Neurosteroid Analogues: Structure-Activity Studies of Benz[e]indene Modulators of $GABA_A$ Receptor Function. 1. The Effect of 6-Methyl Substitution on the Electrophysiological Activity of 7-Substituted Benz[e]indene-3-carbonitriles

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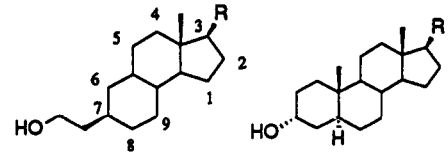
Received May 3, 1993®

The effect of 6-methyl substitution on the ability of 7-(2-hydroxyethyl)benz[e]indene-3-carbonitriles to potentiate GABA-mediated chloride current and to directly gate a chloride current in the absence of GABA in cultured rat hippocampal neurons was investigated. Structurally analogous steroid 17-carbonitriles that either contained or did not contain a 19-methyl group were also investigated. Compounds were evaluated at 1 μM for their ability to potentiate GABA-mediated currents and at 10 μM for current activation in the absence of GABA. The benz[e]indene 3(*R*)-carbonitriles and analogous steroid 17α-carbonitriles had no effects in either assay. The benz[e]indene-3(*S*)-carbonitriles and analogous steroid 17β-carbonitriles were active in both assays. Relative to the 6-unsubstituted benz[e]indene 3(*S*)-carbonitrile, the following effects of 6-methyl substituents were observed: a 6(a)-methyl group increased both activities; a 6(e)-methyl group decreased both activities; and 6,6-dimethyl substitutents had opposing effects so that both activites remained similar to those of the 6-unsubstituted compound. The activities of the steroid 17β-carbonitriles were not affected significantly by the presence or absence of a 19-methyl group. A conformational analysis using molecular modeling methods was also performed for the benz[e]indene 3*S*-carbonitriles and the steroid 17β-carbonitriles. The ability of the different 6-methyl substituents to differentially effect the conformations of the flexible benz[e]indenes and the inability of the steroid 19-methyl group to alter the conformations of the rigid steroid 17β-carbonitriles are suggested to explain the results.

The major inhibitory neurotransmitter in the mammalian central nervous system is γ-aminobutyric acid (GABA). Binding of this neurotransmitter to its recognition site on the postsynaptic $GABA_A$ receptor/chloride channel complex results in the opening of the channel and, in most neurons, the passage of chloride ions into the neuron. This increase in intracellular chloride concentration makes the membrane potential of the postsynaptic cell more negative thereby decreasing its ability to respond to an excitatory stimulus.

There are additional binding sites for other ligands on the $GABA_A$ complex. The most studied of these are the benzodiazepine,[1,2] barbiturate,[3] picrotoxin,[4] and steroid[5-9] binding sites. Ligands for these sites may either modify the response of the complex to GABA or, in the case of the barbiturates and steroids, alter the gating properties of the channel in the absence of GABA. Compounds acting at these sites either to facilitate or to produce an increase in chloride flux may have anxiolytic, sedative-hypnotic, anticonvulsant, muscle relaxant, and anesthetic properties.

We are interested in the development of new ligands for the steroid binding site on the $GABA_A$ complex. In this regard, we recently reported that benz[e]indenes 1 and 2 are ligands for the site.[10,11] A comparison of the electrophysiological effects of benz[e]indene 1 and steroid 3 (a prototypic ligand for the steroid binding site[12]) in cultured rat hippocampal neurons showed that benz[e]indene 1 was more potent than steroid 3 (EC_{50} = 0.2 ± 0.03 and 1.2 ± 0.2 μM, respectively) at increasing currents mediated by 1 μM GABA, but less potent than steroid 3 at gating current in the absence of GABA. For this latter effect, the threshold for gating of current by steroid 3 is 1 μM, whereas this gating threshold was found to be above 10 μM (in some cells it was above 100 μM) for benz[e]indene 1. Thus, if the observed dose-response relationships for the two effects caused by steroid 3 are used as reference standards, these parameters are shifted in opposite directions for benz[e]indene 1. We believe, for the reasons discussed below, that the shifted dose-response relationships observed for benz[e]indene 1 have significant practical importance.



1 R = $COCH_3$ 3 R = $COCH_3$
2 R = CN 4 R = CN

Schulz and Macdonald[13] have provided evidence for a hypothesis to explain why phenobarbital is useful as an anticonvulsant, whereas pentobarbital is useful as an anesthetic. This hypothesis correlates the anticonvulsant activity of a barbiturate with its ability to potentiate GABA-mediated chloride currents and the anesthetic activity with its ability to gate chloride currents at the $GABA_A$ receptor complex in the absence of GABA. Since both dose-response curves are close together for pentobarbital, the anesthetic effect of this drug will be manifest at nearly all doses so this drug is useful primarily as an anesthetic. On the other hand, these dose-response curves are more widely separated for phenobarbital and it can be

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® Abstract published in *Advance ACS Abstracts*, September 1, 1993.

0022-2623/93/1836-3956$04.00/0

used at doses where its GABA potentiating effects are dominant so that the drug is a useful anticonvulsant.

Application of this hypothesis to ligands for the steroid binding site on the $GABA_A$ complex predicts that a compound like benz[*e*]indene **1** should be more like phenobarbital than pentobarbital with regard to its biological activity, and *in vivo* studies with this compound are in progress to address this prediction. The study reported here is concerned with further evaluating the importance of structural differences between steroids and benz[*e*]indenes in relation to their effects on the above discussed dose–response relationships. Specifically, we have studied the effect of the 19-methyl group in steroids and the analogous methyl groups at C^6 in benz[*e*]indenes on the electrophysiological activities of the compounds at the $GABA_A$ receptor complex present in cultured rat hippocampal neurons.

Chemistry

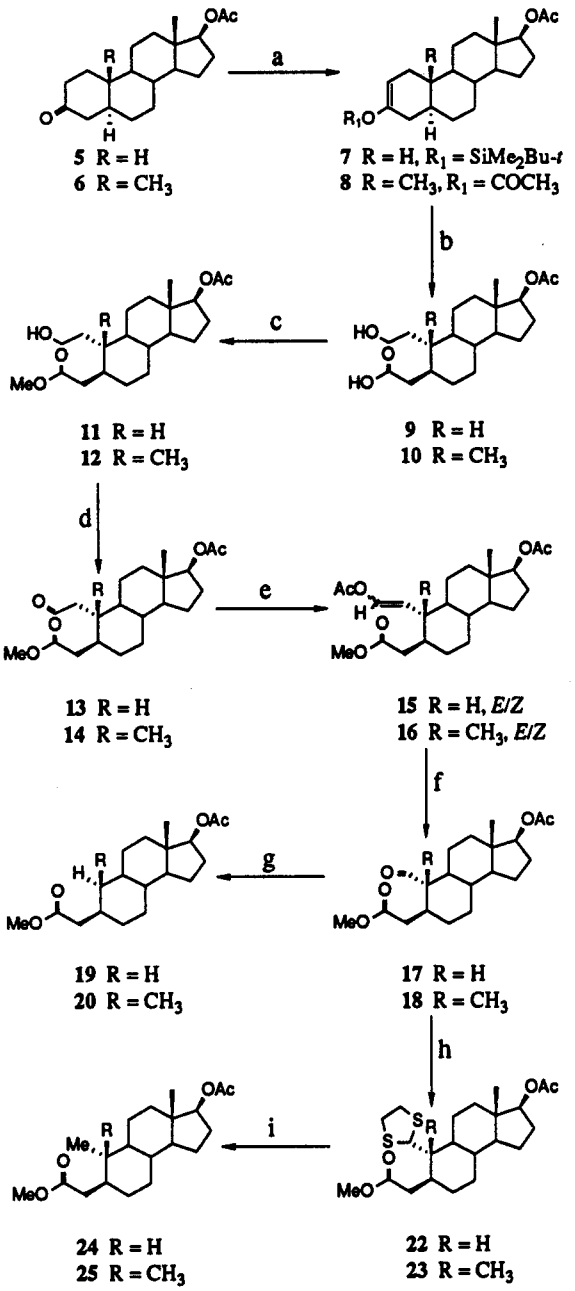
Other investigators have shown that both steroids **3** and **4**, which differ only in their 17β-substituents, have very similar activities as potentiators of muscimol-stimulated $^{36}Cl^-$ uptake in rat brain synaptoneurosomes.[14] Likewise, we have shown that benz[*e*]indenes **1** and **2**, which differ similarly in their analogous 3*S* substituents, have comparable potencies as potentiators of 1 μM GABA-mediated chloride current in cultured rat hippocampal neurons.[11] Thus, it seems likely that congeneric steroids and benz[*e*]indenes having a nitrile group instead of an acetyl group at these same positions will have high biological activities. Accordingly, we chose to evaluate analogues of benz[*e*]indene **2** and steroid **4** . The decision is of practical significance since the nitrile-substitued compounds are more readily prepared.

The desired benz[*e*]indenes **2** and **34-39** were prepared from either 17β-acetoxy-5α-estran-3-one (**5**) or 17β-acetoxy-5α-androstan-3-one (**6**) as indicated in Schemes I and III. The reaction sequence **5** → **7** → **9** → **11** → **13** → **15** → **17** → **19** (Scheme I) has been reported previously.[15] In general, this sequence is useful for the preparation of benz[*e*]indenes having either equatorial substituents or no substituents at C^6. The analogous reaction sequence **6** → **8** → **10** → **12** → **14** → **16** → **18** → **20**, which is reported here, is of general use for the preparation of benz[*e*]indenes having an axial methyl substituent with or without equatorial substitutents at C^6.

The Δ^2-enol acetate **8** was prepared by reacting steroid **6** with isopropenyl acetate in the presence of *p*-TsOH as described previously.[16] Although the literature does not indicate that the isomeric Δ^3-enol acetate is formed in this reaction, the proton NMR spectrum of our reaction product indicated (by integration of the peak area of the vinyl protons) that it was a ca. 8:2 mixture of the Δ^2- and Δ^3-enol acetates. This mixture of isomeric enol acetates was reacted with ozone in dichloromethane–HOAc at −78 °C. Following reduction with dimethyl sulfide and hydrolysis, the crude product was reduced with $NaBH_4$ and purified by recrystallization to give pure hydroxy acid **10** in 61% yield.

Treatment of compound **10** with diazomethane yielded (94%) the corresponding methyl ester **12**, which was oxidized to compound **14** with PCC.[17] Compound **14**, which was obtained as a crude oil, was not purified and characterized because it rapidly converted upon standing into multiple products. Hence, as soon as compound **14** was prepared, it was immediately reacted with isopropenyl acetate–*p*-TsOH and converted into the stable enol acetate **16** (an 8:2 mixture of *E*/*Z* isomers as determined by proton NMR analysis). The overall yield for the oxidation and enol acetylation steps was 36%.

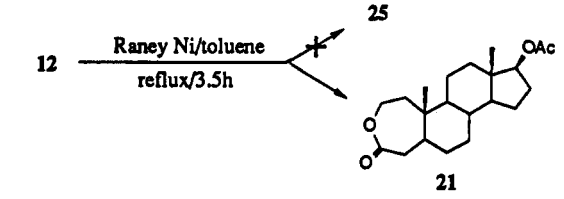
Scheme I[a]



[a](a) TBDMS-OTf, NEt_3 for **5**, isopropenyl acetate, TsOH for **6**; (b) O_3, $NaBH_4$; (c) CH_2N_2; (d) PCC; (e) isopropenyl acetate, TsOH; (f) O_3; (g) Wilkinson's catalyst; (h) $HSCH_2CH_2SH$, BF_3·EtOEt; (i) Raney Ni.

