1] The discovery that these steroids are allosteric modulators of $GABA_A$ receptor (γ-aminobutyric acid type A receptor) function has led to the hypothesis that the anesthetic activity is explained by interactions with $GABA_A$ receptors.[2] Other studies have shown that steroids which enhance GABAergic function also have anxiolytic,[3] analgesic,[4] and anticonvulsant activity.[3f,5]

We previously reported that benz[e]indenes **1** and **2**, which are related structurally to the anesthetic 5α-steroids **5** and **6** (see next paragraph for a discussion of benz[e]indene and steroid nomenclature), are potent allosteric modulators of $GABA_A$ receptors found in rat hippocampal neurons.[6] We now report the synthesis and electrophysiological evaluation of the benz[e]indenes **3** and **4**, which are congeners of anesthetic steroids having the 5β-configuration (**7** and **8**). Comparative studies demonstrating that benz[e]indenes **3** and **4** are more potent than benz[e]indenes **1** and **2** are also reported.

The nomenclature used for the assignment of the α and β stereodescriptors for benz[e]indenes and steroids is different. For the benz[e]indenes, the α-side of the reference plane is that side on which the preferred substituent lies at the lowest-numbered stereogenic position.[7] Accordingly, the C-3 substituent defines the α-side of the plane and groups on the same side of the plane as the C-3 substituent are assigned α descriptors. A different rule governs the nomenclature of steroids wherein substituents below the plane are assigned α descriptors and substituents above the plane are assigned β descriptors.





1: R_1 = β-H; R_2 = α-CH_3CO
2: R_1 = β-H; R_2 = α-CN
3: R_1 = α-H; R_2 = α-CH_3CO
4: R_1 = α-H; R_2 = α-CN
5: R_1 = α-H; R_2 = β-CH_3CO
6: R_1 = α-H; R_2 = β-CN
7: R_1 = β-H; R_2 = β-CH_3CO
8: R_1 = β-H; R_2 = β-CN

Chemistry

The starting material (Scheme 1) for the synthesis of the required benz[e]indenes **3** and **4** is the known 7*H*-benz[e]inden-7-one **9**,[8] which was prepared as described previously.[9] Reduction of the double bond using Li in liquid NH_3 gave saturated ketone **10** in 44% yield. Based on literature precedents for the reduction of the double bond of similar 7*H*-benz[e]inden-7-ones by this method,[8a,10] H-5a was predicted to have the β-configuration. This prediction was verified at a later stage of the synthesis by conversion of a portion of a synthetic intermediate (**13a**, *vide infra*) derived from ketone **10** into a known compound. Carbethoxyolefination[9,11] of ketone **10** using (carbethoxymethylene)triphenylphosphorane (without solvent at 160 °C, ca. 17 h) provided an ~1:1 isomeric mixture of (***E***)-**11** and (***Z***)-**11** in high yield (98%). For characterization purposes, a portion of this isomeric mixture was separated by HPLC on a silica gel column to obtain pure (***E***)-**11** and (***Z***)-**11**.[12] Catalytic hydrogenation (Pd–$CaCO_3$; ~50 psi H_2, room temperature) of the remaining (***E,Z***)-**11** mixture in *N*-methylpyrrolidine[13] produced in 98% yield an inseparable mixture of the C-7 diastereomeric esters **12a** and **12b**. Separation of the corresponding diastereomeric

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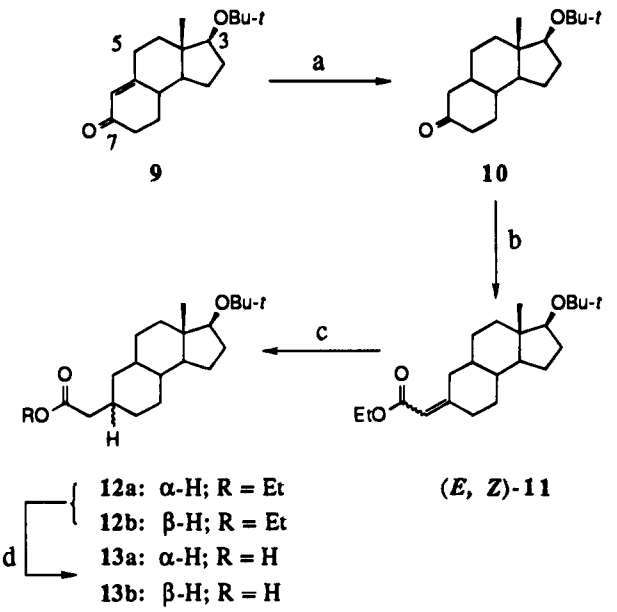
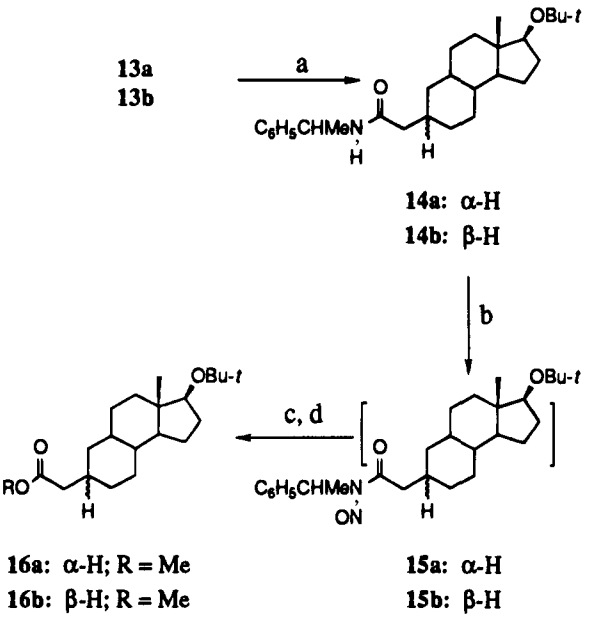
Scheme 1[a]



[a] (a) Li in liquid NH_3, THF, toluene; (b) Ph_3P=CHCOOEt, 160 °C; (c) Pd–$CaCO_3$, H_2; (d) NaOH, aqueous EtOH.

Scheme 2[a]

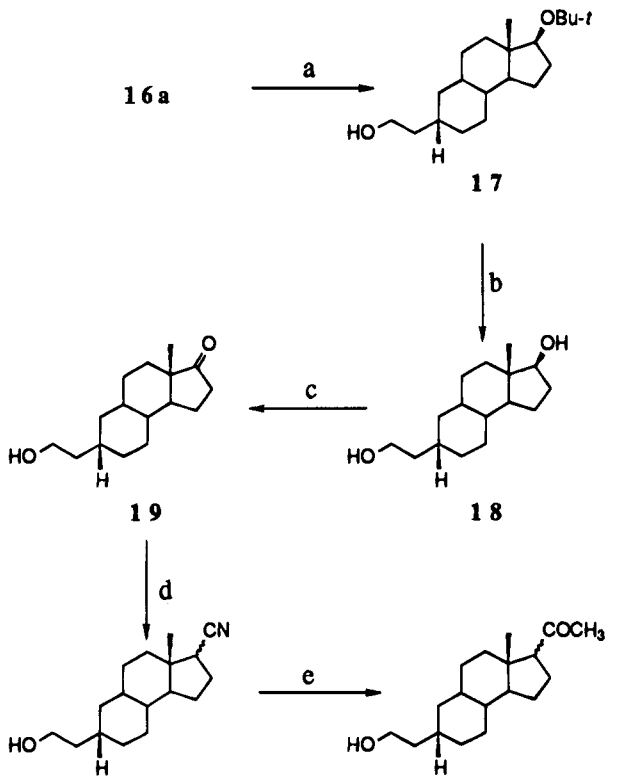


[a] (a) (*S*)-α-methylbenzenemethamine, 1,1′-carbonylbis(1*H*-imidazole); (b) $NaNO_2$, HOAc, AcOAc, 0 °C; (c) NaOH, aqueous EtOH; (d) CH_2N_2, EtOAc, EtOH.

acids **13a** and **13b**, prepared in 98% yield by hydrolysis (20% aqueous NaOH in EtOH) of the carbethoxy group of esters **12a** and **12b**, also was unsuccessful using silica gel chromatography (column or HPLC).

