Synthesis of B-Ring Homologated Estradiol Analogues that Modulate Tubulin Polymerization and Microtubule Stability

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2-Methoxyestradiol is a cytotoxic human metabolite of estradiol with the ability to bind to the colchicine site of tubulin and inhibit its polymerization, and its 2-ethoxy analogue is even more potent. On the basis of a hypothetical relationship between the structures of colchicine and 2-methoxyestradiol, a B-ring-expanded 2-ethoxyestradiol analogue was synthesized in which the B-ring of the steroid is replaced by the B-ring of colchicine. The synthesis relied on the B-ring expansion of available 6-keto estradiol derivatives as opposed to a total synthesis of the homologated steroid framework. The relative configurations of the acetamido substituents in both epimers of the final product were determined by NOESY NMR and confirmed by X-ray crystallography. The epimer having the 6α-acetamido substituent was more active as an inhibitor of tubulin polymerization, and it was also more cytotoxic than the 6β-epimer. These results are consistent with the proposed structural resemblance of 2-methoxyestradiol and colchicine. Several of the synthetic intermediates proved to be potent inhibitors of tubulin polymerization. On the other hand, a 3,17β-diacetylated, B-ring-expanded analogue of 2-ethoxyestradiol having a ketone at C-6 resembled paclitaxel (Taxol) in its ability to enhance tubulin polymerization and stabilize microtubules. The corresponding 3-acetate and the 17β-acetate were both synthesized, and it was determined that the 17β-acetate, but not the 3-acetate, conferred on the steroid derivative its paclitaxel-like activity.

Introduction

The human metabolite 2-methoxyestradiol (**1**) inhibits angiogenesis[1–8] and the growth of solid tumors in vivo,[1,9] both of which have been demonstrated in several animal models. In cancer cell cultures, 2-methoxyestradiol (**1**) produces cytotoxic effects that are associated with inhibition of DNA synthesis and mitosis, uneven chromosome distribution, faulty spindle formation, and an increase in the number of abnormal metaphases.[10,11] The available evidence indicates that these effects result from inhibition of tubulin polymerization by 2-methoxyestradiol, which binds to the colchicine binding site.[12] Other possible mechanisms have also been suggested, including those involving p53,[13–18] nitric oxide synthase,[6] stress-activated protein kinase,[5] p34cdc2,[19] and proliferating cell nuclear antigen (PCNA).[19] There is current interest in the development of 2-methoxyestradiol (**1**) as an anticancer agent, and several studies have appeared describing the synthesis of 2-methoxyestradiol (**1**) analogues, including several compounds that are more potent as inhibitors of tubulin polymerization and/ or as cytotoxic agents than the parent compound.[20–23]

Since both 2-methoxyestradiol (**1**) and colchicine (**2**) bind to the same site on β-tubulin, it is logical to ask

how these two structures are related. On the basis of the observation that the dimethoxylated compounds **3** and **4** were inactive, as were the corresponding congeners containing single methoxyl groups at positions 3 or 4, D'Amato et al. proposed that the A-ring of 2-methoxyestradiol (**1**) is functionally equivalent to the C-ring of colchicine (**2**) and that the CD rings of the steroid correspond to the trimethoxybenzene A-ring of colchicine.[12] On the basis of this hypothesis, Macdonald et al. synthesized a series of 2-methoxyestradiol (**1**) analogues in which the A-ring of the steroid was replaced by variously substituted tropone rings.[22,23] Most of the resulting compounds, including the estratropone **5**, were more potent as inhibitors of tubulin polymerization than 2-methoxyestradiol (**1**) itself.
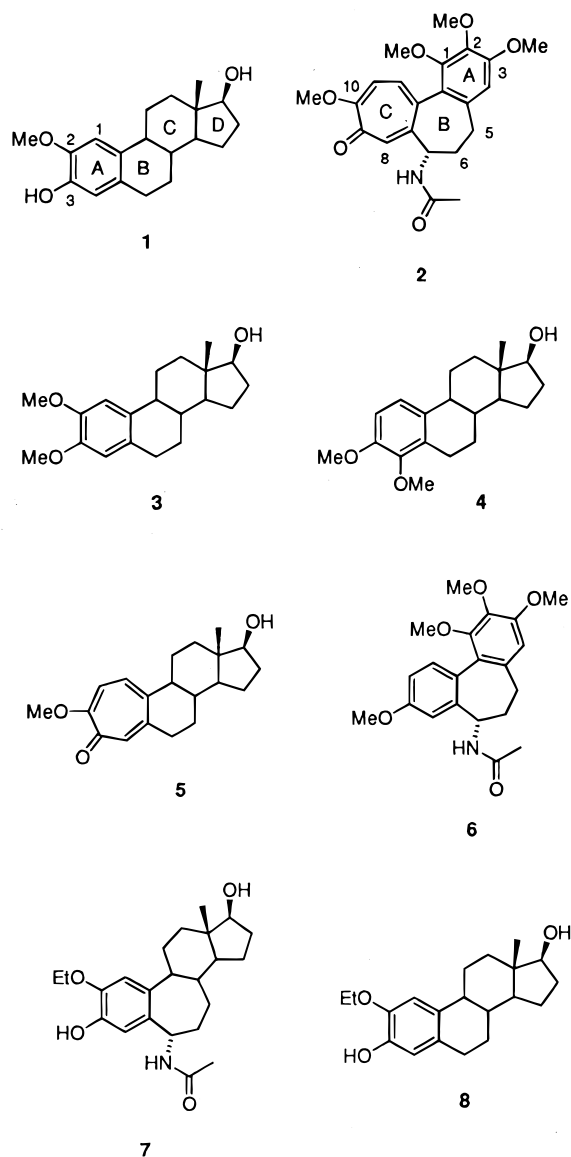
Encouraged by these results and also by the fact that the allocolchicinoids, such as compound **6**,[24] which have a 7-membered B-ring and a 6-membered C-ring, are often more active than the corresponding colchicinoids,[25] we decided to synthesize a 2-methoxyestradiol congener **7** in which the B-ring is replaced by the corresponding B-ring of colchicine. An ethoxyl group was used in **7** instead of a methoxyl group because of our previous finding that 2-ethoxyestradiol (**8**) is significantly more cytotoxic in cancer cell cultures than 2-methoxyestradiol (**1**) itself.[20] This strategy has resulted in a series of B-ring-expanded steroid analogues that modulate tubulin polymerization and microtubule stability. As described herein, several of these compounds resemble