The conversion of enol acetate **16** into either compounds **20** or **23** via aldehyde **18** was carried out without purification of aldehyde **18** because this aldehyde, like aldehyde **14**, was unstable. Hence, ozonolysis of compound **16** in dichloromethane–HOAc at −78 °C followed by decarbonylation of the resulting aldehyde **18** using Wilkinson's catalyst[18-20] in benzonitrile at 160 °C gave the 6(a)-methyl-substituted benz[*e*]indene **20** (18% from **16**). Alternatively, ozonolysis of compound **16** followed by treatment of aldehyde **18** with ethanedithiol–boron trifluoride etherate[21] gave thioacetal **23** (88% from **16**). Desulfurization[22] of compound **23** gave the 6,6-dimethyl-substituted benz[*e*]indene **25** (77%). The 6(e)-methyl-substituted benz[*e*]indene **24** was prepared from aldehyde **17** via thioacetal

Scheme II



22 using a similar thioacetalization (85%)/desulfurization (80%) sequence.

We also considered preparing benz[*e*]indenes **24** and **25** from compounds **11** and **12**, respectively, using Raney nickel in refluxing toluene to effect a dehydroxymethylation reaction as described by Krafft et al.[23] However, when this reaction was tried with compound **12** (Scheme II), the product was found to be the seven-membered ring lactone **21** (93%). No investigations were made into altered reaction conditions that might favor dehydroxymethylation over lactonization.

The remaining steps in the synthesis of benz[*e*]indenes **2** and **34-39** are shown in Scheme III. Diester compounds **19, 20, 24,** and **25** were reduced by DIBALH[24] in toluene at 0–5 °C to their corresponding diols **26-29** in high yields (93–98%). The secondary hydroxy group of each of these diols was oxidized selectively with 5.25% NaOCl in glacial AcOH[25] to yield compounds **30-33** in moderate yields (64–76%). Reaction of the keto groups of compounds **30, 32, 33** with 1.0 M *t*-BuOK in dimethoxyethane and tosylmethyl isocyanide (TosMIC)[26,27] gave a mixture of the corresponding diastereomeric 3(*S*)-carbonitriles (**2, 37,** and **39**) and 3(*R*)-carbonitriles (**34, 36,** and **38**) (27–63% yields); whereas, compound **33** yielded only the 3(*S*)-carbonitrile **35** (36%). The final purification and/or separation of benz[*e*]indenes **2** and **34–39** was accomplished by HPLC on silica as described in the Experimental Section.

The stereochemical assignments for the nitrile groups of compounds **2** and **34-39** were based on the ^{1}H NMR spectra of the compounds. Because of the dihedral angles involved, the coupling constants of the C^3 protons of benz[*e*]indenes having the 3*S* configuration would be 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 protons of benz[*e*]indenes having the 3*R* configuration would be expected to be unequal for vicinal coupling to the adjacent protons on C^2. Consequently, nitriles **2, 35, 37,** and **39** whose ^{1}H NMR spectra showed a resonance at δ 2.25 ± 0.3 as an apparent triplet (J = 9.6 Hz) were assigned as the 3(*S*)-carbonitriles. Compounds **34, 36,** and **38** whose ^{1}H NMR spectra showed a resonance at δ 2.53 ± 0.3 as a doublet of doublets (J = 7.0, 2.0 Hz) were assigned as the 3(*R*)-carbonitriles. The ^{13}C chemical shifts of the carbons of the nitrile groups also correlate well with the stereochemistry of the nitrile groups. The 3(*S*)-carbonitrile carbons are observed at δ 121.2 ± 0.2 and the 3(*R*)-carbonitrile carbons are observed at δ 122.2 ± 0.2.

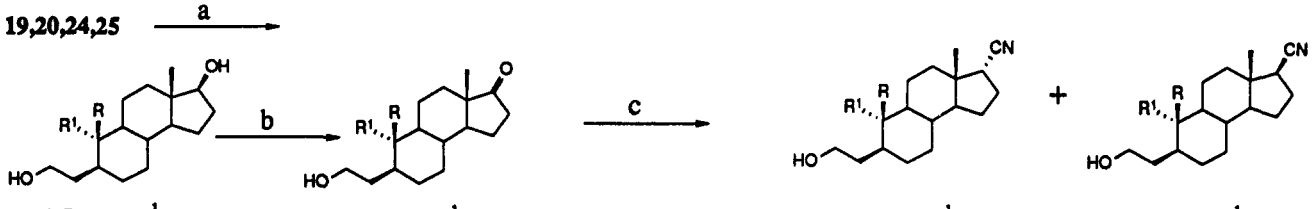
A similar synthetic strategy was used for the preparation of the desired steroid 17-carbonitriles (Scheme IV). Although steroid **41** is commercially available, it is prohibitively expensive. Since potassium tri-*sec*-butylborohydride in tetrahydrofuran (K-Selectride) has been shown to selectively and stereospecifically reduce 3,17-diketo-substituted androgens to 3α-hydroxy-17-keto-substituted products,[28,29] we investigated the reduction of 19-norsteroid **40** with this reagent. Even though the absence of a 19-methyl group increases the accessibility of K-Selectride to the β-face of 19-norsteroid **40**, the only reduction product obtained was the 3α-hydroxy-19-norsteroid **41** (86%). The ^{1}H NMR and ^{13}C NMR spectra of crude compound **41** showed the resonances of the proton on C^3 as an apparent triplet at δ 4.06 and C^3 at δ 66.19. No resonances for the corresponding proton (m, δ 3.5) and carbon (δ 71.26) resonances of an authentic sample of 3β-hydroxy-5α-estran-17-one were observed.

The reaction of 19-norsteroid **41** with TosMIC yields (52%) a 1.2:1 mixture of the 17β-carbonitrile **43** and the 17α-carbonitrile **44**, respectively. As reported previously,[14] reaction of steroid **42** with TosMIC yields (59%) a 1.5:1 mixture of the 17β-carbonitrile **4** and the 17α-carbonitrile **45**, respectively. These diastereomeric mixtures were separated by HPLC on silica as reported in the Experimental Section. As described above for the analogous benz[*e*]indenes (**2** and **34-39**), the configurations of the 17-carbonitrile groups of compounds **4** and **43-45** were assigned from the ^{1}H NMR and ^{13}C NMR spectra of the compounds.

Electrophysiology

Voltage clamp recordings were obtained from cultured postnatal rat hippocampal neurons using whole-cell patch clamp methods.[30] 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 directly gate a current in the absence of GABA (Table I). The two responses were studied at a 10-fold difference in compound concentration so that structural modifications having effects on each activity could be readily identified. The concentrations selected for these experiments were based on our previous experience with compounds **1-3**.[10] Recordings that are representative of the responses observed for the benz[*e*]indenes are shown for compounds **2, 34,**

Scheme III[a]



26 R = H, R^1 = H
27 R = CH$_3$, R^1 = H
28 R = H, R^1 = CH$_3$
29 R = CH$_3$, R^1 = CH$_3$

30 R = H, R^1 = H
31 R = CH$_3$, R^1 = H
32 R = H, R^1 = CH$_3$
33 R = CH$_3$, R^1 = CH$_3$

34 R = H, R^1 = H
36 R = H, R^1 = CH$_3$
38 R = CH$_3$, R^1 = CH$_3$

2 R = H, R^1 = H
35 R = CH$_3$, R^1 = H
37 R = H, R^1 = CH$_3$
39 R = CH$_3$, R^1 = CH$_3$

[a](a) DIBALH; (b) 5.25% NaOCl, HOAc; (c) TosMIC, *t*-BuOK.

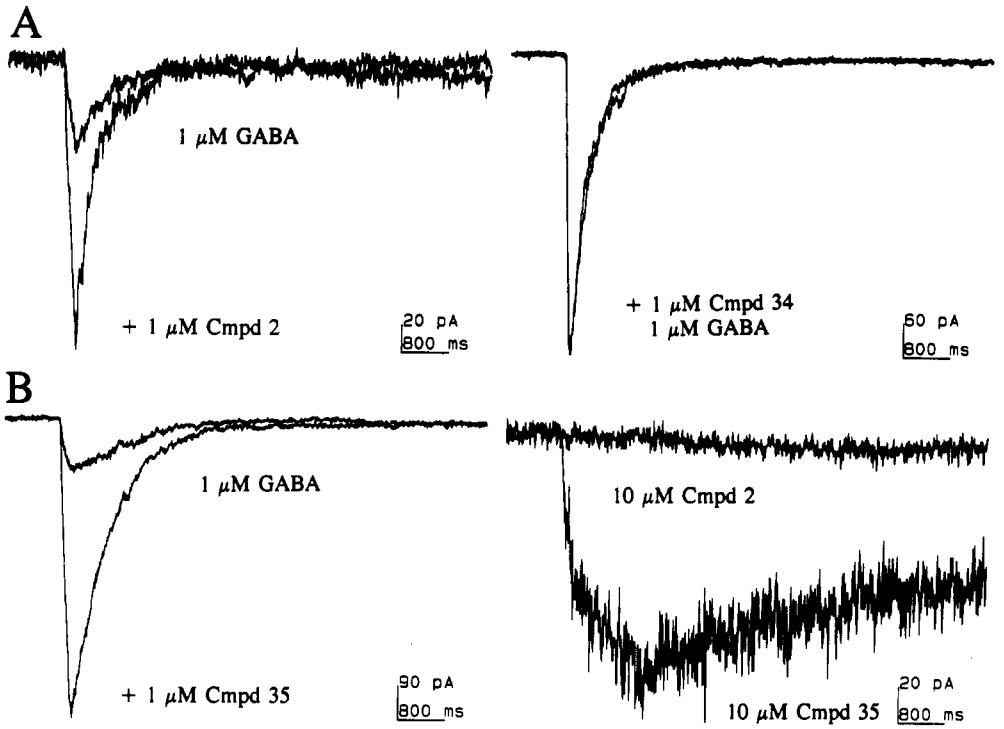
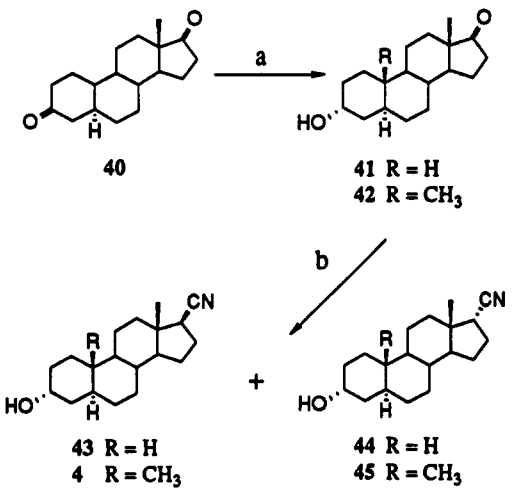


Figure 1. Effects of benz[*e*]indenes on $GABA_A$ receptor-mediated currents in cultured rat hippocampal neurons: (A) The traces show the response of two neurons to 1 μM GABA in the absence and presence of 1 μM **2** (left) and 1 μM **34** (right). (B) The left trace shows the response of a neuron to 1 μM GABA in the absence and presence of 1 μM **35**. The right traces show the response of a neuron to 10 μM **2** and 10 μM **35**. The prolonged nature of the current activated by **35** is typical of currents gated by benz[*e*]indenes (or steroids) at concentrations where these agents activate a response. All traces were obtained at a holding potential of −60 mV and GABA and/or the benz[*e*]indenes were applied for 200 ms.