Reaction of a mixture of acids **13a** and **13b** with (*S*)-α-methylbenzenemethamine in DMF containing 1,1′-carbonylbis(1*H*-imidazole)[14] gave in 89% yield the diastereomeric amides **14a** and **14b** (Scheme 2). The asymmetric interactions of the chiral center in the N-substituted side chain with the *R,S* chiral center at C-7 in the benz[*e*]indene portion of compounds **14a** and **14b** produced large differences in the chromatographic properties of these diastereomeric amides. Hence, these diastereomers (~1:1 ratio) were separated readily by preparative scale HPLC into pure **14a** and **14b**. Hydrolysis of these amides to acids **13a** and **13b** was more difficult than expected. Amides **14a** and **14b** were resistant to hydrolysis under relux conditions by 3 N KOH in water or methanol, 6 N HCl in MeOH (1:1), or 4.6 N H_2SO_4. Acids **13a** or **13b** were obtained using a reaction sequence reported by White in his studies on the chemistry of *N*-alkyl-*N*-nitrosoamides.[15] Treatment of either **14a** or **14b** with $NaNO_2$ in HOAc/AcOAc at 0 °C overnight gave the corresponding intermediate *N*-nitroso compounds **15a** or **15b** which were initially refluxed overnight in dioxane and subsequently refluxed in 20% aqueous NaOH in EtOH (1:1) to give acids **13a** or **13b** in yields of 90% and 80%, respectively. The corresponding esters **16a** or **16b** were obtained in 96% and 93% yield, respectively, by reacting acids **13a** or **13b** with diazomethane in EtOAc/EtOH.

Scheme 3



[a] (a) DIBALH; 0 °C; (b) 6N HCl, aqueous EtOH, reflux; (c) NaOCl, AcOH; (d) TosMIC, *t*-BuOK; (e) CH_3MgCl, THF.

Reduction of ester **16a** (Scheme 3) using DIBALH gave alcohol **17** (89% yield), which was subsequently converted into diol **18** by removal of the *t*-Bu group at C-3 with 6 N HCl in EtOH at reflux (98% yield).[16] Selective oxidation of the secondary hydroxy group of diol **18** with NaOCl in HOAc[6c,18] gave ketone **19** in 80% yield. Reaction of ketone **19** with *t*-BuOK in dimethoxyethane/EtOH followed by the addition of tosylmethyl isocyanide (TosMIC)[6c,19] gave a mixture of diastereomeric carbonitriles which was separated by column chromatography to give 3(*R*)-carbonitrile **20** (17%) and 3(*S*)-carbonitrile **4** (26%). A mixture of compounds **21** and **3** was obtained in 94% yield by reacting a mixture of carbonitriles **20** and **4** with CH_3MgCl in THF. The products **21** and **3** were separated by HPLC and obtained in isolated yields of 18% and 69%, respectively, as described in the Experimental Section

Table 1. Electrophysiological Effects of Benz[e]indenes and Steroids on $GABA_A$ Receptor Function

compd	N[a]	compd (1 μM) potentiation % response relative to current produced by GABA[b]	compd (10 μM) gated current[c]
1[d]	12	489 ± 19[e]	NR[f]
2[d]	6	346 ± 2	NR
3	6	312 ± 34	113 ± 21
4	5	497 ± 14	115 ± 11
5	5	523 ± 87[g]	30 ± 6[d]
6[h]	10	443 ± 55	49 ± 4
7	12	521 ± 43	144 ± 16
8	5	514 ± 27	166 ± 16
17	5	114 ± 7	NR
18	4	93 ± 3	NR
19	6	197 ± 21	NR
20	5	104 ± 6	NR
21	5	213 ± 5	43 ± 5

[a] N = Number of cells examined. [b] To calculate the percentage response, the magnitude of the peak current produced by 1 μM GABA plus 1 μM compound was normalized with respect to the peak current produced by 1 μM GABA alone on the same cell. A percentage response of 100% reflects no change in the current compared to 1 μM GABA alone. 1 μM GABA is a concentration at the foot of the dose–response curve in cultured postnatal rat hippocampal neurons. These experiments were conducted at −60 mV and compounds were applied by pressure ejection for 500 ms. [c] The compound gated current reflects the peak current directly gated by 10 μM compound in the absence of GABA compared to the response obtained from the same cell in response to 1 μM GABA alone. [d] Values reported for this compound are from ref 6b. [e] Values are the mean ± SEM. [f] NR denotes no response. [g] Value reported is from ref 9. [h] Values reported for this compound are from ref 6c.

The stereochemical assignments for the nitrile groups of compounds **4** and **20** and the acetyl groups of compounds **3** and **21** were based on the ^{1}H NMR spectra of the compounds. Because of the dihedral angles involved, the coupling constants of the C-3 proton of the benz[e]indenes having the 3*S* configuration are expected to be approximately the same for vicinal coupling to both of the adjacent protons on C-2. By contrast, the coupling constants of the C-3 proton of the benz[e]indenes having the 3*R* configuration are expected to be unequal for vicinal coupling to the adjacent protons on C-2. Hence, compounds **20** and **21**, the ^{1}H NMR spectra of which showed a resonance appearing as a doublet of doublets at δ 2.57 and 2.80, respectively, were assigned the 3*R* configuration. Compounds **3** and **4**, the ^{1}H NMR spectra of which showed a resonance appearing as an apparent triplet at δ 2.55 and 2.28, respectively, were assigned the 3*S* configuration.

Electrophysiology

Voltage clamp recordings were obtained from cultured postnatal rat hippocampal neurons using whole-cell patch clamp methods.[20] Each compound was evaluated at 1 μM for its ability to potentiate 1 μM GABA-mediated currents and also evaluated at 10 μM for its ability to initiate (gate) a current in the absence of GABA (Table 1). We have shown previously that currents directly gated in hippocampal neurons by benz[e]indene **1** and steroid **5** result from actions on $GABA_A$ receptor chloride channels.[6a] The concentrations selected for the experiments reported herein were chosen so that the results from this study could be compared to our earlier reported results.[6,9] Recordings that are representative of the responses observed for benz[e]indenes and 5β-steroids are shown for compounds **3** and **7** in Figure 1. Dose–response curves for the potentiation of 1 μM GABA-mediated currents by compounds **1**, **3**, **5**, and **7** that have been normalized to the response observed for 10 μM of the compound in the potentiation assay are shown in Figures 2 and 3, and a comparison of the results obtained at 10 μM for each compound which has been normalized to the response obtained with 10 μM steroid **5** is presented in Figure 4.