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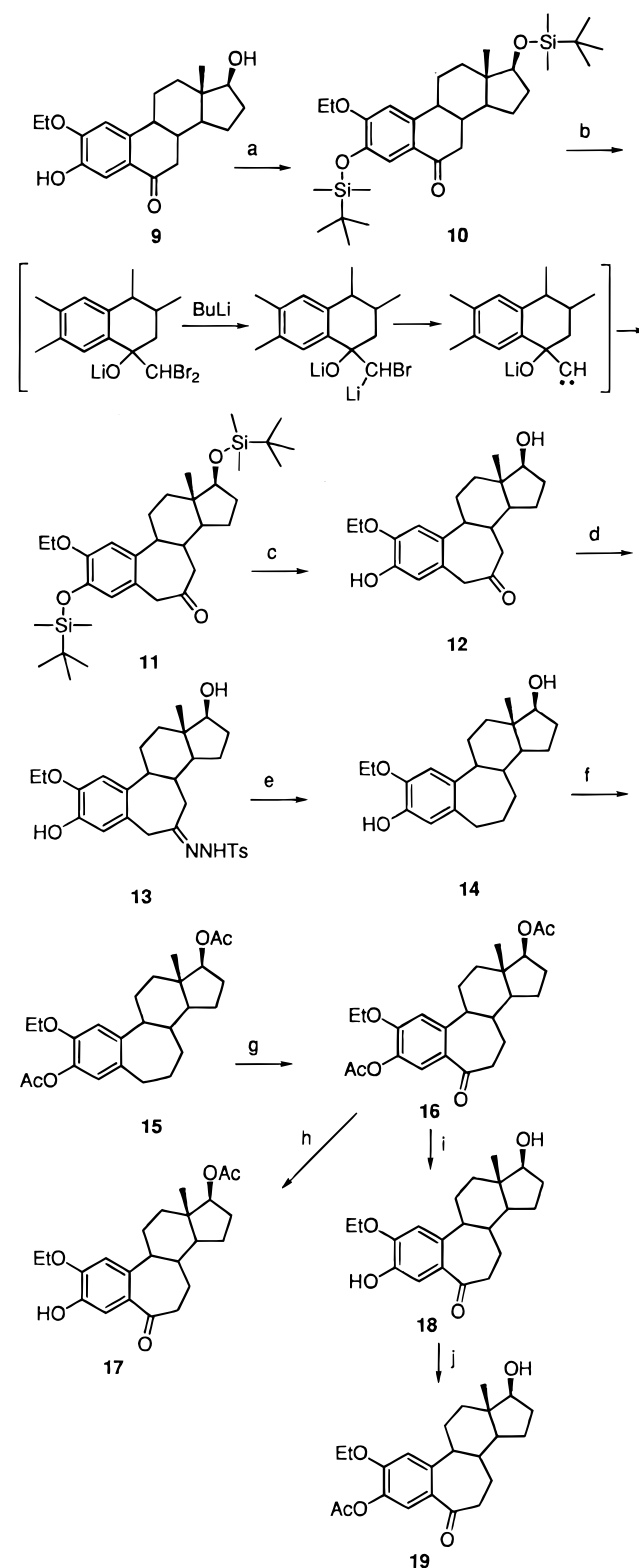§ Frederick Cancer Research and Development Center.

Scheme 1[a]

a Reagents and conditions: (a) TBDMSCl, imidazole, DMF, 23 °C (18 h); (b) (1) CH_2Br_2, LDA, THF, −78 °C (3 h), (2) BuLi, −78 to 0 °C (2.5 h); (c) Bu_4NF, THF, 23 °C (6 h); (d) $TsNHNH_2$, MeOH, 23 °C (24 h); (e) (1) catecholborane, $CHCl_3$, (2) NaOAc, H_2O, reflux (6 h); (f) Ac_2O, pyridine, 23 °C (24 h); (g) CrO_3, AcOH, 12−14 °C (40 min); (h) $KHCO_3$, CH_3OH, 65 °C (1 h); (i) KOH, MeOH, −5 to 23 °C (3.5 h); (j) 1-acetyl-1H-1,2,3-triazolo[4,5-b]pyridine, 1 N NaOH, THF, 23 °C (1.5 h).

paclitaxel (Taxol) in their effects on tubulin and micro-tubules, while others are similar to colchicine.[26]

Results and Discussion

Synthesis. A review of the literature revealed two references for the preparation of the B-homoestrane system, both of which involved total syntheses of the B-homosteroid ring system.[27,28] Limiting our endeavors to routes starting from readily available steroids, we decided to investigate an alternative strategy based on the B-ring expansion of a suitably substituted estradiol derivative.

2-Ethoxy-6-ketoestradiol (**9**) was synthesized from estradiol as previously described[21] and then protected as the bis(*tert*-butyldimethylsilyl) ether **10** (Scheme 1). After many unfruitful attempts involving the well-studied Tiffeneau−Demjanov rearrangement[29] and the diazomethane ring expansion reaction,[30] the key transformation of the six-membered B-ring ketone **10** to the corresponding seven-membered ring ketone **11** was successfully carried out by employing Taguchi's method.[31] This method relies on the nucleophilic addition of dibromomethyllithium to a carbonyl group and smooth decomposition of a β-oxido carbenoid, generated by the

action of *n*-butyllithium on the dibromomethyl inter-mediate, to afford the lithium enolate of the ring-

expanded carbonyl compound. The present case employed a one-pot modification of the Taguchi method in which lithium diisopropylamide was used to generate dibromomethyllithium from methylene bromide. Treatment of the initial adduct with *n*-butyllithium and workup of the reaction mixture afforded the desired product **11** in 62% yield based on consumption of starting material. The starting material was recovered in 25−30% yields even when an excess of the reagents was employed, so proton abstraction from the carbonyl compound may compete effectively with anion addition to the carbonyl. It is noteworthy that the rearrangement of the β-oxido carbenoid intermediate proceeded in a highly regioselective fashion, affording the 7-oxo product **11** instead of the corresponding 6-oxo ring-expanded product. The observed regioselectivity of the reaction might be expected, because in β-oxido carbenoids derived from 2-cyclohexenone, the main product results from migration of the sp² hybridized alkene carbon as opposed to the sp³ hybridized alkane carbon.[32]

The following reactions were performed in order to relocate the carbonyl from the 7-position in **11** to the 6-position in **16** (Scheme 1). Deprotection of **11** with TBAF afforded the diol **12**. Reaction of the carbonyl group in **12** with tosylhydrazine afforded the corresponding tosylhydrazone **13**. Reduction of **13** with catecholborane and thermal decomposition of the intermediate diazine provided the desired product **14**.[33] Acetylation of the diol **14** yielded the diacetate **15**, which was oxidized at the benzylic position with chromium trioxide in acetic acid to afford intermediate **16**. The phenolic acetate of **16** could be selectively deacetylated with potassium bicarbonate in methanol to provide the phenol **17**, while treatment of **16** with potassium hydroxide in methanol resulted in deacetylation of both acetates to produce the diol **18**. The phenolic hydroxyl group of **18** was selectively acetylated with 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine with sodium hydroxide as the base in THF, resulting in the formation of the monoacetate **19**.[34]