Scheme IV[a]



[a](a) K-Selectride; (b) TosMIC, *t*-BuOK.

and **35** in Figure 1. For the steroids, similiar representative recordings are shown for compounds **4** and **45** in Figure 2.

The benz[*e*]indene-3(*R*)-carbonitiriles **34**, **36**, and **38** and the steroid 17α-carbonitriles **44** and **45** were inactive as either potentiators (even at concentrations of 10 μM) or direct activators of current. The benz[*e*]indene-3(*S*)-carbonitriles **2**, **35**, **37**, and **39** and the steroid 17β-carbonitriles **4** and **43** were all potentiators of GABA-mediated currents. Benz[*e*]indene-3(*S*)-carbonitrile **37** was not an effective potentiator at 1 μM, but it was effective when evaluated at 10 μM. For potentiation of GABA-mediated current the potency of the compounds increased as follows: **37** < **39** ≃ **2** < **35** ≃ **43** ≃ **4**. Of these compounds, only 10 μM benz[*e*]indene **35** and 10 μM steroids **43** and **4** directly gated a current. At 1 μM, steroids

Table I. 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]
34	4	NR[d,e]	NR
36	4	NR[e]	NR
38	4	NR[e]	NR
44	8	NR[e]	NR
45	4	NR[e]	NR
2	12	255 ± 8[f]	NR
35	6	382 ± 47	26 ± 4
35	5		NR (1 μM)[g]
37	5	NR	NR
37	5	244 ± 56 (10 μM)[h]	
39	10	220 ± 17	NR
43	7	372 ± 49	38 ● 5
43	7		15 ± 4 (1 μM)[g]
4	10	443 ± 55	49 ● 4
4	6		22 ± 5 (1 μM)[g]

[a] *N* = Number of cells examined. [b] To calculate the % 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 % response of 100% reflects no change in the current compared to 1 μM GABA alone. At the foot of the dose–response curve in cultured postnatal rat hippocampal neurons is a GABA concentration of 1 μM. These experiments were conducted at −60 mV and compounds were applied by pressure ejection for 200 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] NR denotes no response. Compounds that increased current by ≤6% of the response to 1 μM GABA in the same cells were considered to give no response. [e] This compound at 10 μM also did not potentiate 1 μM GABA-mediated currents. [f] Values are the mean ± SEM. [g] This table entry indicates the direct gating effect of this compound at 1 μM. [h] This table entry indicates the potentiating effect of this compound at 10 μM on currents mediated by 1 μM GABA.

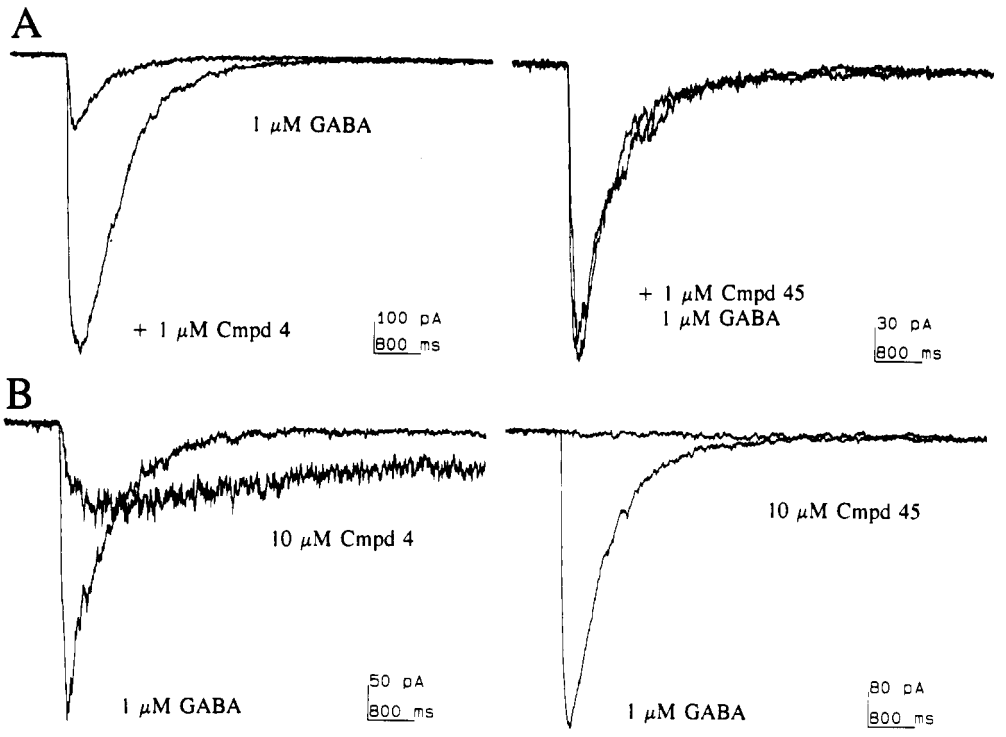


Figure 2. A steroid 17β-carbonitrile potentiates a GABA-mediated response and directly gates a chloride currents whereas its 17α-carbonitrile congener does not: (A) The traces show the response of two neurons to 1 μM GABA in the absence and presence of 1 μM **4** (left) and 1 μM **45** (right). (B) The traces show the response of two neurons to 1 μM GABA or 10 μM **4** (left) and 1 μM GABA or 10 μM **45** (right). Note the prolonged nature of the steroid-gated current compared to current gated by 1 μM GABA. All recording were done at −60 mV and agents were applied for 200 ms.

43 and **4** still had the ability to directly activate a current, whereas benz[*e*]indene **35** did not. The order of potency for the compounds displaying channel activating activity in the concentration range of 1–10 μM was **35** < **43** ≃ **4**.

Discussion

Previous studies have demonstrated that the steroids which potentiate muscimol-stimulated $^{36}Cl^-$ uptake in rat brain synaptoneurosomes and/or which possess anesthetic activity are 17β-substituted. The corresponding 17α-substituted analogues of these steroids are devoid of these activities.[12,14] We found that steroids **43** and **4**, which are 17β-carbonitriles, are effective potentiators of GABA-mediated current. These steroids also directly gate a chloride current. The corresponding steroid 17α-carbonitriles, **44** and **45**, had neither activity. Thus, our electrophysiological results for steroids **4** and **43-45** establish that both potentiation of GABA-mediated current and the direct gating of a chloride current is significant only for steroids having an appropriate 17-substituent in the β-configuration. Similar results were found for the benz[*e*]indenes. The benz[*e*]indene 3(*R*)-carbonitriles (**34**, **36**, and **38**), which are the analogues of the steroid 17α-carbonitriles, also had no effect on either of the measured activities. The benz[*e*]indene 3(*S*)-carbonitriles (**2**, **35**, **37**, and **39**), which are the analogues of the steroid 17β-carbonitriles, were active compounds, and their effects were further modified by the C^6 substitution pattern.

The concept that methyl group substitution at the benz[*e*]indene C^6 position might have electrophysiological consequences occurred to us because the initially prepared benz[*e*]indenes lacked two structural features found in previously well-studied anesthetic steroids. These benz[*e*]indenes lacked the two carbons in the steroid A-ring (C^1 and C^2), and they lacked the 19-methyl group. Thus, we reasonably questioned how the potency of benz[*e*]indene **2** as a potentiator of GABA current and/or a direct activator of chloride current would be altered by methyl group substitution at C^6.

Because it was not feasible for us to generate complete dose–response curves for both activities of all the compounds, we were not able to determine if the different benz[*e*]indene C^6 methyl group substitution patterns affect the dose–response relationships for the two activites in a strictly equivalent manner. However, the results do indicate that each C^6 substitution pattern caused similar rather than opposing shifts in the dose–response curves for the two activities. Relative to compound **2**, benz[*e*]indene **35**, which contains a 6(a)-methyl group, exhibited not only enhanced GABA potentiation at 1 μM, but also direct gating at 10 μM. In this respect, benz[*e*]indene **35** is like steroids **43** and **4**. However, unlike these steroids, compound **35** does not directly gate a current at 1 μM. Thus, the major effect of the 6(a)-methyl group is not to diminish markedly the separation between the dose–response curves for the two activities of compound **35**, but to shift both dose–response curves to the left. This is an important conclusion since it indicates that the presence or absence of a methyl group in this position is not the controlling factor explaining why these dose–response curves are separated more for the 7-substituted benz[*e*]indenes than they are for the congeneric anesthetic steroids.

Benz[*e*]indene **37**, which contains a 6(e)-methyl group, is ineffective both as a potentiator of GABA currents at 1 μM and as a direct channel activator at 10 μM. Compound **37**, however, does potentiate GABA currents at 10 μM indicating that the agent is not inactive, but only less potent than benz[*e*]indene **2**. Clearly, the equatorial substituent has shifted the dose–response curve for potentiation of GABA-mediated current to the right. Most

likely, the dose–response curve for direct activation has also been shifted to the right.

The opposing effects of the axial- and equatorial-methyl groups act to nullify each other when both groups are present in the same benz[e]indene. Thus, compound **39**, which contains both methyl groups at C^6, has electrophysiological activities that are not notably different than those of compound **2**. The relative separation of the dose–response curves for the two types of modulatory effects appears to be unaffected (relative to compound **2**) in any major way by this methyl substitution pattern.

The active steroids **43** and **4**, which differ by substitution with a 19-methyl group, do not differ significantly in the electrophysiological parameters we measured. This result is consistent with the earlier report that the anesthetic activities of 3α-hydroxy-5α-pregnane-11,20-dione and 3α-hydroxy-19-nor-5α-pregnane-11,20-dione are nearly identical.[12] Nevertheless, in view of the results discussed for the 6-methylbenz[e]indenes, one might have expected that the presence or absence of a steroid 19-methyl group might have some notable effect on the electrophysiological parameters we measured. A possible reason for why the activities of the benz[e]indenes, but not the steroids, are significantly altered by methyl substituents that are similarly located in each class of compounds is suggested by molecular modeling studies.

All comparisons of three-dimensional structures were carried out on molecules whose minimum energy conformations were first determined by a molecular mechanics program. There are two energetically equivalent global minimum energy conformations for steroid **4**. These conformations differ only in the orientation of the hydrogen on the oxygen of the 3α-hydroxyl group. The conformation used for molecular modeling is shown in Figure 3A. The conformation of steroid **4** that was not chosen for modeling studies has the hydrogen of the hydroxyl group rotated ~120° in a counterclockwise direction. The conclusions of the reported modeling study were not changed when this alternate minimum energy conformation of steroid **4** was used as the reference conformation for the comparison of the three-dimensional shapes of the different molecules. This does not infer that the directionality of this hydrogen atom is not important in optimizing the interactions of these molecules with their receptor binding site. It is just that the current studies do not provide any basis for preferring either orientation of the hydroxyl group in the modeling studies.