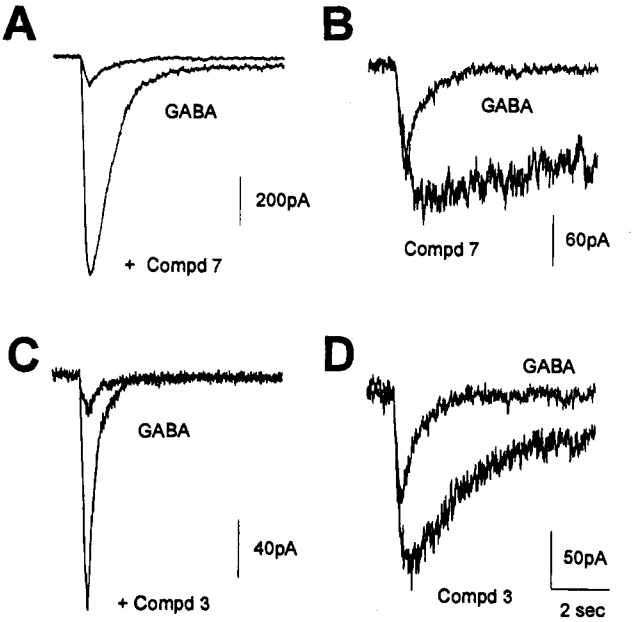


Figure 1. The traces show responses of neurons voltage clamped at −60 mV to 500 ms applications of 1 μM GABA alone and in the presence of 1 μM **7** (A) and 1 μM **3** (C). In panels B and D, currents gated by 1 μM GABA are compared with those gated by 10 μM **7** (B) or 10 μM **3** (D) in the same neurons.

Discussion

Based on previous structure–activity studies of neuroactive steroids[1,19c] and benz[e]indene analogues of 5α-steroids,[6] it was anticipated that the activities determined in the assays reported in Table 1 for the new benz[e]indene analogues would be highly dependent on the type of C-3 functional group, the stereochemistry of this group, and the distance between this group and the hydroxyl group in the side chain at C-7. It was expected that compounds **3** and **4** would have high activity because the hydrogen-bond-accepting group at C-3 is above the plane of the rings and located at an optimal distance from the hydrogen-bond-donating group located in the side chain at C-7. Compound **19** was expected to have weak activity because the distance and relative position of the hydrogen bonding groups is less than optimal. Compounds **17** and **18** were expected to have little or no activity because, in addition to the less than optimal distance between the hydrogen bonding groups found in these analogues, either the hydrogen bond acceptor group at C-3 is sterically inaccessible (compound **17**) or replaced with a group having increased hydrophilicity that can also function as a hydrogen bond donor (compound **18**). Compound **20** was expected to be inactive because the hydrogen bond acceptor group at C-3 is located below the plane of the rings.

Only compound **21**, which contains a 3(*R*)-acetyl group and was unexpectedly as active as benz[e]indene **19** in the potentiation assay and as active as steroids **5**

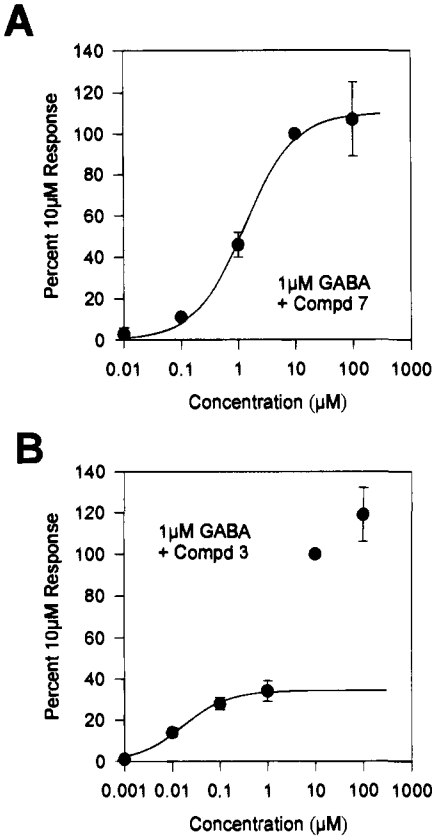


Figure 2. The graphs show dose–response curves for the potentiation of 1 μM GABA currents by **7** (A) and **3** (B). The data are normalized with respect to the response obtained with 1 μM GABA plus 10 μM compound. The solid lines represent the fit of the data to a dose–response equation: response_{max} $([\text{compd}]^n/[\text{compd}]^n + EC_{50}^n$, where response_{max} is the maximal response, [compd] is the concentration of the compound, EC_{50} is the half-maximal concentration, and n is the Hill coffficient. For **7** (A), the concentrations that potentiate GABA currents overlap with those that directly gate chloride currents in the absence of GABA producing a monophasic dose–response relationship where response_{max} = 110%, EC_{50} = 1.3 μM, and n = 1.0. For **3** (B), there is a separation in the concentrations that potentiate GABA currents compared to those that directly gate chloride currents resulting in a biphasic dose–response relationship. For the solid line in B, response_{max} = 34%, EC_{50} = 0.017 μM, and n = 0.9. This EC_{50} gives an estimate of the half-maximal concentration for pure potentiation of GABA currents by **3**.

and **6** in the gating assay, had activities different than those anticipated. Molecular mechanics calculations indicate that the minimum energy conformation of the acetyl side chain in compound **21** has the methyl group below the five-membered ring and the carbonyl group pointing away from the ring in the same direction as the carbonyl group found at C-3 in compound **19**. Moreover, in this conformation the distance (9.48 Å) between the hydroxyl oxygen and the carbonyl oxygen in compound **21** is essentially identical to the distance (9.45 Å) between the hydroxyl oxygen and carbonyl oxygen at C-3 in compound **19**. Indeed, a superimposition of these groups in three dimensional space is readily achieved for these molecules and this may explain the similar activities of both compounds in the potentiation assay. Since in this alignment the rings of compound **21** are located above the rings of compound **19** and also displaced toward the C-7 position of compound **19**, it may be that the different regions of space occupied by the rings in compound **21** explain the gating actions of

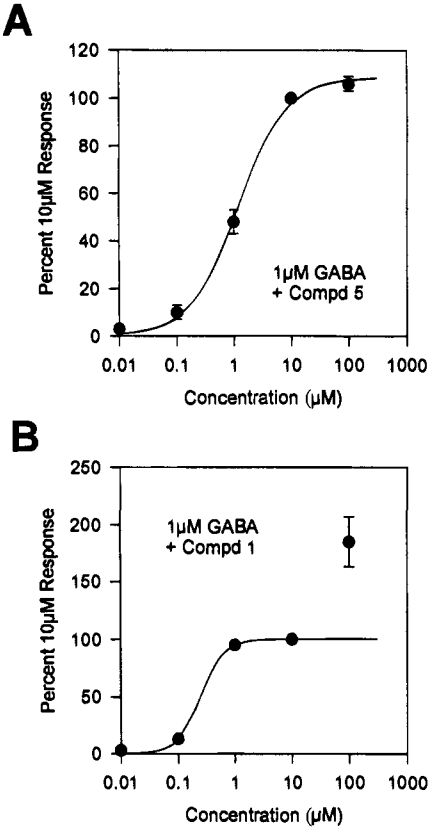


Figure 3. The graphs show dose–response relationships for **5** and 1 for potentiation of 1 μM GABA currents. Data are displayed in a fashion similar to Figure 2. For **5**: response_{max} = 109%, EC_{50} = 1.2 μM, and n = 1.0. For 1: response_{max} = 100%, EC_{50} = 0.25 μM, and n = 2.1. Compound **5** showed significant overlap in concentrations that potentiated GABA currents and those that directly gated chloride currents, resulting in a monophasic dose–response curve. In contrast, only concentrations of 1 >10 μM gated significant chloride currents in the absence of GABA.