As displayed in Scheme 2, an alternative ring enlargement strategy was investigated, resulting in a one-pot conversion of the diacetate **20**[21] to the two possible ketones **12** and **18**.[35] Treatment of **20** with trimethylsilyldiazomethane−boron trifluoride etherate in methylene chloride afforded a mixture of α-trimethylsilyl ketones **23** and **24**, which was converted to the corresponding desilylated mixture of ketones **25** and **16** by hydrochloric acid in the presence of silica gel in ether. The ¹H NMR spectrum of the reaction product indicated a 1:1 mixture of both diacetates **25** and **16**, which could not be separated chromatographically. However, after basic hydrolysis of the mixture of diacetates **25** and **16**, the resulting mixture of diols **12** and **18** could be separated chromatographically to afford both products in 31% and 24% yields, respectively. The mechanism of the critical ring-expansion reaction most likely involves axial attack of trimethylsilyldiazomethane on the 6-keto group in **20** to afford a mixture of adducts **21** and **22**. Migration of the bond antiperiplanar to the diazonium group in **21** and **22** then results in both silylated ketones **23** and **24**, respectively. In comparison to the B-ring expansions performed with these reagents on A-ring saturated steroids, which were highly regioselective,[35]

Scheme 2[a]
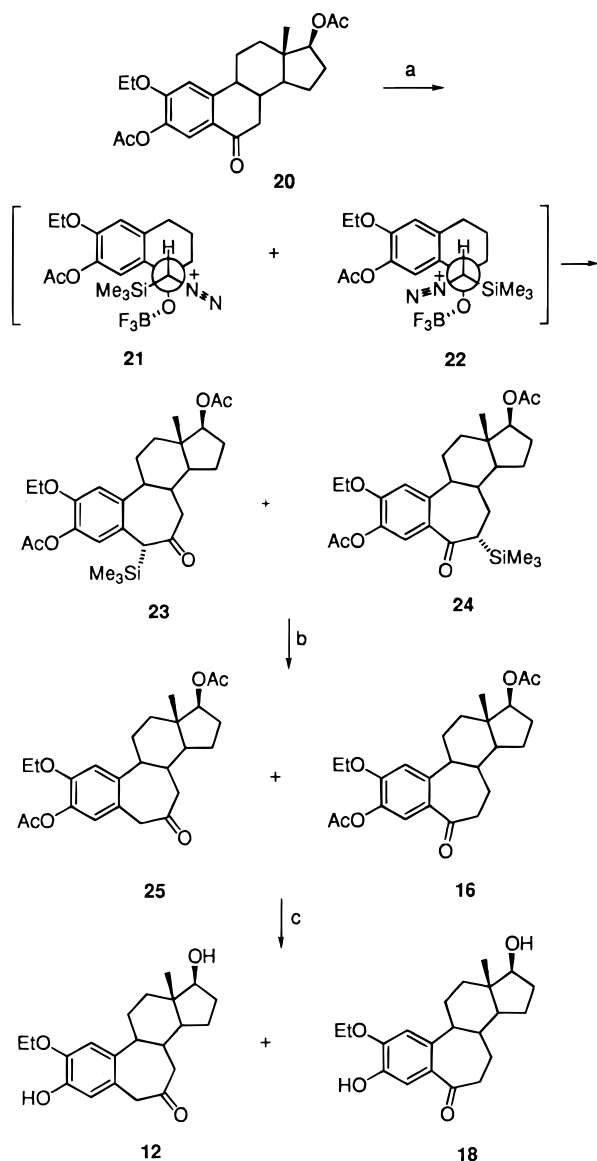
[a] Reagents and conditions: (a) BF₃·OEt₂, TMSCHN₂, CH₂Cl₂, −20 °C (3 h); (b) HCl, SiO₂, Et₂O, 23 °C (4 h); (c) (i) KOH, MeOH, 23 °C (4 h), (ii) HCl.

the present reaction on steroid **20**, having an aromatic ring, proceeded with poor regioselectivity. The poor regioselectivity in the present case might possibly result from the less sterically demanding aromatic A-ring in comparison with the saturated steroid A-ring in the example reported in the original study of the reaction, so that intermediate **21** forms in addition to **22**.[35] Alternatively, assuming that the initial addition of trimethylsilyldiazomethane is reversible, the migratory aptitude of the aromatic A-ring in the present case might be greater than the saturated steroidal A-ring reported in the original example.

The conversion of intermediate **16** to the desired product **7**, in which the B-ring of 2-ethoxyestradiol **8** is replaced by the colchicine (**6**) B-ring, is outlined in Scheme 3. The reductive amination of intermediate **16**, followed by acetylation, yielded a mixture of the acetamides **26** and **27** in a ratio of 1:2, respectively.[36] The 6α- and 6β-epimers, **26** and **27**, were separated by careful chromatography on silica gel. The two acetates

Scheme 3[a]
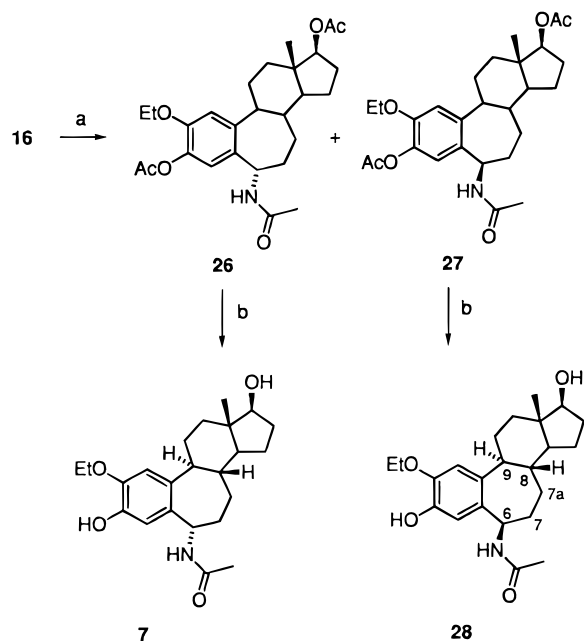
[a] Reagents and conditions: (a) (1) NH₄OAc, NaCNBH₃, MeOH, 65–70 °C (48 h), (2) Ac₂O, pyridine, 23 °C (14 h); (b) NaOH, MeOH, 23 °C (5 h).
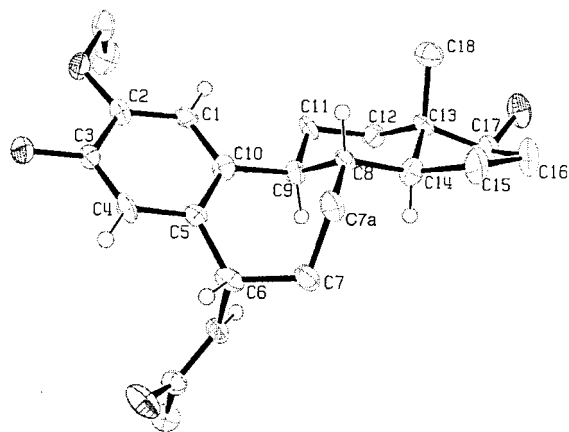
Figure 1. ORTEP diagram resulting from X-ray crystallography of **7**. Most of the hydrogen atoms and a water molecule were omitted for clarity.

of both **26** and **27** were saponified with sodium hydroxide in methanol to afford the desired product **7** and its C-6 epimer **28**. A detailed NMR analysis was performed in order to elucidate the configuration at C-6 in each epimer, as well as to determine the preferred conformation of the 7-membered ring. Although the proton coupling constants were not very informative as far as the assignment of the relative configuration of the substituent at C-6 was concerned, the NOESY spectrum revealed a very strong, diagnostic NOE between the methine protons at H-6 and H-9 in diastereomer **28** that was absent in its epimer **7**. This structure was confirmed by X-ray analysis (Figure 1).