Figure 3A shows in stick drawings the result obtained when the carbon atoms of the steroid B, C, and D rings, the 18-methyl group, and the atoms in the 17β-carbonitrile group from steroid **4** are superimposed using a least-squares fitting program on the corresponding atoms in steroid **43**. Orientation of the steroids relative to each other in this way makes it clear that the alleviation of the 1,3-diaxial interactions between the 19-methyl group and the 6β-, 8β- and 11β-axial hydrogens of steroid **4** causes a downward displacement of the A ring. The oxygen atom of the essential 3α-hydroxyl group, which on the basis of previous structure–activity studies of anesthetic steroids[12] has been postulated to serve as a hydrogen bond donor when these compounds are bound to the receptor, is displaced 0.73 Å away from the position it occupies in three-dimensional space in steroid **43**. This displacement is more readily observed in Figure 3D which shows an orthogonal ball and stick drawing of only the A-ring atoms of the steroids as fit to each other in Figure 3A. The orthogonal view has C^3 in front of the plane of the page and C^{10} and its respective axial-hydrogen or axial-methyl substituent behind the plane of the page.

The described difference in the relative position of the 3α-hydroxy group of steroids **4** and **43** is not large. Moreover, one needs to consider that only a slight change in alignment of steroids **4** and **43** is needed to bring about the superimposition of the oxygen atoms of the 3α-hydroxyl groups and the nitrogen atoms of the 17β-carbonitirile groups of the two steroids. Thus, it is reasonable to conclude that these rigid steroids should bind similarly to the receptor site and have similar electrophysiological activities. This is what we observed in the electrophysiological evaluation of the compounds.

Conformational analysis of the benz[e]indenes is more complex. The different methyl group substitution patterns at position C^6 of the benz[e]indenes differentially affect the conformations available to the hydroxy group located in the flexible C^7 side chain. Figure 3B shows the minimum energy conformations of benz[e]indenes **2**, **35**, **37**, and **39** that are most similar to the modeled conformations of steroids **4** and **43**. An alternate orthogonal view of partial ball and stick structures is shown in Figure 3E. The compounds are superimposed on each other in the same way that the steroids shown in Figure 3, parts A and D, are superimposed. Figure 3, parts C and F, shows the superimpositon of both the steroids and the benz[e]indenes thereby demonstrating how similar both classes of molecules are to each other when viewed in a common alignment.

The conformation shown for benz[e]indene **2** is one of 27 minimum energy conformations that were found by our conformational search strategy. The energies of these 27 conformations differed by as much as 3.09 kcal/mol. The conformer shown has an energy that is 0.17 kcal/mol above that found for the global minimum energy conformer.[31] Hence, the modeled conformation of benz[e]indene **2** will be significantly populated even in the absence of additional binding interactions from the receptor that might tend to further stabilize this conformation because of its similarity to the conformation of the active steroid analogues. As aligned in Figure 3, parts C and F, the oxygen atoms of compounds **43** and **2** are separated in space by 0.11 Å. Thus, this analysis indicates that benz[e]indene **2** is an excellent conformational mimic of steroid **43**, and as reported, this benz[e]indene has a potent electrophysiological effect.

The modeled conformation of benz[e]indene **35**, which contains a 6(a)-methyl group,is one of 33 minimum energy conformations that were found for this molecule. The energies of these 33 conformations differed by as much as 8.17 kcal/mol. The conformer shown has an energy that is 0.16 kcal/mol above that found for the global minimum energy conformer.[31] In the alignment shown in Figure 3, parts C and F, the oxygen atoms of compounds **43** and **35** are separated in space by 0.40 Å. Hence, like compound **2**, benz[e]indene **35** is also an excellent conformational mimic of steroid **43** and this flexible steroid analogue also has potent electrophysiological effects.

Notice also that the1,3-diaxial interaction of the 6(a)-methyl group and the axial-hydrogens at C^5, C^8,and C^{9a} of benz[e]indene **35** are alleviated, as discussed earlier for steroid **4**, by a leftward bending of the axial-methyl group (Figure 3B). However, since the hydroxyl group of benz[e]indene **35** resides in a flexible side chain, whereas the 3α-hydroxyl group of steroid **4** is on a semirigid ring, the

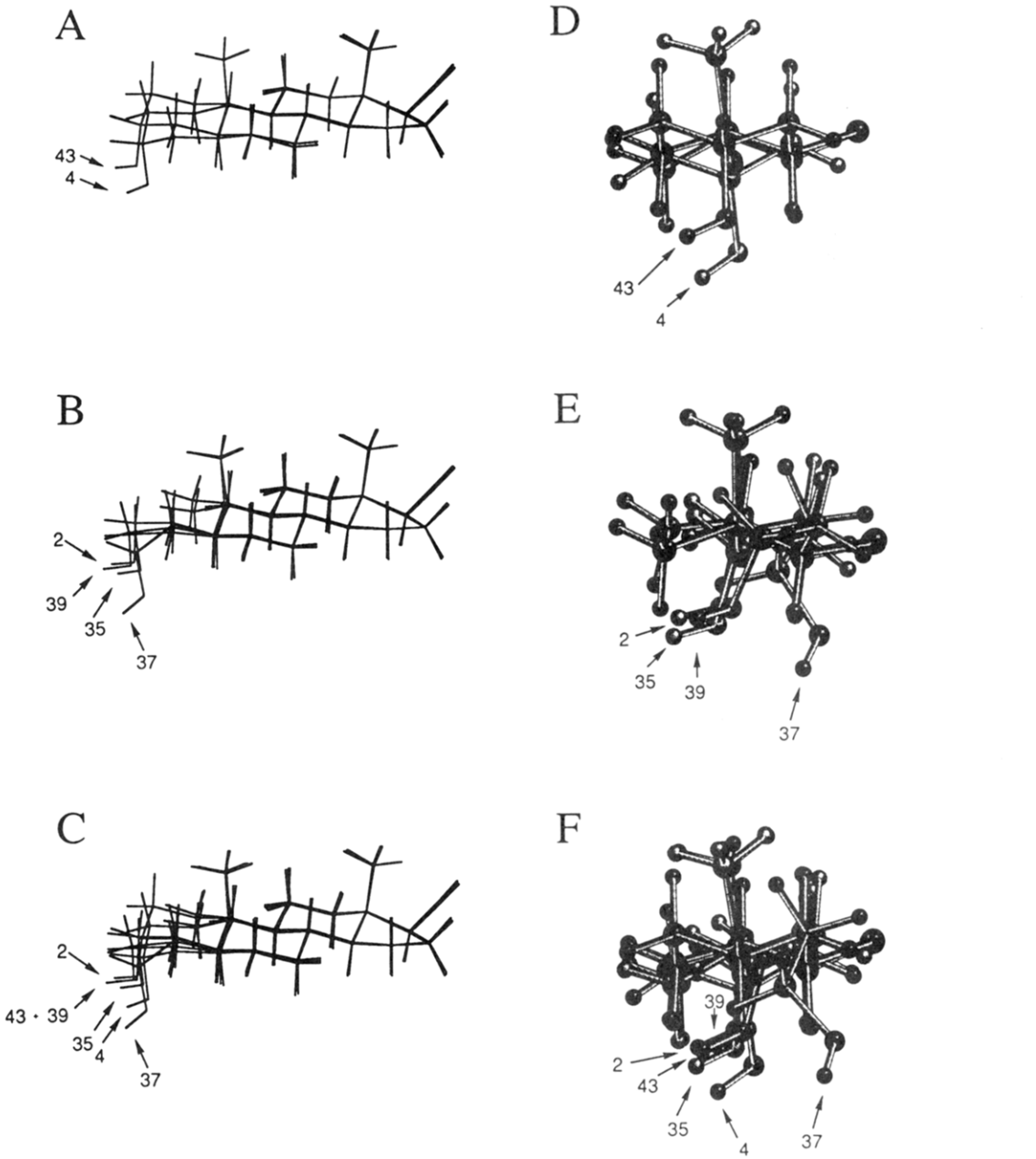


Figure 3. Molecular modeling results. Numbering of the arrows in the figure corresponds to the numbering of the compounds: (For ease of identification of the molecules, the numbered arrows point to the hydrogen atom of the hydroxyl group in each molecule.) (A) Stick drawings of minimum energy conformations of steroids **4** and **43**.The molecules have been superimposed by aligning the carbon atoms of the steroid B, C, and D rings, the 18-methyl group, and the atoms in the 17β-carbonitrile group of each steroid. (B) Stick drawings of analogously superimposed minimum energy conformations of benz[*e*]indenes **2**, **35**, **37**, and **39**. (C) The superimposition of the molecules present in panels A and B. (D)An orthogonal ball and stick drawing of only the A-ring atoms of the steroids as fit to each other in panel A. The orthogonal view has C^3 in front of the plane of the page and C^{10} and its respective axial-hydrogen or axial-methyl substituent behind the plane of the page. (E) An orthogonal ball and stick drawing of partial benz[*e*]indene structures as fit to each other in panel B. This view is analogous to that shown in panel D for the partial steroid structures. (F) The superimposition of the partial molecules present in panels D and E.

leftward bending of the axial-methyl group in compound **35** does not cause as large a downward movement of the hydroxyl group in benz[*e*]indene **35** as it causes for the hydroxyl group in steroid **4**. Figure 3E readily demonstrates that the hydroxyl groups of compounds **2** and **35** remain close to each other in three-dimensional space even though the latter compound contains an axial-methyl group. Thus, it may be that the axial-methyl group in benz[*e*]indene **35** provides an additional favorable hydrophobic interaction with the receptor binding site without requiring an unfavorable movement of the hydroxyl group in the side chain. This could explain the observed increase in potency found for compound **35** relative to compound **2**.

The modeled conformation of benz[*e*]indene **37**, which contains a 6(e)-methyl group, is one of 29 minimum energy conformations that were found for this molecule. The energies of these 29 conformations differed by as much as 9.12 kcal/mol. The conformer shown in Figure 3, parts B and E, has an energy that is 2.50 kcal/mol above that found for the global minimum energy conformer,[32] indicating that the modeled confomation will not be highly populated unless additional binding interactions from the receptor make this conformation more energetically favorable.

The minimization of a steric interaction between the *pro-R* hydrogen of the hydroxymethylene group in the side chain and the 6(e)-methyl group is responsible for the observed rightward movement of the side chain shown

for benz[e]indene **37** in Figure 3E. In the alignment shown in Figure 3, parts C and F, the oxygen atoms of compounds **43** and **37** are separated in space by 1.86 Å. The diminished activities of benz[e]indene **37** in the electrophysiological evaluation can be readily explained by the fact that this compound, even if stabilized in the conformation shown by its binding interactions with the receptor, is not as close a conformational mimic of the active steroids as the other more potent benz[e]indene analogues.

The modeled conformation of benz[e]indene **39** (Figure 3, parts B and E), which contains the 6,6-dimethyl groups, is one of 30 minimum energy conformations that were found for this molecule. In the alignment shown in Figure 3, parts C and F, the oxygen atoms of compounds **43** and **39** are separated in space by 0.22 Å. The energies of the 30 conformers found for benz[e]indene **39** differed by as much as 4.87 kcal/mol. The conformer shown has an energy that is 3.82 kcal/mol above that found for the global minimum energy conformer.[33] Clearly, this conformer, which is conformationally quite similar to the modeled conformation of benz[e]indene **2**, will not be heavily populated in the absence of an energetically favorable binding interaction from the receptor. If, however, the hydroxyl group does indeed form a hydrogen bond with the receptor as the earlier structure–activity studies[12] of anesthetic steroids suggest, this interaction would supply the stabilization energy necessary to maintain this receptor bound conformation of compound **39**. Consequently, this line of reasoning would suggest that it is not surprising that compounds **39** and **2** have similar electrophysiological activities.