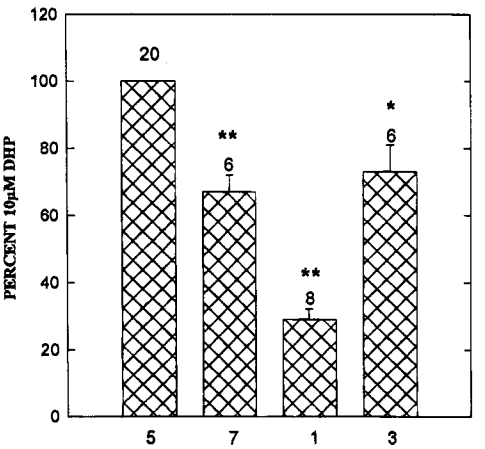


Figure 4. The bar graph shows a direct comparison of the effects of 10 μM **5**, **7**, 1, and **3** on 1 μM GABA currents. Responses are normalized with respect to the response to **5**. The numbers at the top of the bars show the number of cells studied. $^*p < 0.05$, $^{**}p < 0.01$ by two-tailed paired t-test.

compound **21**. Clearly, a rigorous evaluation of these structure–activity rationalizations requires future studies with additional benz[*e*]indenes and steroids containing a 17α-acetyl group.

Comparison of the results reported in Table 1 for compounds **1** and **3** shows that under the conditions chosen for the potentiation and gating assays, compound

1 potentiated GABA-mediated current to a greater extent than compound 3. However, compound 3 directly gated a current, whereas compound 1 did not. The analogous steroids 5 and 7 were comparable to benz[e]indene 1 as potentiators of current. Steroid 5 was less effective at directly gating a current than benz[e]indene 3, and steroid 7 was slightly more effective than this benz[e]indene. Similar comparisons of the results obtained for compounds 2, 4, 6, and 8 yield similar conclusions.

A more complete evaluation of the magnitude of the differences in activity observed for compounds 1, 3, 5, and 7 was made possible by obtaining dose–response relationships for the potentiation of 1 μM GABA-mediated currents by these compounds (Figures 2 and 3). The results shown indicate that the neuroactive steroids and their benz[e]indene counterparts have high potency and efficacy as modulators of GABA-mediated chloride currents in hippocampal neurons. Previous results[6a] as well as the results presented in Table 1 and Figures 2 and 3 indicate that both sets of compounds can, at certain concentrations, directly gate chloride currents via $GABA_A$ channels. In the case of steroids 5 and 7, the concentrations that potentiate GABA-mediated currents overlap with those which directly gate chloride currents. This results in the monophasic dose–response curves for the potentiation of 1 μM GABA-mediated currents shown in Figures 2 and 3. By contrast, the dose–response curves for GABA potentiation and direct channel gating are separated for benz[e]indenes 1 and 3. This difference is likely to contribute to the peculiar biphasic dose–response curves shown for these compounds in Figures 2 and 3. Whether these differences in effects on GABA currents influence the actions of the steroids and benz[e]indenes as anesthetic, anticonvulsant, or anxiolytic agents remains to be determined.

The dose–response studies indicate that benz[e]indenes 1 and 3 are more potent than steroids 5 and 7 at potentiating GABA-mediated currents. On the basis of the results shown in Figure 4, which compare steroid 5 with compounds 7, 1, and 3, it appears that steroid 5 is the most effective agent at a concentration of 10 μM. It is not possible to make an absolute statement regarding the relative effectiveness of these compounds because the solubility of the benz[e]indenes limits the ability to study these agents at concentrations that are likely to gate larger chloride currents. However, it is worth noting that even 10 μM is known to be an unphysiologically high concentration of endogeneous neuroactive steroids *in vivo*.[21]

$GABA_A$ receptors exist as hetero-oligomers of different subunits.[22] Since rat hippocampal neurons are known to contain multiple forms of $GABA_A$ receptors,[23] the responses to the compounds evaluated in this study represent the average responses recorded from multiple forms of $GABA_A$ receptors. Additionally, there is evidence for the presence of multiple steroid binding sites on $GABA_A$ receptors.[24] This diversity of receptor subtypes and steroid binding sites limits interpretation of our results. Thus, although the finding that there are different dose–response relationships for the potentiating and gating effects of the benz[e]indenes suggests to us the hypothesis that these two activities occur at different steroid binding sites on the same receptor, conclusions regarding this hypothesis require additional studies in which the effects of receptor diversity have been minimized using the methods of molecular biology.

In summary, the results of this study along with those from earlier studies of benz[e]indenes 1 and 2,[6] and 5*H*-benz[e]inden-5-one analogues of 3α-hydroxy-5α-pregnane-11,20-dione (alfaxalone)[9] provide some information for comparing the GABAergic effects of benz[e]indene analogues having structural features similar to those found in the most thoroughly studied neuroactive steroids (5, 7, and alfaxalone). The electrophysiological evaluations of the benz[e]indene analogues reveal not only clear differences on GABAergic function between the individual series of analogues, but also clear differences between a particular benz[e]indene and the corresponding structurally related neuroactive steroid. The pharmacological significance of these differences remains to be established in future studies.

Experimental Section

General Methods. Melting points were determined on a Kofler micro hot stage and are uncorrected. NMR spectra were recorded at ambient temperature in $CDCl_3$ (unless noted otherwise) with a 5 mm probe on a Varian Gemini-300 operating at 300 MHz (^1H) or 75 MHz (^{13}C). For ^1H NMR and ^{13}C NMR spectra; the internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.00), respectively. IR spectra were recorded as films on a NaCl plate (unless noted otherwise) with a Perkin-Elmer 1710 FT-IR spectrophotometer. Molecular modeling was performed on a SiliconGraphics Iris Indigo Elan 4000 computer using the Sybyl Molecular Modeling Software, version 6.0, from Tripos Associates, Inc., St. Louis, MO. Preparative scale HPLC was performed on a Waters PrepLC/System 500A instrument using dual silica P/N 50041 cartridges connected in tandem. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Solvents were used either as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32–63 μm) purchased from Scientific Adsorbants, Atlanta, GA. 3α-Hydroxy-5β-pregnan-20-one was purchased from Sigma Chemical Co., St. Louis, MO. (*S*)-α-Methylbenzenemethamine was purchased from Aldrich Chemical Co., Milwaukee, WI. 3α-Hydroxy-5α-pregnan-20-one was purchased from Steraloids, Wilton, NH. The Econosil HPLC column was purchased from Alltech Associates, Inc., Deerfield, IL.