Because of the potent biological activity previously observed for the 6-oximino derivative of 2-ethoxyestradiol (**8**),[21] the two methoximes **29** and **30**, as well as the oxime **31**, were prepared in the present series. The methoxime **29** was synthesized by treatment of **18** with methoxylamine hydrochloride in pyridine. Acetylation

Table 1. Cytotoxicities of B-Ring Homologated Estradiol Analogues

| compd	MGM[a] (μM)	compd	MGM[a] (μM)
1	1.3	**18**	14.4
7	7.0	**28**	90.4
8	0.076	**29**	24.0
9	0.13	**31**	51.3
12	11.5	**32**	1.32
13	15.5	**33**	42.7
14	2.48	**34**	0.079
15	14.5	**35**	0.27
16	>100		

[a] Mean graph midpoint for growth inhibition of all human cancer cell lines tested.

of **29** with acetic anhydride in pyridine then afforded the diacetate **30**. The oximes **31** and **33** were obtained in the usual way by reaction of **18** and **12** with hydroxylamine hydrochloride in pyridine. The alkene **32** was synthesized by treatment of the tosylhydrazone **13** with an excess of methyllithium in THF.

29 R = H
30 R = Ac

31

32

33

34

35
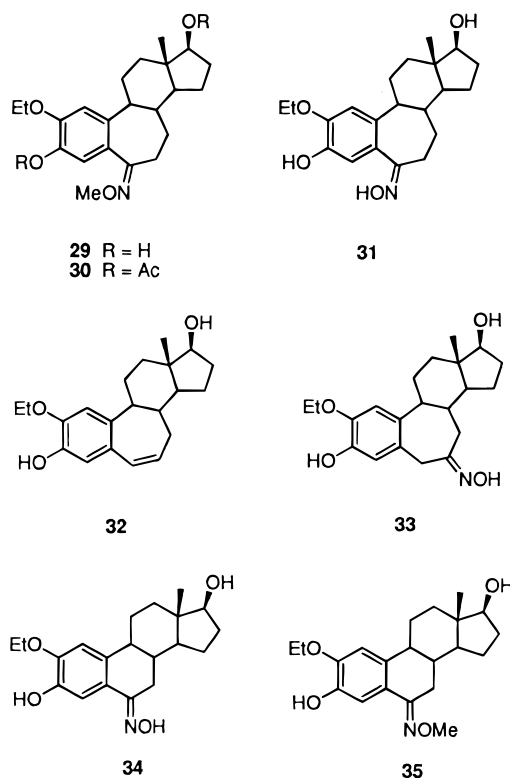
Biological Results. Most of the new compounds were tested in the National Cancer Institute's screen of approximately 55 different human cancer cell cultures of diverse tumor origins. The resulting mean graph midpoints (MGMs) for 50% growth inhibition are listed in Table 1. The MGM is based on a calculation of the average GI_{50} for all of the cell lines tested in which GI_{50} values above and below the test range (10^{-4}–10^{-8} M) are taken as the maximum (10^{-4} M) and minimum (10^{-8} M) drug concentrations in the screening test.[37] The table includes the previously published values for 2-methoxyestradiol (**1**), 2-ethoxyestradiol (**8**), 2-ethoxy-6-ketoestradiol (**9**), 2-ethoxy-6-oximinoestradiol (**34**), and 2-ethoxy-6-methoximinoestradiol (**35**).[21]

The most interesting compound in the series proved to be **16**, which unexpectedly accelerated tubulin polymerization and stabilized the microtubules toward
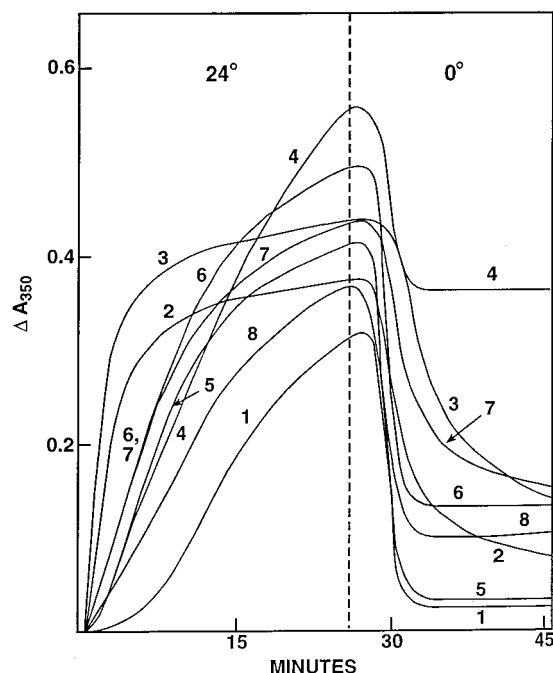
Figure 2. Effects of weakly active stimulatory steroid derivatives on tubulin polymerization. Reaction mixtures were prepared as described in the text and contained the described components as well as the following: curve 1, no further addition; curve 2, 7.5 μM **16**; curve 3, 100 μM **16**; curve 4, 400 μM **15**; curve 5, 200 μM **28**; curve 6, 400 μM **20**; curve 7, 400 μM **25**; and curve 8, 200 μM **30**. The figure is a composite of several experiments.

Table 2. Interactions of Steroid Derivatives with Tubulin

Part I. Apparent Stimulators of Assembly[a]

compound	enhancement of assembly rate EC$_{50}$ (μM) $\pm$ SD
paclitaxel	0.83 ± 0.1
15	>400
16	5.5 ± 1
17	8.2 ± 1
19	360 ± 7
20	>400
25	>400
28	>200
30	>200

Part II. Inhibitors of Tubulin Polymerization[b]

compd	inhibition of polymerization IC$_{50}$ (μM) $\pm$ SD	inhib of colchicine binding[c] % inhib $\pm$ SD	
		expt I[d]	expt II[e]
1	1.4 ± 0.2	19 ± 3	60 ± 10
7	6.9 ± 0.1		
8	0.66 ± 0.06	51 ± 6	87 ± 4
9	3.0 ± 0.05	19 ± 6	
12	8.1 ± 0.7		
13	80 ± 20		
14	0.97 ± 0.3		56 ± 7
18	130 ± 40		
29	30 ± 4		
31	57 ± 10		
32	0.85 ± 0.1		
33	99 ± 30		

[a] Stimulators of tubulin polymerization were evaluated as described in the Experimental Section. [b] Inhibitors of tubulin polymerization were evaluated as described in the Experimental Section. [c] Reaction mixtures contained 1.0 μM tubulin, 5.0 μM [^{3}H]colchicine, and 50 μM inhibitor. [d] Expt I data are from ref 21. [e] Expt II data are from ref 27.

cold-induced depolymerization.[26] These effects resemble those produced by the potent anticancer agent paclitaxel (Taxol). The monoacetate **17**, in which the C-17 hydroxyl group is acetylated, proved to be almost as potent as **16**, but the alternative monoacetate **19**, in which the phenolic hydroxyl group was acetylated, displayed minimal taxoid activity. Thus, the 17-acetate, but not the 3-acetate, appears to be necessary for the taxoid activity of **16**. Both **16** and **17** were less potent than paclitaxel in their effects on tubulin and as cytotoxic agents in cancer cell cultures, but the possibility exists that they could serve as lead compounds for the development of steroid derivatives having more potent and more useful taxoid activities.