Finally, some discussion of why compound **39** is better able to mimic the conformation of steroid **43** than is compound **37** is in order. As noted earlier the presence of a 6(a)-methyl group causes 1,3-diaxial interactions that are alleviated by a leftward bending of the axial-methyl group. For compound **39** this bending of the axial-methyl group also results in a downward movement of the 6(e)-methyl group. Consequently, the unfavorable steric interactions that occurred between the 6(e)-methyl group and the *pro-R* hydrogen of the hydroxymethylene group of compound **37** are not as severe in compound **39** and a large rightward movement of the side chain is not needed to minimize this steric interaction in compound **39**.

In conclusion, the studies reported provide new information regarding the conformations of 6-methyl-substituted 7-(2-hydroxyethyl)benz[e]indenes and the activites of these compounds at $GABA_A$ receptors. Additional studies are needed to explain why the dose–response curves for the two different $GABA_A$ receptor modulatory activites of 7-(2-hydroxyethyl)benz[e]indenes are further separated for these compounds than they are for the anesthetic steroids.

Experimental Section

General Methods. All melting points were determined with a capillary melting point apparatus and are uncorrected. NMR spectra were recorded at ambient temperature in $CDCl_3$ (unless noted otherwise) with a 5-mm probe on either a Varian Gemini-300 operating at 300 (^1H) or 75 MHz (^{13}C). For ^1H 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 with a Perkin-Elmer 1710 FT-IR spectrophotometer. 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-5α-androstan-17-one was purchased from Sigma Chemical Co., St. Louis, MO. 17β-Hydroxyandrostan-3-one was purchased from United States Biochemical Corp., Cleveland, OH. The Econosil HPLC column was purchased from Alltech Associates, Inc., Deerfield, IL.

[3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-6-(2-hydroxyethyl)dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetic Acid (10). A solution of an approximately 2:8 mixture of 3,17β-diacetoxyandrost-3-ene and 3,17β-diacetoxyandrost-2-ene (**4**) (9.7 g, 26.9 mmol) in dichloromethane (400 mL) and acetic acid (30 mL) was treated with O_3 at −78 °C until a blue color persisted. Excess O_3 was discharged by an O_2 stream and then methyl sulfide (ca. 20 μL) was added. The dichloromethane was removed on a rotary evaporator, and water (90 mL) and acetic acid (200 mL) were added to the remaining solution. After the mixture was stirred overnight to hydrolyze the anhydride group generated during ozonolysis, water (200 mL) and diethyl ether (500 mL) were added. The diethyl ether layer was repeatedly washed with water to remove acetic acid and then dried over $MgSO_4$. Solvent removal yielded a slightly yellow solid (10.7 g) that was dissolved in methanol (220 mL), cooled to 0 °C, and reacted with slowly added portions of $NaBH_4$ (14.3 g, 378 mmol). After 15 min, 10% aqueous HCl was added until the solution became acidic. Water (250 mL) was added, and methanol removal on a rotary evaporator was accompanied by the precipitation of the steroid product. The precipitate was filtered and dried by air to give the crude product as a white solid (8.2 g, 87% crude yield). This material was combined with crude product (4.0 g) from a similar smaller scale reaction and recrystallized from acetone to give pure product **10** (7.4 g, 61%) as white crystals: mp 182–184.5 °C; IR 3319, 1247, 1214, 1729, 1692 cm^{-1}; ^1H NMR δ 4.58 (t, J = 10.8 Hz, 1H, C*H*OAc), 3.72 (m, 2H, CH_2OH), 2.62 (d, J = 12.9 Hz, 1H of CH_2COOH), 2.04 (s, 3H, OCOCH_3), 0.77 (s, 6H, CH_3 on C^{3a} and CH_3 on C^6). Anal. ($C_{21}H_{34}O_5$) C, H.

Methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-6-(2-hydroxyethyl)dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (12). To a solution of compound **10** (4.0 g, 10.9 mmol) in ethyl acetate (200 mL) and absolute ethanol (20 mL) was added diazomethane in diethyl ether until a yellow color persisted. The solution was allowed to stir for an additional 15 min. Excess diazomethane was destroyed by addition of several drops of formic acid. The mixture was washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (25 mL). The organic layer was dried over Na_2SO_4. The solvent was removed to give a solid, which was purified by column chromatography (silica gel, 30% ethyl acetate in hexane) to give 3.9 g (94%) of pure product **12** as white crystals: mp 115.5–117.5 °C (from diethyl ether–hexane); IR 3453, 2934, 1732, 1438, 1373, 1246 cm^{-1}; ^1H NMR δ 4.58 (t, J = 8.5 Hz, 1H, C*H*OAc), 3.73 (m, 1H of CH_2OH), 3.68 (s, 3H, COOCH_3), 3.63 (m, 1H of CH_2OH), 2.56 (dd, J = 14.4 Hz, 2.4 Hz, 1H of CH_2COOCH_3), 2.04 (s, 3H, OCOCH_3), 0.77 (s, 6H, CH_3 on C^{3a} and CH_3 on C^6). Anal. ($C_{22}H_{36}O_5$) C, H.

Methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-6-[2(*E*)-(acetyloxy)ethenyl]dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (*E*-16) and Methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-6-[2(*Z*)-(acetyloxy)ethenyl]-dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (*Z*-16). A solution of compound **12** (4.7 g, 12.3 mmol) in dichloromethane (50 mL) was added rapidly to a suspension of pyridinium chlorochromate (10.8 g, 50.2 mmol) in dichloromethane and stirred at room temperature under nitrogen. After 3 h, the volume of dichloromethane was reduced to 40 mL on a rotary evaporator and then poured into diethyl ether (750 mL). The diethyl ether was passed through a small column of Florisil, and the Florisil was washed with additional diethyl ether. The solvent was removed on a rotary evaporator to give crude methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(acetyloxy)-6-(2-oxoethyl)-dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (**14**) as an oil (4.6 g) which was used immediately without further purification or characterization.

A solution of crude compound **14** (4.6 g) and *p*-toluenesulfonic acid (0.5 g) in isopropenyl acetate (60 mL) was refluxed for 16 h. The solution was gently distilled for 1 h until about 10 mL of distillate was collected and then cooled to room temperature. The solution was poured into dichloromethane and washed with water (50 mL), 5% aqueous $NaHCO_3$ (50 mL), water (3 × 100 mL), and dried over $MgSO_4$. The solvent was removed on a

rotary evaporator to give slightly yellow crystals (5.6 g), which were purified by chromatography (silica gel eluted with 4% ethyl acetate in dichloromethane) to give 3.7 g of product. Recrystallization from ethyl acetate–hexane gave 1.83 g (36%) of a mixture of the *E* and *Z* enol acetates. The enol acetate isomers were separated by HPLC (Econosil 5 μm, 250 mm × 4.6 mm, eluted at 2.0 mL/min with 10% ethyl acetate in hexane).

The major 6-[2(*E*)-(acetyloxy)ethenyl] isomer *E*-**16** had experimental data as follows: mp 191–192 °C; IR 2918, 1756, 1733, 1666, 1451, 1373, 1227 cm^{-1}; ^{1}H NMR δ 6.98 (d, *J* = 12.7 Hz, 1H, AcOC*H*=CH), 5.15 (d, *J* = 12.7 Hz, 1H, AcOCH=C*H*), 4.56 (t, *J* = 8.5 Hz, 1H, C*H*OAc), 3.63 (s, 3H, $COOCH_3$), 2.41 (dd, *J* = 15.2 Hz, *J* = 2.8 Hz, 1H of CH_2COOCH_3), 2.03 (s, 3H, $OCOCH_3$), 2.11 (s, 3H, $OCOCH_3$), 0.87 (s, 3H, CH_3 on C^6), 0.76 (s, 3H, CH_3 on C^{3a}). Anal. ($C_{24}H_{36}O_6$) C, H.

The minor 6-[2(*Z*)-(acetyloxy)ethenyl] isomer *Z*-**16** had experimental data as follows: mp 109.5–112 °C; IR 2936, 1759, 1735, 1668, 1437, 1371, 1246, 1217 cm^{-1}; ^{1}H NMR δ 6.70 (d, *J* = 7.6 Hz, 1H, AcOC*H*=CH), 4.58 (t, *J* = 8.5 Hz, 1H, C*H*OAc), 4.39 (d, *J* = 7.5 Hz, 1H, AcOCH=C*H*), 3.64 (s, 3H, $COOCH_3$), 2.44 (d, *J* = 14.3 Hz, 1H of CH_2COOCH_3), 2.11 (s, 3H, $OCOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 1.06 (s, 3H, CH_3 on C^6), 0.79 (s, 3H, CH_3 on C^{3a}). Anal. ($C_{24}H_{36}O_6$) C, H.

Methyl [3*S*-(3α,3aα,5aβ,6α,7α,9aα,9bβ)]-3-(Acetyloxy)-dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (20). A solution of compound **16** (200 mg, 0.48 mmol) in dichloromethane (25 mL) and HOAc (0.5 mL) was treated with O_3 at −78 °C until a blue color persisted. The excess O_3 was removed with a stream of O_2 and methyl sulfide (ca. 20 μL) was added. Additional dichloromethane (75 mL) was added and the solution was washed with water, 5% aqueous $NaHCO_3$, and water and dried over Na_2SO_4. The solvent was removed to give the crude methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(acetyloxy)-6-formyldodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (**18**) as a yellow oil (158 mg), which was used immediately without purification or characterization.

Crude aldehyde **18** and Wilkinson's catalyst (3 × 500 mg, added in equal portions, initially, and after 72 and 120 h) in benzonitrile (25 mL) was heated to 150–180 °C for 144 h under nitrogen. After cooling, ethyl acetate (50 mL) was added and the mixture was filtered. Removal of the solvents from the filtrate yielded a brown sludge (2.0 g) which was chromatographed (silica gel eluted successively with 2.5%, 5.0%, and 7.5% ethyl acetate in hexane) to give 25.4 mg (17.5%) of pure product **20** as a white solid: mp 61–63 °C; IR 2922, 1737, 1437, 1373, 1248 cm^{-1}; ^{1}H NMR δ 4.61 (t, *J* = 7.8 Hz, 1H, C*H*OAc), 3.67 (s, 3H, $COOCH_3$), 2.56 (s, 3H, $OCOCH_3$), 0.78 (s, 3H, CH_3 on C^{3a}). Anal. ($C_{20}H_{32}O_4$) C, H.