[3*S*-(3α,3aα,5aβ,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-7*H*-benz[*e*]inden-7-one (10). Liquid ammonia (ca. 800 mL) was condensed in a 2 L, three-necked round bottom flask at −78 °C. Toluene (160 mL) and THF (160 mL) were added with stirring, and Li wire (1.52 g, 219 mmol) was added. When all the Li was dissolved, a solution of [3*S*-(3α,3aα,9aα,9bβ)]-3-(1,1-dimethylethoxy)-1,2,3,-3a,4,5,8,9,9a,9b-decahydro-3a-methyl-7*H*-benz[*e*]inden-7-one (**9**)[9] (13.31 g, 48.1 mmol) in toluene (80 mL) and THF (80 mL) was added slowly. The deep blue reaction mixture was stirred for another 40 min and then 1,2-dibromoethane was added dropwise to discharge the blue color. A solution of HOAc (20 mL) in MeOH (80 mL) was added dropwise over 20 min and the NH_3 was allowed to evaporate. The mixture was diluted with water (800 mL) and EtOAc (400 mL), and the organic layer was separated. The water layer was extracted with EtOAc (400 mL). The combined organic layers were washed with brine (2 × 400 mL) and dried over Na_2SO_4. The solvent was removed to give an oil which was purified by chromatography (silica gel, 15% EtOAc in hexane) to give the product **10** (5.84 g, 44%) as colorless crystals: mp 98–99 °C (from absolute EtOH); IR 2967, 2869, 1711, 1454, 1387, 1361, 1195, 1072, 1026 cm^{-1}; ^1H NMR δ 3.41 (t, J = 8.2 Hz, 1H, C*H*OC-$(CH_3)_3$), 1.13 (s, 9H, $C(CH_3)_3$), 0.80 (s, 3H, CH_3); ^{13}C NMR δ 211.61 (C=O), 80.44 (C-3), 72.18 ($C(CH_3)_3$), 28.67 ($C(CH_3)_3$), 11.65 (CH_3), 49.27, 48.06, 44.46, 43.13, 41.39, 40.09, 36.59, 30.93, 30.66, 29.74, 23.51. Anal. ($C_{18}H_{30}O_2$) C, H.

[3S-(3α,3aα,5aβ,7Z,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-7-[(ethoxycarbonyl)methylene]-3a-methyl-1H-benz[e]indene [(Z)-11] and [3S-(3α,3aα,5aβ,7E,9aα,-9bβ)]-3-(1,1-Dimethylethoxy)dodecahydro-7-[(ethoxycarbonyl)methylene]-3a-methyl-1H-benz[e]indene [(E)-11]. Compound **10** (5.54 g, 19.9 mmol) and (carbethoxymethylene)triphenylphosphorane (13.87 g, 39.8 mmol) were heated to 160 °C overnight (ca. 17 h) under nitrogen with stirring. The resultant brown liquid was cooled to room temperature and EtOAc (200 mL) was added. The solution was washed with water (200 mL) and brine (200 mL) and dried over Na_2SO_4. The organic layer was evaporated under reduced pressure to yield a gum which was purified by chromatography (silica gel, 5% EtOAc in hexane) to give **(Z)**-11 and **(E)**-11 (6.8 g, 98%) as an oil. A portion of the product was separated by HPLC (Alltech Econosil silica column, 250-mm × 10-mm, 2.5% EtOAc in hexane, 3 mL/min) to give **(Z)**-11 (first fraction) and **(E)**-11 (second fraction).

Compound **(Z)**-11 was obtained as white crystals: mp 56–58 °C; IR 2974, 2928, 1717, 1647, 1447, 1379, 1362, 1199, 1155, 1043, 903, 865 cm^{-1}; ^1H NMR δ 5.60 (s, 1H, CH=), 4.14 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 3.38 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 1.27 (t, J = 7.1 Hz, 3H, CH_3CH_2), 1.12 (s, 9H, $C(CH_3)_3$), 0.77 (s, 3H, CH_3); ^{13}C NMR δ 166.84 (C=O), 162.94 (C=), 112.89 (C=), 80.62 (C-3), 72.14 ($C(CH_3)_3$), 59.43 (OCH_2), 28.70 ($C(CH_3)_3$), 11.72 (CH_3), 49.63, 45.22, 43.16, 41.20, 37.54, 36.94, 35.84, 32.19, 31.07, 29.66, 23.34, 14.28. Anal. ($C_{22}H_{36}O_3$) C, H.

Compound **(E)**-11 was obtained as an oil: IR 2974, 2922, 1718, 1650, 1446, 1380, 1361, 1199, 1173, 1045, 894, 851 cm^{-1}; ^1H NMR δ 5.59 (s, 1H, CH=), 4.14 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 3.37 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 1.27 (t, J = 7.1 Hz, 3H, CH_3CH_2), 1.13 (s, 9H, $C(CH_3)_3$), 0.77 (s, 3H, CH_3); ^{13}C NMR δ 166.89 (C=O), 162.91 (C=), 113.10 (C=), 80.62 (C-3), 72.14 ($C(CH_3)_3$), 59.44 (OCH_2), 28.70 ($C(CH_3)_3$), 11.72 (CH_3), 49.52, 45.97, 44.25, 43.12, 41.22, 36.88, 31.49, 31.06, 29.48, 29.21, 23.40, 14.29. Anal. ($C_{22}H_{36}O_3$) C, H.

[3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-1H-benz[e]indene-7-acetic Acid Ethyl Ester (12a) Containing [3S-(3α,3aα,5aβ,7α,9aα,-9bβ)]-3-(1,1-Dimethylethoxy)dodecahydro-3a-methyl-1H-benz[e]indene-7-acetic Acid Ethyl Ester (12b). An unseparated mixture of **(Z)**-11 and **(E)**-11 (5.96 g) dissolved in *N*-methylpyrrolidine (150 mL) and Pd–$CaCO_3$ (5.96 g, Pd content 5%) was hydrogenated (room temperature, overnight, ca. 40–50 psi) in a Parr hydrogenation apparatus. The catalyst was filtered off, the solvent was moved under reduced pressure, and the product was purified by chromatography (silica gel, 2.5% EtOAc in hexane) to give an inseparable mixture of products **12a** and **12b** (5.86 g, 98%) as an oil: IR 2906, 1733, 1447, 1361, 1276, 1199, 1074, 1031, 906 cm^{-1}; ^1H NMR; δ 4.12 (q, J = 7.1 Hz, 2H, OCH_2), 3.38 (t, 1H, J = 8.2 Hz $CHOC(CH_3)_3$), 1.25 (t, J = 7.2 Hz, 3H, CH_3CH_2), 1.12 (s, 9H, $C(CH_3)_3$), 0.73 (s, 3H, CH_3); ^{13}C NMR δ 173.60 (C=O), 173.17 (C=O), 80.82 (C-3), 72.07 ($C(CH_3)_3$), 60.04 (OCH_2CH_3), 49.83 (CH_2COOEt), 28.71 ($C(CH_3)_3$), 11.70 (CH_3), 43.81, 43.17, 42.19, 41.75, 40.97, 39.55, 38.24, 37.36, 37.17, 37.11, 36.89, 35.05, 32.78, 31.12, 30.59, 30.28, 29.81, 29.58, 29.42, 28.71, 25.20, 23.37, 23.23, 14.25. Anal. ($C_{22}H_{38}O_3$) C, H.