In evaluating the newly synthesized compounds described here for their effects on tubulin polymerization, we noted that a number of other agents also appeared to have slight activity as stimulators of tubulin assembly. Since their relative activities could provide useful structure−activity information, we decided to design a more quantitative assay to compare the activities of these compounds. We continued to use glutamate- and GTP-induced assembly of purified tubulin as the basic assay system, and we found that the difference between the reaction without steroid and that with compound **16** was maximal at a reaction temperature of 24 °C (see Figure 2). The reaction parameter we decided to use for compound comparisons was the maximum rate of turbidity change (in terms of ΔA_{350} units) in the presence of 100 μM **16** (see below for further details; in the absence of steroid this rate was 18 milliunits of absorbance/min, and with 100 μM **16**, 160 milliunits/min). Compound concentrations that

yielded 50% of this rate were determined (EC$_{50}$ values). The data we obtained are tabulated in part I of Table 2.

Compound **16** itself yielded an EC$_{50}$ value of 5.5 μM, while that for **17** was 8.2 μM. For comparison, paclitaxel had an EC$_{50}$ value of 0.83 μM. The only other apparently stimulatory compound that yielded an EC$_{50}$ value within the concentration ranges we could study was compound **19**, with an EC$_{50}$ value of 360 μM. The remaining five compounds listed in part I of Table 2 all seemed to have some ability to enhance tubulin assembly, and turbidity curves obtained at the highest concentrations we could examine (based on the concentrations of the stock solutions) are shown in Figure 2. This figure also shows typical curves obtained without drug (curve 1) and with compound **16** near its EC$_{50}$ value (curve 2) and at 100 μM (curve 3).

Besides enhancement of tubulin assembly, another important property of paclitaxel, shared by compound **16**, is stabilization of polymer to cold-induced disassembly. Although in the current studies the stabilizing effect of **16** was less pronounced than observed previously,[26] a variety of stabilization patterns was observed with the stimulatory steroid derivatives. Two parameters, relative to the control without drug, are apparent from the reaction curves shown in Figure 2. First, upon reaching a depolymerizing temperature, the control disassembles rapidly. Second, the disassembly of the control polymer is essentially complete (electron micrographs rarely show surviving polymer, and the slight change in the baseline is generally interpreted as representing partial denaturation of tubulin during the course of the reaction).

In reaction mixtures containing compound **16**, the rate of depolymerization was markedly reduced, and the reversal of the assembly reaction was not complete. Only compounds **17** (not shown, but see ref 26) and **25** (Figure 2, curve 7) yielded similar stability patterns. Compound **17** is the analogue of **16** lacking only the C-3 acetyl group. Compound **25** had both acetyl groups, but the ketone group was at C-7 (rather than at C-6 in **16**).

The most common pattern observed with weakly stimulatory analogues was a rapid disassembly reaction, differing little from that without drug, but with incomplete reversal. The extent of reversal was minimal with compound **15** (Figure 2, curve 4) and more extensive with compounds **20** (curve 6) and **30** (curve 8). With **20** and **30**, the reversibility was similar to what was observed with **16**. These three compounds were all acetylated at both positions C-3 and C-17. In addition, compound **15** had an unsubstituted 7-membered B-ring, **20** had a 6-membered B-ring but a ketone substituent at C-6, and **30** was a close analogue of **16**, but with a methoxime replacing the ketone at C-6. These results would appear to emphasize the importance of the 7-membered B-ring and the ketone substituent at C-6 for taxoid-like activity in a steroid derivative.

Two compounds showed no apparent stabilization of polymer, in that reaction mixtures containing these compounds displayed disassembly patterns essentially superimposable on the control reaction. These were compounds **19** (data not shown) and **28** (Figure 2, curve 5). Compound **19** is structurally identical to compound **16**, except that it lacks the C-17 acetyl group. Compound **28**, however, was a diastereomer of the target compound **7**, which inhibits the polymerization reaction (see below). It thus retains the 7-membered B-ring but lacks the acetate and ketone groups observed in most of the other stimulatory compounds.

The remaining compounds in the series displayed the expected ability to inhibit tubulin polymerization, although a number of them were only marginally active (**13**, **18**, **29**, **31**, and **33**). The target compound **7**, in which the acetamide at C-6 has the same absolute configuration at the corresponding group in colchicine (assuming the correctness of the proposed structural analogy between these two compounds), proved to be significantly more active than its C-6 epimer **28** as a cytotoxic agent. Compound **7** also inhibited tubulin polymerization, as opposed to the stimulation observed with **28**. However, in contrast to the enhanced activity observed with expanded A-ring analogues,[22,23] compound **7** was about 10-fold less potent than 2-ethoxyestradiol (**8**) as an inhibitor of both tubulin polymerization and cell growth. The complete removal of the acetamide from **7/28** resulted in the more potent analogue **14**. This compound was almost as potent as 2-ethoxyestradiol (**8**) as an inhibitor of tubulin polymerization, but **14** was less cytotoxic than **8**. The biological profile produced by the alkene **32** was very similar to that observed with its saturated analogue **14**.

Several of the compounds in the series were striking in their biological inactivities. For example, both the 6-oximino and 7-oximino compounds **31** and **33** were minimally active as inhibitors of tubulin polymerization and as cytotoxic agents, even though the corresponding oxime **34**, having a six-membered B-ring, displayed an

MGM of 0.079 μM and had an IC$_{50}$ of 1.1 μM for inhibition of tubulin polymerization.[21] Similarly, the methoxime **29** displayed an MGM of 24 μM and an IC$_{50}$ value for inhibition of tubulin polymerization of 30 μM, even though the closely related methoxime **35**, having a six-membered B-ring, had an MGM of 0.27 μM and an IC$_{50}$ value for inhibition of tubulin polymerization of 1.4 μM. These differences underscore a high degree of structural specificity attached to the biological effects of the oxime **34** and the methoxime **35**.