17β-(Acetyloxy)-3-oxa-*A*-homo-5α-androstan-4-one (21). A mixture of compound **12** (378 mg, 1.0 mmol) and Raney nickel (1.2 g) in toluene (10 mL) was refluxed for 3.5 h under nitrogen. The mixture was cooled and filtered, and the Raney nickle was washed with EtOH (10 mL). The filtrates were combined and evaporated to give a solid which was recrystallized from EtOH to give pure compound **21** as white crystals (335 mg, 96.8%): mp 230–233 °C (lit.[34] mp 218–223 °C); IR 2924, 2846, 1728, 1452, 1374, 1259 cm^{-1}; ^{1}H NMR δ 4.57 (t, *J* = 8.5 Hz, 1H, C*H*OAc), 4.27 (t, *J* = 13 Hz, 1H of $COOCH_2$), 4.16–4.18 (m, 1H of $COOCH_2$), 2.85 (dd, *J* = 11.4 Hz, *J* = 2.9 Hz, 1H of CH_2COO), 2.02 (s, 3H, $OCOCH_3$), 0.93 (s, 3H, CH_3 on C^{19}), 0.77 (s, 3H, CH_3 on C^{18}); ^{13}C NMR: δ 175.80 (CH_2COO), 171.10 (CH_3COO), 82.54 (C^3), 64.55 ($COOCH_2$), 12.06 (CH_3 on C^{18}or C^{19}), 12.03 (CH_3 on C^{18}or C^{19}), 53.62, 50.47, 43.26, 42.34, 41.61, 37.89, 37.46, 36.70, 34.33, 31.29, 30.30, 27.45, 23.37, 21.13, 20.73 ppm. Anal. ($C_{21}H_{32}O_4$) C, H.

Methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-dodecahydro-6-(1,3-dithiolan-2-yl)-3a-methyl-1*H*-benz[*e*]indene-7-acetate (22). A solution of compound **17** (1.7 g, 5.0 mmol) and ethanedithiol (690 mg, 7.5 mmol) in dry dichloromethane (20 mL) was treated with boron trifluoride etherate (1.0 mL). After the mixture was stirred for 2 h at room temperature, additional dichloromethane (50 mL) was added and the mixture was washed with cold 10% aqueous NaOH (50 mL) and brine (50 mL) and dried over Na_2SO_4. Evaporation of the solvent gave an oil, which was purified by chromatography (silica gel eluted with 15% EtOAc in hexane) to yield compound **22** as a pure colorless oil (1.75 g, 85%): IR 2923, 2851, 1734, 1435, 1373, 1245, 1036 cm^{-1}, ^{1}H NMR δ 5.00 (s, 1H, SCHS), 4.54 (t, *J* = 8.4 Hz, 1H, C*H*OAc), 3.62 (s, 3H, $COOCH_3$), 2.00 (s, 3H, $OCOCH_3$), 0.76 (s, 3H, CH_3); ^{13}C NMR δ 173.4 ($COOCH_3$), 170.98 (CH_3COO), 82.58 (C^3), 55.77 (SCHS), 37.58 (SCH_2CH_2S), 11.92 (C^{3a}), 51.22, 49.81, 47.24, 45.91, 42.22, 40.53, 39.96, 37.64, 36.68, 31.92, 29.22, 27.37, 26.64, 23.23.

Methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-dodecahydro-6-(1,3-dithiolan-2-yl)-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (23). To a solution of crude aldehyde **18** prepared by ozonolysis (see preparation of compound **20** for details) of compound **16** (1.2 g, 2.7 mmol) was added ethanedithiol (2.0 mL) and boron trifluoride etherate (1.0 mL). After the mixture was stirred for 1 h at room temperature, dichloromethane (50 mL) was added and the solution was washed with cold 10% aqueous NaOH (100 mL) and brine (100 mL), and dried over Na_2SO_4. Evaporation of the solvent give an oil, which was purified by chromatography (silica gel eluted with 25% EtOAc in hexane) to yield compound **23** as a pure colorless oil (1.10 g, 88%): IR 2927, 2851, 1734, 1435, 1373, 1293, 1039 cm^{-1}; ^{1}H NMR δ 4.92 (s, 1H, SCHS), 4.43 (t, *J* = 8.4 Hz, 1H, C*H*OAc), 3.51 (s, 3H, $COOCH_3$), 1.89 (s, 3H, $OCOCH_3$), 0.93 (s, 3H, CH_3 on C^6), 0.76 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR δ 173.67 ($COOCH_3$), 170.41 (CH_3COO), 82.13 (C^3), 62.87 (SCHS), 37.61 (SCH_2CH_2S), 15.03 (CH_3 on C^6), 11.92 (CH_3 on C^{3a}), 50.98, 50.57, 50.18, 41.62, 39.61, 36.91, 36.49, 35.48, 30.26, 28.54, 27.12, 23.17, 21.28, 20.71.

Methyl [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-(Acetyloxy)-dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-acetate (24). A suspension of compound **22** (1.75 g, 4.1 mmol), Raney nickle (20 g, thoroughly washed with 3 × 30 mL of distillated water and 3 × 50 mL of absolute EtOH) in absolute ethanol (100 mL) was refluxed for 3 h under nitrogen. Then the mixture was filtered, and the Raney nickle was washed with EtOH (2 × 30 mL). The combined solution was evaporated to give an oil, which was purified by chromatography (silica gel eluted with 1:1:8 EtOAc/CH_2Cl_2/hexane) to yield pure product **24** (1.1 g, 80%) as white crystals which had: mp 83–85 °C (from EtOEt–hexane); IR 2922, 2862, 1739, 1437, 1372, 1246, 1041 cm^{-1}; ^{1}H NMR δ 4.59 (t, *J* = 8.5 Hz, 1H, C*H*OAc), 3.66 (s, 3H, $COOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 0.79 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR δ 174.10 ($COOCH_3$), 171.17 (CH_3COO), 82.83 (C^3), 16.42 (CH_3 on C^6), 12.01 (CH_3 on C^{3a}), 51.36, 49.84, 49.33, 42.49, 41.46, 41.06, 40.52, 39.70, 36.92, 32.24, 30.15, 27.50, 26.07, 23.25, 21.16. Anal. ($C_{20}H_{32}O_4$) C, H.

Methyl [3*S*-(3α,3aα,5aβ,7α,9aα,9bβ)]-3-(Acetyloxy)-dodecahydro-3a,6,6-trimethyl-1*H*-benz[*e*]indene-7-acetate (25). By using the same desulfurization procedure as above, a solid was obtained from compound **23** (1.1 g, 2.5 mmol) which was purified by chromatography (silica gel eluted with 4% EtOAc in hexane) to yield pure product **25** (675 mg, 77%) as white crystals: mp 98.5–99.5 °C (from EtOEt–hexane); IR 2943, 2877, 2850, 1733, 1437, 1371, 1246, 1036 cm^{-1}; ^{1}H NMR δ 4.58 (t, *J* = 7.9 Hz, 1H, C*H*OAc), 3.66 (s, 3H, $COOCH_3$), 2.03 (s, 3H, $OCOCH_3$), 0.91 (s, 3H, ax-CH_3 on C^6), 0.77 (s, 3H, CH_3 on C^{3a}), 0.72 (s, 3H, eq-CH_3 on C^6); ^{13}C NMR δ 174.49 ($COOCH_3$), 171.01 (CH_3COO), 82.68 (C^3), 21.27 (eq-CH_3 on C^6), 14.67 (ax-CH_3 on C^6), 11.93 (CH_3 on C^{3a}), 52.89, 51.37, 50.65, 44.60, 42.21, 36.90, 36.20, 35.81, 35.21, 31.00, 27.95, 27.47, 26.39, 23.38, 21.06. Anal. ($C_{21}H_{34}O_4$) C, H.

[3*S*-(3α,3aα,5aβ,7α,9aα,9bβ)]-3-Hydroxydodecahydro-3a-methyl-1*H*-benz[*e*]indene-7-ethanol (26). To a stirred solution of compound **19** (0.65 g, 2.0 mmol) in dry dichloromethane (50 mL) was added diisobutyl aluminum hydride (1.0 M solution in toluene, 12 mL, 12 mmol) at 0 °C. After 2.0 h, dichloromethane–methanol (1:1, 4 mL) and then 10% aqueous HCl (10 mL) were added. The mixture was washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a solid, which was recrystallized from ethyl alcohol to yield pure product **26** (0.47g, 93%) as white crystals: mp 145–147 °C; IR 3279, 2914, 2870, 2858, 2841, 1469, 1443, 1381, 1348, 1067, 1056 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.73–3.65 (m, 3H, CH_2OH and C*H*OH), 0.76 (s, 3H, CH_3); ^{13}C NMR (CD_3OD) δ 82.63 (C^3), 60.78 (CH_2OH), 11.71 (C^{3a}), 51.36, 45.35, 44.84, 42.91, 41.29, 41.23, 37.98, 35.64, 34.28, 31.72, 30.74, 30.56, 23.93. Anal. ($C_{16}H_{28}O_2$) C, H.

[3*S*-(3α,3aα,5aβ,6α,7α,9aα,9bβ)]-3-Hydroxydodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-ethanol (27). By using the procedure reported for the preparation of compound **26**, a solid was obtained from compound **20** (294 mg, 1.0 mmol) which was

purified by chromatography (silica gel eluted with 2:2:1 EtOAc/hexane/CH_2Cl_2) to yield pure product **23** (253 mg, 95%) as white crystals: mp 126–128 °C (from CH_2Cl_2–hexane); IR 3343, 2920, 2872, 1445, 1386, 1055, 1028 cm^{-1}; ^1H NMR δ 3.69–3.61 (m, 3H, CH_2OH and CHOH), 0.76 (s, 3H, CH_3 on C^6), 0.73 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR δ 81.87 (C^3), 61.10 (CH_2OH), 11.06 (CH_3 on C^{3a}), 7.41 (CH_3 on C^6), 50.84, 48.19, 43.38, 38.58, 37.29, 36.73, 36.11, 33.83, 31.50, 30.46, 26.48, 26.19, 23.23. Anal. ($C_{17}H_{30}O_2$) C, H.

[3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-3-Hydroxydodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-7-ethanol (28). By using the procedure reported for the preparation of compound **26**, a solid was obtained from compound **24** (1.0 g, 3.0 mmol), which was purified by chromatography (silica gel eluted with 50% EtOAc in hexane) to yield pure product **24** (756 mg, 96%) as white crystals: mp 133–134.5 °C (from EtOH–EtOEt); IR 3339, 2918, 2865, 1445, 1379, 1066, 1029 cm^{-1}; ^1H NMR δ 3.73–3.61 (m, 3H, CH_2OH and CHOH), 0.75 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR (CD_3OD) δ 82.54 (C^3), 60.99 (CH_2OH), 17.01 (CH_3 on C^6), 11.73 (CH_3 on C^{3a}), 51.58, 51.44, 49.56, 44.05, 43.02, 42.34, 42.01, 38.18, 38.11, 33.08, 31.72, 30.67, 27.59, 24.16. Anal. ($C_{17}H_{30}O_2$) C, H.

[3*S*-(3α,3aα,5aβ,7α,9aα,9bβ)]-3-Hydroxydodecahydro-3a,6,6-trimethyl-1*H*-benz[*e*]indene-7-ethanol (29). By using the procedure reported for the preparation of compound **26**, a solid was obtained from **25** (600 mg, 1.7 mmol), which was purified by chromatography (silica gel eluted with 50% EtOAc in hexane) to yield pure product **29** (470 mg, 98%) as white crystals: mp 175–177 °C (from EtOH–EtOEt); IR 3358, 2938, 2869, 1471, 1367, 1054 cm^{-1}; ^1H NMR δ 3.71–3.55 (m, 3H, CH_2OH and CHOH), 0.91 (s, 3H, eq-CH_3 on C^6), 0.73 (s, 3H, ax-CH_3 on C^6), 0.72 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR (CD_3OD) δ 82.49 (C^3), 62.31 (CH_2OH), 22.73 (eq-CH_3 on C^6), 15.24 (ax-CH_3 on C^6), 11.66 (CH_3 on C^{3a}), 55.08, 52.47, 45.57, 43.84, 38.23, 37.06, 34.90, 32.70, 30.70, 29.03, 27.02, 24.32. Anal. ($C_{18}H_{32}O_2$) C, H.