[3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-N-[(S)-1-phenylethyl]-1H-benz[e]indene-7-acetamide (14a) and [3S-(3α,3aα,5aβ,7α,9aα,-9bβ)]-3-(1,1-Dimethylethoxy)dodecahydro-3a-methyl-N-[(S)-1-phenylethyl]-1H-benz[e]indene-7-acetamide (14b). Unseparated compounds **12a** and **12b** (7.30 g, 20.8 mmol) were refluxed in 10% aqueous NaOH (20 mL) and EtOH (30 mL) for 1 h. The solution was cooled with ice water, acidified with 3 M HCl to pH = 2–3, and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (2 × 200 mL) and water (100 mL) and dried with Na_2SO_4. The solvent was evaporated under reduced pressure to give an inseparable mixture of acids **13a** and **13b** as an oil (6.6 g, 98%) which was not characterized further.

To unseparated acids **13a** and **13b** (3.89 g, 12.1 mmol) dissolved in dry benzene (100 mL) and dry DMF (8 mL) was added 1,1′-carbonylbis(1*H*-imidazole) (3.94 g, 24.3 mmol) under N_2. The reaction was stirred at room temperature for 1 h, then HOAc (732 mg, 12.2 mmol) was added. After 10 min, (S)-α-methylbenzenemethamine (4.44 g, 36.6 mmol) was added. After an additional 2 h, the solution was evaporated to ~10 mL and chromatographed (silica gel, 25% EtOAc in hexane) to give products **14a** and **14b** as an oil (4.55 g, 89%). A portion of this oil (3.0 g) was separated by preparative scale HPLC (silica gel, 35.6% EtOAc in hexane, 250 mL/min) to give products **14a** (1.36 g, first fraction) and **14b** (1.41 g, second fraction).

Compound 14a was obtained as colorless crystals: mp 175–177 °C (from EtOAc/hexane); IR 3277, 3061, 2973, 2852, 1637, 1542, 1361, 1199, 1134, 1082, 908, 735 cm^{-1}; ^1H NMR δ 7.39–7.27 (m, 5H, C_6H_5), 5.72 (d, J = 9.0 Hz, 1H, NH), 5.17–5.13 (m, 1H, $NCHCH_3$), 3.36 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 2.24 (d, J = 7.6 Hz, 2H, $NCOCH_2$), 1.48 (d, J = 6.8 Hz, 3H, $NCHCH_3$), 1.12 (s, 9H, $C(CH_3)_3$), 0.73 (s, 3H, CH_3); ^{13}C NMR δ 171.87 (C=O), 143.20 (ArC), 128.61 (ArC), 127.29 (ArC), 126.14 (ArC), 80.81 (C-3), 72.10 ($C(CH_3)_3$), 28.71 ($C(CH_3)_3$), 11.74 (CH_3 on C-3a), 49.93, 48.47, 43.19, 41.78, 39.67, 38.48, 37.21, 36.75, 31.11, 29.71, 29.61, 25.35, 23.24, 21.67. Anal. ($C_{28}H_{43}NO_2$) C, H, N.

Compound **14b** was obtained as colorless crystals: mp 145–146 °C (EtOAc/hexane); IR 3277, 3063, 2914, 1638, 1542, 1361, 1199, 1141, 1074, 909, 732, 700 cm^{-1}; ^1H NMR δ 7.33–7.25 (m, 5H, C_6H_5), 5.86 (d, J = 7.3 Hz, 1H, NH), 5.16–5.11 (m, 1H, $NCHCH_3$), 3.37 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 2.05–2.02 (m, 2H, $NCOCH_2$), 1.48 (d, J = 7.0 Hz, 3H, $NCHCH_3$), 1.12 (s, 9H, $C(CH_3)_3$), 0.72 (s, 3H, CH_3); ^{13}C NMR δ 171.57 (C=O), 143.21 (ArC), 128.57 (ArC), 127.28 (ArC), 126.17 (ArC), 80.81 (C-3), 72.08 ($C(CH_3)_3$), 28.72 ($C(CH_3)_3$), 11.71 (CH_3 on C-3a), 49.79, 48.60, 44.85, 43.74, 43.16, 41.05, 39.64, 37.10, 35.57, 32.90, 31.12, 30.26, 29.41, 23.37, 21.70. Anal. ($C_{28}H_{43}NO_2$) C, H, N.

[3S-(3α,3aα,5aβ,7α,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-1H-benz[e]indene-7-acetic Acid (13b). To a stirred solution of compound **14b** (0.63 g, 1.48 mmol) in HOAc (5 mL) and acetic anhydride (25 mL) at 0 °C was added (1 h addition time) $NaNO_2$ (2.61 g, 37.84 mmol). The reaction was kept at 0 °C overnight. The mixture was then extracted with EtOEt (2 × 80 mL), and the combined extracts were washed with water (100 mL), saturated $NaHCO_3$ (100 mL), brine (100 mL), and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a residue. Dioxane (100 mL) was added and the reaction was refluxed overnight. The dioxane was removed under reduced pressure and 20% aqueous NaOH (30 mL) and EtOH (25 mL) were added. The reaction was refluxed for 1 h, cooled with ice–water, acidified with 3 M HCl to pH = 1–2, and extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with water (150 mL) and brine (150 mL) and dried with Na_2SO_4. Solvent removal gave an oil which was purified by chromatography (silica gel, 50% EtOAc and 1% HOAc in hexane) to give the product **13b** (0.42 g, 80%) as white crystals: mp 139–140 °C; IR 2916, 1704, 1447, 1412, 1382, 1359, 1300, 1201, 1076, 1029, 951 cm^{-1}; ^1H NMR δ 3.38 (t, J = 8.3 Hz, 1H, $CHOC(CH_3)_3$), 2.22 (d, J = 7.0 Hz, 2H, CH_2COOH), 1.13 (s, 9H, $C(CH_3)_3$), 0.73 (s, 3H, CH_3); ^{13}C NMR δ 179.43 (C=O), 80.87 (C-3), 72.16 ($C(CH_3)_3$), 28.74 ($C(CH_3)_3$), 11.74 (CH_3), 49.85, 43.83, 43.21, 41.91, 40.96, 39.50, 37.15, 34.84, 32.77, 31.13, 30.28, 29.43, 23.39. Anal. ($C_{20}H_{34}O_3$) C, H.