It is also worth contrasting the deacetylated/diacetate pairs **9/20** and **18/16**. As noted previously,[21] compound **9** has reasonable activity as an inhibitor of tubulin polymerization (IC$_{50}$ value, 3.0 μM in the current evaluation) and is among the more cytotoxic steroid derivatives we have prepared, whereas **20** has minimal activity as a stimulator of tubulin polymerization (EC$_{50}$ value > 400 μM). In contrast, **18** is a weak inhibitor of assembly (IC$_{50}$ value, 130 μM), while **16** is the most potent of the stimulatory steroid derivatives. In addition, shifting the ketone moiety to the C-7 carbon versus the C-6 carbon results in substantial enhancement of activity as an inhibitor of assembly (cf. **12** vs **18**) but substantial loss of activity as a stimulator of assembly (cf. **25** vs **16**).

In conclusion, the synthesis of a 2-ethoxyestradiol analogue has been executed in which the B-ring is replaced by the corresponding 7-membered B-ring present in colchicine. An investigation of the biological activities of the synthetic intermediates led to the unexpected observation that two compounds in the series resemble paclitaxel in that they accelerate tubulin polymerization and stabilize microtubules, whereas other members of the series show the expected ability to inhibit tubulin polymerization. One possibility is that the taxoid compounds **16** and **17** may "cross over" from the colchicine binding site on tubulin to the paclitaxel binding site, or at least partially occupy the paclitaxel binding site. The recent determination of the structure of the tubulin dimer in docetaxel-stabilized, antiparallel zinc protofilaments by electron crystallography has revealed the structure of the protein complex containing GTP, GDP, and docetaxel.[38] In addition, the available information on colchicine binding, in conjunction with the structure of the protein, has allowed a hypothetical model to be constructed of the binding of colchicine to tubulin.[39] Although it is clear how the structure of the 2-ethoxyestradiol−colchicine hybrid **7** would be overlapped with the proposed structure of colchicine in the colchicine binding pocket, it is not obvious on first inspection how the structure of the taxoid-like compounds **16** and **17** resembles that of paclitaxel. In any case, compounds **16** and **17** represent the first compounds in the steroid series to have demonstrable paclitaxel-mimetic properties. Further work is needed to define the structure−activity relationships of the compounds in this series.

Experimental Section

General. Melting points are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a 300 MHz spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard. For data reporting, s = singlet, d = doublet, m = multiplet, br = broad, bs = broad singlet.

Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions. Column chromatography was carried out using Merck silica gel (230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on silica gel GF (Analtech) glass-coated plates (2.5 × 10 cm with 250 μM layer and prescored), and spots were visualized with UV light at 254 nm or with 5% H_2SO_4 in ethanol. Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Company (Milwaukee, WI).

3,17β-Di-*O*-(*tert*-butyldimethylsilyl)-2-ethoxy-6-oxoestradiol (10). Under argon, a solution of compound **9** (4.92 g, 14.9 mmol), imidazole (12.3 g, 178.4 mmol), and *tert*-butyldimethylchlorosilane (13.5 g, 89.6 mmol) in DMF (100 mL) was stirred for 18 h at room temperature. The reaction mixture was poured into an ice-cold sodium bicarbonate solution (250 mL), and the product was extracted with ethyl acetate (200 mL, 150 mL, 100 mL). The organic layers were washed with water (200 mL) and brine (2 × 100 mL), dried over sodium sulfate, and evaporated to dryness. Crystallization of the residue from methanol gave compound **10** (7.6 g, 91%), which was obtained as white crystals: mp 150−151 °C; ^{1}H NMR (CDCl$_3$) δ 7.51 (s, 1 H), 6.78 (s, 1 H), 4.19 (q, J = 6.9 Hz, 2 H), 3.65 (t, J = 8.5 Hz, 1 H), 2.85−2.48 (m, 2 H), 2.45−1.1 (m, 14 H), 0.98 (s, 9 H), 0.86 (s, 9 H), 0.72 (s, 3 H), 0.15 (s, 6 H), 0.01 (s, 6 H); CIMS (isobutane) m/z (rel intensity) 559 (MH$^+$, 100). Anal. (C$_{32}$H$_{54}$O$_4$Si$_2$) C, H.

B-Homo-3,17β-di-*O*-(*tert*-butyldimethylsilyl)-2-ethoxy-7-oxoestradiol (11). Under argon, a well-stirred solution of **10** (6.38 g, 11.41 mmol) and dibromomethane (11.9 g, 68.5 mmol) in dry THF (190 mL) was cooled to −78 °C, and then the solution was treated with lithium diisopropylamide mono-(tetrahydrofuran) (30.4 mL, 1.5 M solution in cyclohexane, 45.6 mmol) dropwise over a period of 1.5 h. The mixture was stirred for 3 h, and *n*-butyllithium (60 mL, 1.6 M solution in hexane, 96 mmol) was added to the mixture over a period of 1.5 h. The resulting red solution was stirred for another 1 h at −78 °C and 10 min at 0 °C, quenched by pouring into ice (150 g), and extracted with ethyl acetate (3 × 100 mL). The organic layers were washed with brine (3 × 100 mL), combined, dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue (silica gel 230−400 mesh, methylene chloride:hexane 7:3 by volume) yielded the starting material **10** (1.98 g) and pure compound **11** as an oil (2.78 g, 61.6% based on the consumption of **10**): IR (KBr, cm^{-1}) 2955, 2930, 1709 (7-C=O), 1511; ^{1}H NMR (CDCl$_3$) δ 6.78 (s, 1 H), 6.60 (s, 1 H), 4.04 (qd, J = 7.0 Hz, J = 1.5 Hz, 2 H), 3.66 (t, J = 8.5 Hz, 1 H), 3.60 (d, J = 20.1 Hz, 1 H), 3.28 (d, J = 20.3, 1 H), 2.68 (dd, J = 11.3 Hz, J = 8.5 Hz, 1 H), 2.34 (m, 1 H), 2.2−1.20 (m, 14 H), 0.98 (s, 9 H), 0.88 (s, 9 H), 0.78 (s, 3 H), 0.13 (s, 6 H), 0.05 (s, 6 H); CIMS (isobutane) m/z (rel intensity) 573 (MH$^+$, 100). Anal. Calcd for (C$_{33}$H$_{56}$O$_4$Si$_2$) C, H.

B-Homo-2-ethoxy-7-oxo-3,17β-estradiol (12). Under nitrogen, a mixture of **11** (3.42 g, 5.97 mmol) and a 1.0 M solution of tetrabutylammonium fluoride in THF (50 mL, 50 mmol) was stirred at room temperature for 6 h. The reaction mixture was poured into an ice-cold sodium bicarbonate solution (200 mL) and extracted with ethyl acetate (3 × 100 mL). The organic phase was washed with brine (2 × 100 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on silica gel, eluting with ethyl acetate:hexane 1:1 by volume, gave compound **12** as white crystals (1.8 g, 87%): mp 210−212 °C; IR (KBr, cm^{-1}) 3403 (br), 2970, 2855, 1688, 1582, 1510; ^{1}H NMR (CDCl$_3$ + DMSO-d_6) δ 6.82 (s, 1 H), 6.69 (s, 1 H), 4.14 (q, J = 7.0 Hz, 2 H), 3.69 (t, J = 8.5 Hz, 1 H), 3.62 (d, J = 20.1 Hz, 1 H), 3.32 (d, J = 19.8, 1 H), 2.68 (dd, J = 11.3 Hz, J = 8.5 Hz, 1 H), 2.34 (m, 1 H), 2.10−1.70 (m, 7 H), 1.50−1.20 (m, 7 H), 0.88 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 345 (MH$^+$, 100). Anal. (C$_{21}$H$_{28}$O$_4$) C,H.