[3a*S*-(3aα,5aβ,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)dodecahydro-3a-methyl-3*H*-benz[*e*]inden-3-one (30). To a stirred solution of compound **26** (254 mg, 1.0 mmol) in glacial acetic acid (5 mL) was added dropwise at room temperature over ~10 min a 5.25% aqueous solution of sodium hypochlorite (1.5 mL, 1.05 mmol). After 1.0 h, 2-propanol (2.0 mL) was added to quench any excess oxidant and water (5.0 mL) was added. The mixture was extracted with EtOAc (2 × 25 mL). The combined organic layer was washed with water (25 mL), saturated aqueous $NaHCO_3$ (25 mL), water (25 mL), and brine (25 mL) and dried over Na_2SO_4. The solvent was removed to give an oil, which was purified by column chromatography (silica gel eluted with 1:1 ethyl acetate–hexane) to give pure product **30** (161 mg, 64%) as white crystals: mp 38–40 °C (from EtOEt–hexane); IR: 3435, 2917, 1739, 1452, 1406, 1373, 1258, 1097, 1046 cm^{-1}; ^1H NMR δ 3.71 (t, J = 6.5 Hz, 2H, CH_2OH), 0.87 (s, 3H, CH_3); ^{13}C NMR δ 222.05 (C=O), 60.07 (CH_2OH), 13.44 (C^{3a}), 50.15, 48.15, 43.55, 10.10, 39.80, 39.34, 35.46, 33.88, 32.46, 31.13, 29.38, 28.63, 21.06. Anal. ($C_{16}H_{28}O_2$) C, H.

[3a*S*-(3aα,5aβ,6α,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)dodecahydro-3a,6-dimethyl-3*H*-benz[*e*]inden-3-one (31). By using the procedure reported for the preparation of compound **30**, an oil was obtained from **27** (1.6 g, 6.0 mmol) which was purified by column chromatography (silica gel eluted with 50% ethyl acetate in hexane) to give pure product **31** (1.2 g, 76%) as a colorless oil: IR 3435, 2922, 2860, 1740, 1455, 1407, 1387, 1053, 1004 cm^{-1}; ^1H NMR δ 3.58 (t, J = 6.8 Hz, 2H, CH_2OH), 0.80 (s, 3H, CH_3 on C^{3a}), 0.71 (s, 3H, CH_3 on C^6); ^{13}C NMR δ 221.55 (C=O), 60.53 (CH_2OH), 13.55 (CH_3 on C^{3a}), 7.19 (CH_3 on C^6), 51.16, 48.01, 38.32, 36.92, 35.81, 35.68, 33.13, 31.40, 30.61, 26.07, 25.58, 21.47. Anal. ($C_{17}H_{28}O_2$) C, H.

[3a*S*-(3aα,5aβ,6β,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)dodecahydro-3a,6-dimethyl-3*H*-benz[*e*]inden-3-one (32). By using the procedure reported for the preparation of compound **30**, an oil was obtained from **28** (700 mg, 2.63 mmol) which was purified by column chromatography (silica gel eluted with 50% EtOAc in hexane) to give pure product **32** (489 mg, 70%) as a colorless oil: IR 3433, 2921, 2863, 1740, 1454, 1407, 1376, 1050 cm^{-1}; ^1H NMR δ 3.73–3.64 (m, 2H, CH_2OH), 0.93 (s, 3H, CH_3 on C^6), 0.88 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR δ 221.61 (C=O), 60.92 (CH_2OH), 16.46 (CH_3 on C^6), 13.74 (CH_3 on C^{3a}), 50.58, 49.84, 47.73, 41.55, 40.56, 40.25, 37.09, 35.85, 31.69, 31.60, 29.67, 25.89, 21.56. Anal. ($C_{17}H_{28}O_2$) C, H.

[3a*S*-(3aα,5aβ,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)dodecahydro-3a,6,6-trimethyl-3*H*-benz[*e*]inden-3-one (33). By using the procedure reported for the preparation of compound **30**, a solid was obtained from **29** (450 mg, 1.6 mmol) which was purified by column chromatography (silica gel eluted with 40% EtOAc in hexane) to give pure product **33** (335 mg, 75%) as white crystals: mp 150–151 °C; IR 3430, 2942, 1739, 1472, 1454, 1373, 1056 cm^{-1}; ^1H NMR δ 3.72–3.57 (m, 2H, CH_2OH), 0.92 (s, 3H, eq-CH_3 on C^6), 0.86 (s, 3H, CH_3 on C^{3a}), 0.74 (s, 3H, ax-CH_3 on C^6); ^{13}C NMR δ 221.58 (C=O), 61.97 (CH_2OH), 21.10 (eq-CH_3 on C^6), 14.58 (ax-CH_3 on C^6), 13.59 (CH_3 on C^{3a}), 53.36, 51.35, 47.47, 44.06, 36.07, 35.80, 34.98, 33.79, 31.55, 30.55, 27.56, 26.30, 21.62. Anal. ($C_{18}H_{30}O_2$) C, H.

[3*S*-(3α,3aα,5aβ,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)dodecahydro-3a-methyl-1*H*-benz[*e*]indene-3-carbonitrile (2) and [3*R*-(3α,3aβ,5aα,7β,9aβ,9bα)]-7-(2-Hydroxyethyl)dodecahydro-3a-methyl-1*H*-benz[*e*]indene-3-carbonitrile (34). To a stirred solution of compound **30** (320 mg 1.28 mmol) in dimethoxyethane (32 mL) and ethanol (2.0 mL) at room temperature was added a 1.0 M solution of t-BuOK in dimethoxyethane (12.8 mL, 12.8 mmol). A solution of tosylmethyl isocyanide (500 mg, 2.56 mmol) in dimethoxyethane (6.5 mL) was slowly (ca. 20 min) added from a syringe. After 3.0 h, the mixture was quenched with water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with water (2 × 50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was removed to yield an oil which was purified by column chromatography (silica gel eluted with 10% acetonitrile in dichloromethane) to give a 6:4 mixture (200 mg, 60%) of products **2** and **34**, respectively, as a colorless oil. The isomers were separated by HPLC (Ultrasphere-Si, 5 μm, 250-mm × 10-mm column eluted with 30% ethyl acetate in hexane at 3.0 mL/min).

The 3*S* isomer, compound **2**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (90 mg, 27%): mp 82–83 °C; IR 3294, 2917, 2853, 2233, 1470, 1384, 1338, 1056, 1021 cm^{-1}; ^1H NMR δ 3.64–3.61 (m, 2H, CH_2OH), 2.25 (t, J = 9.5 Hz, 1H, CHCN), 0.88 (s, 3H, CH_3); ^{13}C NMR δ 121.43 (CN), 60.30 (CH_2OH), 14.05 (CH_3), 53.12, 44.71, 43.22, 41.34, 39.95, 39.87, 39.29, 36.76, 33.88, 32.62, 30.54, 29.03, 26.17, 23.79. Anal. ($C_{17}H_{27}NO$) C, H, N.

The 3*R* isomer, compound **34**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (55 mg, 17%): mp 44–46 °C; IR 3361, 2920, 2858, 2233, 1474, 1450, 1384, 1211, 1168, 1095 cm^{-1}; ^1H NMR δ 3.66 (t, J = 6.7 Hz, 2H, CH_2OH), 2.55 (dd, J = 6.8 Hz, J = 2.0 Hz, 1H, CHCN), 0.81 (s, 3H, CH_3); ^{13}C NMR δ 122.48 (CN), 60.55 (CH_2OH), 17.87 (CH_3), 50.84, 44.71, 42.83, 41.43, 40.04, 39.82, 39.49, 34.85, 33.95, 32.76, 30.76, 29.17, 26.98, 24.20. Anal. ($C_{17}H_{27}NO$) C, H, N.

[3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)-dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-3-carbonitrile (35). By using the procedure reported for the preparation of compounds **2** and **34**, a yellowish gum was obtained from **31** (900 mg, 3.4 mmol) which was purified by column chromatography (silica gel eluted with dichloromethane) to yield a solid. The ^{13}C NMR spectra showed only the 3*S* isomer, compound **35**, was present. The compound was purified further by HPLC (Ultrasphere-Si, 5 μm, 250-mm × 10-mm column eluted with 30% ethyl acetate in hexane at 3.0 mL/min) and recrystallized from EtOEt and hexane to yield product **32** (338 mg, 36.0%) as white crystals: mp 96–98 °C; IR 3402, 2922, 2877, 1450, 1389, 1056 cm^{-1}; ^1H NMR δ 3.61 (t, J = 6.7 Hz, 2H, CH_2OH), 2.67 (s, 1H, OH), 2.28 (t, J = 9.6 Hz, 1H, CHCN), 0.90 (s, 3H, CH_3 on C^{3a}), 0.76 (d, J = 39.9 Hz, 3H, CH_3 on C^6); ^{13}C NMR δ 121.11 (CN), 60.29 (CH_2OH), 13.99 (CH_3 on C^{3a}), 7.10 (CH_3 on C^6), 53.90, 47.48, 44.48, 39.81, 38.12, 36.85, 36.77, 35.57, 33.86, 31.56, 26.21, 26.05, 25.84, 24.11. Anal. ($C_{18}H_{29}NO$) C, H, N.

[3*R*-(3α,3aβ,5aα,6α,7β,9aβ,9bα)]-7-(2-Hydroxyethyl)-dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-3-carbonitrile (36) and [3*S*-(3α,3aα,5aβ,6β,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)dodecahydro-3a,6-dimethyl-1*H*-benz[*e*]indene-3-carbonitrile (37). By using the procedure reported for the preparation of compounds **2** and **34**, a yellowish gum was obtained from **32** (450 mg, 1.7 mmol) which was purified by column chromatography (silica gel eluted with 10% acetonitrile in dichloromethane) to give a 1:1 mixture (125 mg, 27%) of products **36** and **37** as a colorless oil. The mixture was separated by HPLC

(Ultrasphere-Si, 5 μm, 250-mm × 10-mm column eluted with 30% EtOAc in hexane at 3.0 mL/min).

The 3*R* isomer, compound **36**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (62 mg, 13%): mp 131–133 °C; IR 3433, 2922, 2863, 2233, 1473, 1451, 1383, 1058, 1035 cm^{-1}; ^1H NMR δ 3.66–3.57 (m, 2H, CH_2OH), 2.54 (dd, J = 7.0, J = 2.0 Hz, 1H, CHCN), 0.79 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR δ 122.19 (CN), 60.71 (CH_2OH), 17.94 (CH_3 on C^{3a}), 16.38 (CH_3 on C^6), 51.22, 48.98, 44.11, 41.41, 41.05, 40.40, 39.96, 37.03, 35.10, 31.79, 30.92, 27.18, 26.26, 24.56. Anal. ($C_{18}H_{29}NO$) C, H, N.