[3S-(3α,3aα,5aβ,7α,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-1H-benz[e]indene-7-acetic Acid Methyl Ester (16b). Diazomethane in EtOH and EtOEt at 0 °C was added to a stirred solution of acid **13b** (150 mg, 0.47 mmol) in EtOAc (5 mL) and EtOH (10 mL) until a yellow color persisted. The solution was allowed to stir for an additional 5 min, excess diazomethane was destroyed by the addition of several drops of formic acid, the solution was evaporated to near dryness under reduced pressure, and purified by chromatography (silica gel, 5% EtOAc in hexane) to give product **16b** (150 mg, 96%) as white crystals: mp 47–48 °C; IR 2915, 1741, 1445, 1390, 1361, 1277, 1200, 1156, 1074, 1029, 895 cm^{-1}; ^1H NMR δ 3.66 (s, 3H, CH_3O), 3.37 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 2.19 (d, J = 6.9 Hz, 2H, CH_2COOMe), 1.12 (s,

9H, $C(CH_3)_3$), 0.73 (s, 3H, CH_3); ^{13}C NMR δ 173.45 (C=O), 80.79 (C-3), 71.99 ($C(CH_3)_3$), 28.69 ($C(CH_3)_3$), 11.68 (CH_3), 51.24, 49.82, 43.81, 43.17, 41.89, 40.96, 39.54, 37.11, 35.00, 32.78, 31.12, 30.27, 29.41, 23.36. Anal. ($C_{21}H_{36}O_3$) C, H.

[3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-1H-benz[e]indene-7-acetic Acid Methyl Ester (16a). Using the procedures described above for the preparation of compounds **13b** and **16b** from compound **14b**, compound **14a** (1.74 g, 4.09 mmol) was initially converted into [3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-3-(1,1-dimethylethoxy)-dodecahydro-3a-methyl-1H-benz[e]indene-7-acetic acid (**13a**, 1.19 g, 90%) and then, without isolation, this acid was esterified and purified by chromatography (silica gel, 5% EtOAc in hexane) to give compound **16a** (1.16 g, 93%) as white crystals: mp 55–57 °C; IR 2914, 1743, 1437, 1360, 1276, 1198, 1131, 1083, 1031, 988, 904 cm^{-1}; ^1H NMR δ 3.66 (s, 3H, CH_3O), 3.37 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 2.41–2.34 (m, 2H, CH_2-COOMe), 1.12 (s, 9H, $C(CH_3)_3$), 0.73 (s, 3H, CH_3); ^{13}C NMR δ 173.90 (C=O), 80.76 (C-3), 71.98 ($C(CH_3)_3$), 28.65 ($C(CH_3)_3$), 11.68 (CH_3), 51.29, 49.81, 43.11, 41.69, 38.21, 37.10, 37.04, 36.86, 31.06, 30.49, 29.74, 29.52, 25.14, 23.18. Anal. ($C_{21}H_{36}O_3$) C, H.

[3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-3-(1,1-Dimethylethoxy)-dodecahydro-3a-methyl-1H-benz[e]indene-7-ethanol (17). To a stirred solution of the compound **16a** (1.70 g, 5.05 mmol) in dry CH_2Cl_2 (80 mL) was added DIBALH (1.0 M solution in toluene, 33.6 mL, 33.6 mmol) at 0 °C. After 2 h, CH_2Cl_2/MeOH (1:1, 8 mL) was added, and then aqueous 10% HCl (10 mL) was added. The organic layer was washed with 0.3 N NaOH (170 mL) and brine (200 mL) and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure to give a solid which was purified by chromatography (silica gel, 15% EtOAc in hexane) to give the product **17** (1.51 g, 96%) as white crystals: mp 86–87 °C (from EtOAc/hexane); IR 3328, 2973, 2915, 2862, 1449, 1361, 1197, 1122, 1083, 990 cm^{-1}; ^1H NMR δ 3.65 (t, J = 7.0 Hz, 2H, $HOCH_2$), 3.37 (t, J = 8.2 Hz, 1H, $CHOC(CH_3)_3$), 1.13 (s, 9H, $C(CH_3)_3$), 0.73 (s, 3H, CH_3); ^{13}C NMR δ 80.76 (C-3), 72.10 ($C(CH_3)_3$), 61.79 ($HOCH_2$), 28.71 ($C(CH_3)_3$), 11.76 (CH_3), 49.96, 43.17, 41.90, 38.32, 37.24, 37.11, 34.87, 31.11, 29.93, 29.72, 29.65, 25.31, 23.25. Anal. ($C_{20}H_{36}O_2$) C, H.

[3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-Dodecahydro-7-(2-hydroxyethyl)-3a-methyl-1H-benz[e]inden-3-ol (18). A solution of compound **17** (1.50 g, 4.86 mmol) in EtOH (50 mL) and 6 N HCl (15 mL) was refluxed for 1.5 h. Most of the EtOH was removed under reduced pressure and the solution was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with water (200 mL), saturated $NaHCO_3$ (200 mL), and brine (200 mL) and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure to give a solid which was purified by chromatography (silica gel, 35% EtOAc in hexane) to yield product **18** (1.20 g, 98%) as white crystals: mp 103–104 °C (from EtOAc/hexane); IR (KBr pellet) 3293, 2916, 2856, 1656, 1448, 1382, 1138, 1061, 1022 cm^{-1}; ^1H NMR (CD_3OD) δ 3.69–3.65 (m, 3H, $HOCH_2$, $CHOH$), 0.76 (s, 3H, CH_3); ^{13}C NMR (CD_3OD) δ 82.52 (C-3), 61.70 ($HOCH_2$), 11.81 (CH_3), 51.32, 44.85, 43.51, 39.56, 38.35, 38.07, 35.77, 31.07, 31.02, 30.92, 30.60, 26.46, 23.84. Anal. ($C_{16}H_{28}O_2$) C, H.

[3aS-(3aα,5aβ,7β,9aα,9bβ)]-Dodecahydro-7-(2-hydroxyethyl)-3a-methyl-3H-benz[e]inden-3-one (19). To a stirred solution of compound **18** (0.57 g, 2.26 mmol) in acetic acid (10 mL) at room temperature was added dropwise within 10 min a 5.25% aqueous solution of sodium hypochlorite (3.2 mL, 2.3 mmol). After 30 min, 2-propanol (3 mL) was added to quench any excess oxidant and water (20 mL) was added. The mixture was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with water (100 mL), saturated $NaHCO_3$ (100 mL), water (100 mL), and brine (100 mL) and dried over Na_2SO_4. Solvent removal under reduced pressure gave a solid which was purified by chromatography (silica gel, 30% EtOAc in hexane) to give product **19** (0.45 g, 80%) as white crystals: mp 59–60 °C (from EtOAc/hexane); IR 3418, 2920, 1739, 1453, 1052 cm^{-1}; ^1H NMR δ 3.67 (t, J = 6.9 Hz, 2H, $HOCH_2$), 0.89 (s, 3H, CH_3); ^{13}C NMR δ 221.53 (C=O), 61.61 ($HOCH_2$), 13.90 (CH_3), 50.46, 48.44, 41.32, 38.23, 36.94, 35.79, 34.75, 31.57, 29.65, 29.47, 29.13, 24.58, 21.32. Anal. ($C_{16}H_{26}O_2$) C, H.