B-Homo-2-ethoxy-3,17β-estradiol-7-tosylhydrazone (13). Under nitrogen and at room temperature, a mixture of compound **12** (1.73 g, 5.02 mmol) and *p*-toluenesulfonhydrazide (4.67 g, 25.1 mmol) in methanol (100 mL) was stirred for 24

h. The resultant mixture was filtered and the solid washed with cold methanol to afford tosylhydrazone **13** as white crystals (2.48 g, 96.5%). The analytical sample was recrystallized from methanol: mp 179−181 °C; IR (KBr, cm^{-1}) 3428 (br, OH, NH), 2922, 1624, 1594, 1511; ^{1}H NMR (CDCH$_3$) δ 7.88 (d, J = 8.0 Hz, 2 H), 7.34 (d, J = 8.0 Hz, 2 H), 7.33 (s, 1 H, NH), 6.71 (s, 1 H), 6.68 (s, 1 H), 5.55 (s, 1 H), 4.11 (q, J = 7.0 Hz, 2 H), 3.58 (d, J = 19.1 Hz, 2 H), 3.39 (s, 1 H), 3.45 (t, J = 8.5, 1 H), 3.15 (d, J = 19.1 Hz, 1 H), 2.46 (s, 3 H), 2.10−0.80 (m, 16 H), 0.78 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 360 (MH$^+$, 100), 342 (51). Anal. (C$_{28}$H$_{36}$O$_5$N$_2$) C, H.

B-Homo-2-ethoxy-3,17β-estradiol (14). Under argon, a solution of **13** (2.27 g, 4.43 mmol) in anhydrous chloroform (150 mL) was cooled to 0 °C, and then catecholborane (44.3 mL, 1.0 M solution in THF, 44.3 mmol) was added dropwise. The resultant mixture was stirred for 10 h, and sodium acetate trihydrate (12.06 g, 88.62 mmol) was added in portions. The mixture was allowed to warm to room temperature over 30 min and then heated under reflux for 6 h. The reaction mixture was cooled to room temperature and filtered. The solid material was washed with chloroform (100 mL), and the combined filtrates were evaporated under reduced pressure to dryness. The remaining oil was purified by chromatography on a silica gel column with ethyl acetate−hexane 1:3 by volume. The product was crystallized from ethyl acetate/hexane to give compound **14** as white crystals (1.17 g, 80.1%): mp 86-88 °C; IR (KBr, cm^{-1}) 3490, 3259, 2925, 2861, 1607, 1511; ^{1}H NMR (CDCl$_3$ + DMSO-d_6) δ 6.81 (s, 1 H), 6.67 (s, 1 H), 5.46 (s, 1 H), 4.11 (dq, J = 3.3 Hz, J = 7.0 Hz, 2 H), 3.74 (t, J = 8.2 Hz, 1 H), 2.86 (dt, J = 14.6 Hz, J = 7.3, 1 H), 2.64−2.47 (m, 2 H), 2.14−1.22 (m, 18 H), 0.83 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 330 (M$^+$, 100). Anal. Calcd for (C$_{21}$H$_{30}$O$_3$) C, H.

B-Homo-3,17β-di-*O*-acetyl-2-ethoxyestradiol (15). Acetic anhydride (6 mL, 63 mmol) was added under argon at room temperature to a solution of compound **14** (1.14 g, 3.45 mmol) in anhydrous pyridine (12 mL). The resulting mixture was stirred for 24 h and poured into an ice−water mixture (100 g). The compound was extracted with ethyl acetate (3 × 70 mL). The organic layers were washed with water (100 mL), a solution of sodium bicarbonate (2 × 100 mL), and brine (2 × 100 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on a silica gel (230−400 mesh) column using ethyl acetate−hexane 1:1 by volume gave compound **15** (1.43 g, 100%), which was crystallized from ethyl acetate/hexane to afford white crystals: mp 98−100 °C; IR (KBr, cm^{-1}) 2928, 2870, 1768, 1735, 1510; ^{1}H NMR (CDCl$_3$) δ 6.89 (s, 1 H), 6.75 (s, 1 H), 4.70 (t, J = 8.7 Hz, 1 H), 4.06 (dq, J = 3.3 Hz, J = 7.0 Hz, 2 H), 2.89 (dt, J = 14.6 Hz, J = 7.3, 1 H), 2.64−2.52 (m, 2 H), 2.29 (s, 3 H), 2.07 (s, 3 H), 2.23−1.26 (m, 17 H), 0.88 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 415 (MH$^+$, 48), 355 (100). Anal. Calcd for (C$_{25}$H$_{34}$O$_5$) C, H.

B-Homo-3,17β-di-*O*-acetyl-2-ethoxy-6-oxoestradiol (16). A solution of chromium trioxide (1.34 g, 13.4 mmol) in 90% glacial acetic acid (13 mL) was added dropwise over a period of 20 min at 13−15 °C to a well-stirred solution of compound **15** (1.29 g, 3.11 mmol) in glacial acetic acid (35 mL), and the resulting mixture was stirred at room temperature for 15 min. The mixture was poured into an ice/water mixture (300 g) and extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with brine (2 × 100 mL), a solution of sodium bicarbonate (2 × 100 mL), and brine (2 × 100 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on a silica gel column using 20% ethyl acetate in hexane gave pure compound **16** (925 mg, 69.3%), which was crystallized from ethyl acetate/hexane to afford white crystals: mp 178−180 °C; IR (KBr, cm^{-1}) 2935, 2865, 1774, 1728, 1669, 1500; ^{1}H NMR (CDCl$_3$) δ 7.36 (s, 1 H), 6.84 (s, 1 H), 4.72 (t, J = 8.3 Hz, 1 H), 4.13 (q, J = 7.0 Hz, 2 H), 2.64−2.52 (m, 3 H), 2.29 (s, 3 H), 2.07 (s, 3 H), 2.23−1.26 (m, 15 H), 0.94 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 429 (MH$^+$, 49), 341 (100). Anal. (C$_{25}$H$_{32}$O$_6$) C, H.