The 3*S* isomer, compound **37**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (62 mg, 13%): mp 123–125 °C; IR 3481, 2920, 2867, 2236, 1462, 1450, 1386, 1336, 1055 cm^{-1}; ^1H NMR δ 3.71–3.62 (m, 2H, CH_2OH), 2.27 (t, J = 9.5 Hz, 1H, CHCN), 0.92 (s, 3H, CH_3 on C^{3a}); ^{13}C NMR δ 121.33 (CN), 60.73 (CH_2OH), 16.39 (CH_3 on C^6), 14.21 (CH_3 on C^{3a}), 53.52, 49.36, 41.37, 41.04, 40.38, 40.18, 37.11, 36.95, 31.69, 30.75, 26.47, 26.19, 24.23. Anal. ($C_{18}H_{29}NO$) C, H, N.

[3*R*-(3α,3aβ,5aα,7β,9aβ,9bα)]-7-(2-Hydroxyethyl)dodecahydro-3a,6,6-trimethyl-1*H*-benz[*e*]indene-3-carbonitrile (38) and [3*S*-(3α,3aα,5aβ,7α,9aα,9bβ)]-7-(2-Hydroxyethyl)-dodecahydro-3a,6,6-trimethyl-1*H*-benz[*e*]indene-3-carbonitrile (39). By using the procedure reported for the preparation of compounds **2** and **34**, a yellowish solid was obtained from **33** (332 mg, 1.7 mmol) which was purified by column chromatography (silica gel eluted with 10% acetonitrile in dichloromethane) to give a 3:7 mixture (220 mg, 63%) of products **38** and **39**, respectively, as a solid. The mixture was separated by HPLC (Ultrasphere-Si, 5 μm, 250-mm × 10-mm column eluted with 30% EtOAc in hexane at 3.0 mL/min).

The 3*R* isomer, compound **38**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (70 mg, 20%): mp 157–159 °C; IR 3469, 2924, 2876, 2235, 1475, 1451, 1387, 1367, 1055 cm^{-1}; ^1H NMR δ 3.67–3.54 (m, 2H, CH_2OH), 2.54 (dd, J = 7.0 Hz, J = 2.0 Hz, 1H, CHCN), 0.90 (s, 3H, eq-CH_3 on C^6), 0.78 (s, 3H, CH_3 on C^{3a}), 0.69 (s, 3H, ax-CH_3 on C^6); ^{13}C NMR δ 122.20 (CN), 61.95 (CH_2OH), 21.51 (eq-CH_3 on C^6), 17.82 (CH_3 on C^{3a}), 14.54 (ax-CH_3 on C^6), 52.48, 52.04, 43.89, 39.81, 35.89, 35.77, 35.09, 33.78, 31.77, 27.68, 27.16, 26.30, 24.77. Anal. ($C_{19}H_{31}NO$) C, H, N.

The 3*S* isomer, compound **39**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (150 mg, 43%): mp 175.5–177.5 °C; IR 3508, 2942, 2904, 2845, 2233, 1474, 1448, 1436, 1389, 1368, 1066 cm^{-1}; ^1H NMR δ 3.67–3.52 (m, 2H, CH_2OH), 2.42 (s, 1H, OH), 2.27 (t, J = 9.6 Hz, 1H, CHCN), 0.91 (s, 3H, eq-CH_3 on C^6), 0.88 (s, 3H, CH_3 on C^{3a}), 0.72 (s, 3H, ax-CH_3 on C^6); ^{13}C NMR δ 121.16 (CN), 61.65 (CH_2OH), 21.38 (eq-CH_3 on C^6), 14.47 (ax-CH_3 on C^6), 14.02 (CH_3 on C^{3a}), 54.19, 52.82, 43.96, 43.80, 39.92, 37.01, 35.81, 35.69, 33.60, 31.53, 27.49, 26.34, 26.18, 24.31. Anal. ($C_{17}H_{31}NO$) C, H, N.

3α-Hydroxy-5α-estran-17-one (41). To a cooled (−78 °C), stirred solution of 5α-estrane-3,17-dione (**40**, 274 mg, 1.0 mmol) in dry THF (20 mL) under nitrogen was added a solution of K-Selectride (1.0 M solution in THF, 2.0 mL, 2.0 mmol). The reaction was monitored by TLC (silica gel plate eluted with 30% EtOAc in hexane). After 1.5 h, the reaction was stopped by the addition of 10% aqueous NaOH (10 mL) followed by 30% H_2O_2 (10 mL). The reaction mixture was allowed to warm to room temperature and stirring was continuned for another half hour. The mixture was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (2 × 50 mL) and dried over Na_2SO_4. Evaporation of solvent gave a white solid which was recrystallized from EtOAc to give compound **38** (237 mg, 86%) as white crystals: mp 167–168.5 °C; IR 3309, 2916, 2859, 1724 cm^{-1}; ^1H NMR δ 4.06 (t, J = 2.8 Hz, 1H, CHOH), 0.84 (s, 3H, CH_3); ^{13}C NMR δ 221.63 (C=O), 66.19 (CHOH), 13.73 (CH_3), 50.55, 48.12, 47.83, 46.89, 40.63, 40.40, 35.87, 35.78, 33.31, 32.83, 31.44, 29.72, 24.80, 23.60, 21.54. Anal. ($C_{18}H_{28}O_2$) C, H.

3α-Hydroxy-5α-estrane-17β-carbonitrile (43) and 3α-Hydroxy-5α-estrane-17α-carbonitrile (44). By using the procedure reported for the preparation of compounds **2** and **34**, a yellowish solid was obtained from compound **41** (415 mg, 1.5 mmol) which was purified by column chromatography (silica gel eluted with 30% EtOAc in hexane) to give a 1.2:1 mixture (228 mg, 52%) of products **43** and **44**, respectively, as a solid. The mixture was separated by HPLC (Ultrasphere-Si, 5 μm, 250-mm × 10-mm column eluted with 30% EtOAc in hexane at 3.0 mL/min).

The 17β-carbonitrile, product **43**, was obtained as a solid which was recrystallized from EtOAc as white crystals (118 mg, 27%): mp 193–195 °C; IR 3503, 2917, 2237, 1446, 1386 cm^{-1}; ^1H NMR δ 4.06 (t, J = 2.7 Hz, 1H, CHOH), 2.26 (t, J = 9.6 Hz, 1H, CHCN), 0.89 (s, 3H, CH_3); ^{13}C NMR δ 121.34 (CN), 66.15 (CHOH), 14.23 (CH_3), 47.68, 46.74, 44.42, 41.46, 40.34, 40.22, 36.98, 35.75, 33.35, 32.79, 30.83, 26.42, 25.13, 24.25, 23.58. Anal. ($C_{19}H_{29}NO$) C, H, N.

The 17α-carbonitrile, product **44**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (98 mg, 23%): mp 138–139 °C; IR 3468, 2919, 2862, 2233, 1446, 1384 cm^{-1}; ^1H NMR δ 3.98 (t, J = 2.7 Hz, 1H, CHOH), 2.50 (dd, J = 7.0 Hz, J = 2.0 Hz, 1H, CHCN), 0.74 (s, 3H, CH_3); ^{13}C NMR δ 122.05 (CN), 65.76 (CHOH), 17.79 (CH_3), 51.03, 47.07, 46.65, 44.05, 41.28, 40.22, 39.77, 35.46, 34.81, 33.27, 32.61, 30.83, 26.95, 25.02, 24.43, 23.42. Anal. ($C_{19}H_{29}NO$) C, H, N.

3α-Hydroxy-5α-androstane-17β-carbonitrile (4) and 3α-Hydroxy-5α-androstane-17α-carbonitrile (45). By using the procedure reported for the preparation of compounds **2** and **34**, a yellowish solid was obtained from compound **42** (436 mg, 1.5 mmol) which was purified by column chromatography (silica gel eluted with 30% EtOAc in hexane) to give a 1.5:1 mixture (267 mg, 59%) of products **4** and **45**, respectively, as a solid. The mixture was separated by HPLC (Ultrasphere-Si, 5 μm, 250-mm × 10-mm column eluted with 30% ethyl acetate in hexane at 3.0 mL/min).

The 17β-carbonitrile, product **4**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (160 mg, 35%): mp 159–160.5 °C (lit.[14] mp 156–158 °C); IR 3435, 2923, 2236, 1449, 1387, 1267, 1166 cm^{-1}; ^1H NMR δ 3.99 (t, J = 2.5 Hz, 1H, CHOH), 2.23 (t, J = 9.6 Hz, 1H, CHCN), 0.86 (s, 3H, 18-CH_3), 0.74 (s, 3H, 19-CH_3); ^{13}C NMR δ 121.27 (CN), 66.09 (CHOH), 14.20 (18-CH_3), 11.03 (19-CH_3), 44.26, 40.37, 38.80, 36.97, 35.93, 35.70, 35.56, 32.00, 31.77, 28.76, 28.15, 26.37, 24.37, 20.26.

The 17α-carbonitrile, product **45**, was obtained as a solid which was recrystallized from EtOEt and hexane as white crystals (107 mg, 24%): mp 183–185 °C (lit.[14] mp 183–185 °C); IR 3480, 2926, 2234, 1450, 1386, 1072 cm^{-1}; ^1H NMR δ 4.02 (t, J = 2.5 Hz, 1H, CHOH), 2.54 (dd, J = 7.0 Hz, J = 2.0 Hz, 1H, CHCN), 0.78 (s, 3H, 18-CH_3), 0.76 (s, 3H, 19-CH_3); ^{13}C NMR δ 122.24 (CN), 66.24 (CHOH), 17.98 (18-CH_3), 11.06 (19-CH_3), 53.41, 52.04, 44.14, 39.88, 38.77, 35.97, 35.73, 35.02, 32.03, 31.96, 28.82, 28.24, 27.15, 24.70, 20.35.

Electrophysiology. Hippocampal cultures were prepared from 1–2 day old albino rat pups and maintained as described previously.[35] 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 (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 10 *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES), and 0.001 tetrodotoxin (TTX) with 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.[30] 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 (in mM): 140 CsCl, 4 NaCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, and 5 ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) with pH adjusted to 7.3 using CsOH. Currents were filtered at 1.5 kHz and were digitized at 0.25 kHz using pCLAMP V 5.5 (Axon Instruments). Data were analyzed using pCLAMP V 5.5, Sigmaplot V 4.0 and routines written in Axobasic. The data in this paper represents 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 100–500-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 pipet contents.

Molecular Modeling. 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. Conformational searches were carried out on steroids starting from an initial minimum energy conformation with rotations in 120° increments for the carbon–oxygen bond of the 3α-hydroxyl group. Conformations generated in this way were then minimized using the Powell minimization option (without charges) in the Sybyl program. Conformational searches for the benz[e]indenes were similar except that rotations about the C^7–$C^{1'}$ bond (i.e., the bond connecting the side chain to the ring) were carried out in ~60° increments, whereas the $C^{1'}$–$C^{2'}$ and $C^{2'}$–O bonds in the side chain were rotated in ~120° increments. Conformational searches for the benz[e]indenes started with minimum energy conformations that most closely mimicked a steroid conformation.

Acknowledgment. The authors thank Ms. Ann Betz, Mr. Jianxin Que, and Mr. Richard Skubish for technical assistance. The assistance of Mr. Daniel M. Scolnick for the molecular modeling study is also gratefully acknowledged. We thank Dr. Michael Rafferty of G. D. Searle and Co. for the authentic sample of 3β-hydroxy-5α-estran-17-one. The work was supported by NIH grant GM 47969, a grant from Monsanto Co., and Research Scientist Development Award MH00964 (C.F.Z.). Assistance was also provided by the Washington University High Resolution NMR Facility supported in part by NIH 1 S10 RR00204 and a gift from the Monsanto Co.

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