[3R-(3α,3aβ,5aα,7α,9aβ,9bα)]-Dodecahydro-7-(2-hydroxyethyl)-3a-methyl-1H-benz[e]indene-3-carbonitrile (20) and [3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-Dodecahydro-7-(2-hydroxyethyl)-3a-methyl-1H-benz[e]indene-3-carbonitrile (4). To a stirred solution of compound **19** (450 mg, 1.8 mmol) in dimethoxyethane (62 mL) and EtOH (2.8 mL) at room temperature was added *t*-BuOK (2.02 g, 18.0 mmol). A solution of tosylmethyl isocyanide (703 mg, 3.6 mmol) in dimethoxyethane (9.1 mL) was slowly (ca. 10 min) added from a syringe. After 3 h, the mixture was quenched with water (50 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL) and dried over Na_2SO_4. The solvent was removed to yield an oil which was purified by chromatography (silica gel, 20% EtOAc in hexane) to give products **20** (first fraction, 80 mg, 17%) and **4** (second fraction, 120 mg, 26%).

Compound **20** was obtained as white crystals: mp 97–98 °C (from EtOAc/hexane); IR 3374, 2922, 2234, 1451, 1384, 1333, 1148, 1060, 1059 cm^{-1}; ^1H NMR δ 3.66 (t, J = 6.8 Hz, 2H, $HOCH_2$), 2.57 (dd, J = 6.8 Hz, J = 2.0 Hz, 1H, CHCN), 0.83 (s, 3H, CH_3); ^{13}C NMR δ 122.36 (CN), 61.52 ($HOCH_2$), 50.97 (C-3), 18.12 (CH_3), 44.84, 42.14, 40.01, 37.38, 36.76, 35.08, 34.69, 29.73, 29.52, 29.32, 27.16, 25.78, 24.24. Anal. ($C_{17}H_{27}NO$) C, H, N.

Compound **4** was obtained as white crystals: mp 61–62 °C (from EtOEt/hexane); IR 3374, 2919, 2236, 1450, 1386, 1061 cm^{-1}; ^1H NMR δ 3.65 (t, J = 6.9 Hz, 2H, $HOCH_2$), 2.28 (t, J = 9.6 Hz, 1H, CHCN), 0.93 (s, 3H, CH_3); ^{13}C NMR δ 121.37 (CN), 61.68 ($HOCH_2$), 53.38 (C-3), 14.45 (CH_3), 45.04, 42.19, 40.26, 37.84, 37.12, 36.76, 34.77, 29.76, 29.43, 26.48, 25.67, 23.97. Anal. ($C_{17}H_{27}NO$) C, H, N.

[3R-(3α,3aβ,5aα,7α,9aβ,9bα)]-1-[Dodecahydro-7-(2-hydroxyethyl)-3a-methyl-1H-benz[e]inden-3-yl]ethanone (21) and [3S-(3α,3aα,5aβ,7β,9aα,9bβ)]-1-[Dodecahydro-7-(2-hydroxyethyl)-3a-methyl-1H-benz[e]inden-3-yl]ethanone (3). To a stirred solution of CH_3MgCl (3.0 M solution in THF, 5.0 mL, 15.0 mmol) cooled in an ice–water bath was added under N_2 a solution of a mixture of compounds **20** and **4** (130 mg, 0.5 mmol) in dry THF (20 mL). The solution was refluxed for 24 h and cooled to 0 °C, saturated NH_4Cl solution (20 mL) was added, and the solution was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated to give an oil which was purified by chromatography (silica gel, 30% EtOAc in hexane) to give products **21** and **3** (130 mg, 94%). Separation of the products was accomplished by HPLC [Econosil, 10 μm silica column, 250-mm × 10-mm, EtOAC/hexane/$ClCH_2CH_2Cl$ (3:7:10), 3 mL/min].

Compound **21** (25 mg, 18%) was obtained as a colorless oil: IR 3408, 2918, 1702, 1450, 1357, 1190, 1061 cm^{-1}; ^1H NMR δ 3.64 (t, J = 6.7 Hz, 2H, $HOCH_2$), 2.80 (dd, J = 5.7 Hz, J = 2.5 Hz, 1H, $CHCOCH_3$), 2.12 (s, 3H, $COCH_3$), 0.93 (s, 3H, CH_3); ^{13}C NMR δ 212.96 (C=O), 61.70 ($HOCH_2$), 49.30 (C-3), 21.06 (CH_3), 61.35, 46.48, 42.16, 37.49, 36.95, 35.36, 34.87, 32.85, 29.93, 29.54, 25.97, 25.26, 24.31. Anal. ($C_{18}H_{30}O_2$) C, H.

Compound **3** (95 mg, 69%) was obtained as white crystals: mp 78–79 °C (from hexane); IR 3410, 2918, 1704, 1450, 1358, 1191, 1061 cm^{-1}; ^1H NMR δ 3.66 (t, J = 6.9 Hz, 2H, $HOCH_2$), 2.55 (t, J = 9.0 Hz, 1H, CHCO), 2.12 (s, 3H, $COCH_3$), 0.63 (s, 3H, CH_3); ^{13}C NMR δ 209.81 (C=O), 61.72 ($HOCH_2$), 55.65 (C-3), 13.53 (CH_3), 63.84, 44.89, 41.87, 39.05, 37.92, 36.91, 34.86, 31.50, 29.92, 29.86, 29.54, 25.63, 23.81, 22.72. Anal. ($C_{18}H_{30}O_2$) C, H.

Electrophysiology. Hippocampal cultures were prepared from 1–2 day old albino rat pups and maintained as described previously.[25] Experiments were carried out at room temperature (~22 °C) using cultures that had been maintained *in vitro* for 3–10 days. At the time of an experiment the growth media was exchanged for a solution containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM glucose, 10 mM hydroxyethylpiperazineethanesulfonic acid (HEPES), and 0.001 mM tetrodotoxin (TTX) with the pH adjusted to 7.3. TTX was included to block voltage-gated Na^+ currents and to diminish

spontaneous synaptic currents. Voltage clamp recordings were obtained using whole-cell patch clamp methods.[20] Recording electrodes were fashioned from 1.2 mm borosilicate glass capillaries (World Precision Instruments) using a Flaming-Brown P-87 horizontal pipet puller (Sutter Instruments) and had resistances of 5–8 MΩ when fire-polished and filled with a solution containing 140 mM CsCl, 4 mM NaCl, 4 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM HEPES, and 5 mM ethyleneglycolbis-(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) with the pH adjusted to 7.3 using CsOH. Currents were filtered at 1.5 kHz and were digitized at 0.125 kHz using pCLAMP V 5.5 (Axon Instruments). Data were analyzed using pCLAMP V 5.5, Sigmaplot for Windows V 2.0, and routines written in Axobasic. The data in this paper are presented as the mean ± SEM.

GABA stock solutions were prepared in the extracellular solution. Test compound stock solutions were prepared in DMSO and were diluted with the extracellular solution at the time of an experiment. The final DMSO concentration was <0.2%, a concentration that does not alter GABA currents in hippocampal neurons. Compounds were applied by pressure ejection from pipets positioned within 5 μm of the recorded neuron using a 500 ms jet of compressed air at 10–20 psi. This system allows no discernable drug leakage between applications and affords reliable repeated drug delivery. The concentrations of drugs reported are those in the pipet. The actual concentration at the cell is likely to be less due to diffusion and the fact that the entire cell is not uniformly exposed to the pipette contents.

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