B-Homo-17β-*O*-acetyl-2-ethoxy-3-hydroxyestra-6-ketoestradiol (17). Under nitrogen, a solution of compound **16** (65 mg, 0.15 mmol) in methanol (10 mL) was deoxygenated by bubbling through it a slow stream of nitrogen for 30 min. A similar deoxygenated solution of $KHCO_3$ (152 mg, 1.52 mmol) in water (1 mL) was added, and the reaction mixture was stirred and heated at 65 °C (outer bath) for 1.5 h. The reaction mixture was cooled to room temperature and neutralized to pH = 6 with 6 N HCl. The solvents were removed under reduced pressure, and the residue was dissolved in a mixture of ethyl acetate (50 mL) and water (50 mL). The ethyl acetate layer was separated, dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on silica gel, eluting with ethyl acetate−hexane 1:2 by volume, yielded compound **17** (56 mg, 95%), which was easily crystallized from ethyl acetate−hexane to afford white crystals: mp 240−242 °C; IR (KBr, cm^{-1}) 3385 (OH), 2941, 1721, 1663, 1614, 1511; ^{1}H NMR (CDCl$_3$) δ 7.22 (s, 1 H), 6.75 (s, 1 H), 5.54 (s, 1 H), 4.72 (t, J = 8.5 Hz, 1 H), 4.19 (q, J = 7 Hz, 2 H), 2.55 (m, 1 H), 2.08 (s, 3 H), 1.94−1.27 (m, 14 H), 0.88 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 387 (MH$^+$, 100). Anal. Calcd. for (C$_{23}$H$_{30}$O$_5$) C, H.

B-Homo-2-ethoxy-6-oxo-3,17β-estradiol (18). Under nitrogen, a suspension of compound **16** (0.95 g, 2.2 mmol) in anhydrous methanol (10 mL) was cooled to −5−0 °C, and a 20% solution of KOH in methanol (10 mL, KOH 1 g, 17.8 mmol) was added dropwise. The resulting mixture was allowed to warm to room temperature and stirred for 3.5 h. The mixture was cooled to 0 °C and neutralized with 3 N HCl to pH = 5, and the solid was allowed to precipitate in a refrigerator overnight. The resulting mixture was filtered to afford the white solid **18** (725 mg, 95%), which was recrystallized from methanol to afford analytical sample: mp 198−200 °C; IR (KBr, cm^{-1}) 3497 (OH), 3281 (OH), 2935, 2882, 1650, 1608, 1508; ^{1}H NMR (CDCl$_3$) δ 7.22 (s, 1 H), 6.76 (s, 1 H), 4.19 (q, J = 7 Hz, 2 H), 3.78 (t, J = 8.5 Hz, 1 H), 2.54 (m, 3 H), 2.15−1.21 (m, 15 H), 0.90 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 345 (MH$^+$, 100). Anal. (C$_{21}$H$_{28}$O$_4$) C, H.

B-Homo-3-*O*-acetyl-2-ethoxy-6-oxo-3,17β-estradiol (19). Under argon at room temperature, compound **18** (60 mg, 0.17 mmol) was dissolved in THF (4 mL), and 1 N NaOH (0.19 mL, 0.19 mmol) was added to the solution. After stirring for 20 min, a solution of 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine (31 mg, 0.19 mmol) in THF (2 mL) was added dropwise to the reaction mixture, which was stirred at room temperature for 1.5 h. The reaction mixture was poured into ice (20 g) and neutralized to pH = 6 with 2 N HCl, and it was extracted with ethyl acetate (2 × 30 mL). The ethyl acetate layers were combined, dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on silica gel, eluting with ethyl acetate−hexane, 1:3 by volume, gave compound **19** (61 mg, 90%), which precipitated from hexane to afford stable white foam that was dried under vacuum at 40−50 °C: IR (KBr, cm^{-1}) 3449 (br), 2933, 1766, 1669, 1605, 1502; ^{1}H NMR (CDCl$_3$) δ 7.36 (s, 1 H), 6.86 (s, 1 H), 4.14 (q, J = 7 Hz, 2 H), 3.78 (t, J = 8.5 Hz, 1 H), 2.59 (m, 3 H), 2.30 (s, 3 H), 2.15−1.27 (m, 15 H), 0.89 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 387 (MH$^+$, 100). Anal. (C$_{23}$H$_{30}$O$_5$) C, H.

Homologation by Trimethylsilyldiazomethane: B-Homo-2-ethoxy-7-oxo-3,17β-estradiol (12) and B-Homo-2-ethoxy-6-oxo-3,17β-estradiol (18). A solution of trimethylsilyldiazomethane (4.83 mL, 2 M in hexane, 9.7 mmol) was added dropwise to a solution of intermediate **20**[21] (1 g, 2.41 mmol) in dichloromethane (40 mL) containing BF$_3$·OEt$_2$ (1.5 mL, 12.2 mmol) at −20 °C. After 3 h, the reaction mixture was poured over ice water (50 mL). The organic layer was dried (Na$_2$SO$_4$), and the solvent was removed. The residue was dissolved in diethyl ether (40 mL). Silica gel (20 g) and 6 M HCl (1 mL) were added, and the reaction mixture was stirred at room temperature for 4 h. Usual workup of the reaction mixture gave a sticky residue that was then dissolved in anhydrous methanol (30 mL). A 20% solution of KOH in methanol (15 mL) was slowly added, and the reaction mixture was stirred at room temperature for an additional 4 h. The

mixture was neutralized with 6 M HCl, and the solvent was removed. Extraction of the crude product with ethyl acetate, followed by column chromatographic purification on silica gel, eluting with 1:2 ethyl acetate−hexane, afforded compound **12** (0.26 g, 31%) and compound **18** (0.20 g, 24%). The IR and NMR spectra of these compounds were identical with those of the samples described above.

B-Homo-6α-acetamido-3,17β-di-*O*-acetyl-2-ethoxyestradiol (26) and B-Homo-6β-acetamido-3,17β-di-*O*-acetyl-2-ethoxyestradiol (27). Sodium cyanoborohydride (493 mg, 7.8 mmol) was added to a solution of compound **16** (270 mg, 0.78 mmol) and ammonium acetate (4.0 g, 51.9 mmol) in anhydrous methanol (20 mL) at room temperature under argon. The reaction mixture was stirred at room temperature for 30 min and then heated at reflux for 48 h. The mixture was cooled to about 40 °C, and the solvent was removed under reduced pressure. The residue was treated with saturated aqueous NaHCO$_3$ (50 mL) and ethyl acetate (3 × 50 mL). The ethyl acetate layers were washed with brine (2 × 30 mL), combined, dried over sodium sulfate, and evaporated to dryness. The residue was dissolved in anhydrous pyridine (10 mL), and acetic anhydride (4 mL) was added. The resulting mixture was stirred at room temperature under argon for 14 h. The pyridine and excess acetic anhydride were removed under reduced pressure at 40−45 °C, and the residue was dissolved in ethyl acetate (100 mL). The ethyl acetate solution was washed with saturated NaHCO$_3$ (50 mL) and brine (50 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on a silica gel column, eluting with ethyl acetate−hexane, 7:2 by volume, yielded compound **26** (99 mg, 27%), which was dried under high vacuum at 40−50 °C to provide a stable white foam: IR (KBr, cm^{-1}) 3313, 2935, 1765, 1733, 1655, 1510; ^{1}H NMR (CDCl$_3$) δ 6.95 (s, 1 H, 4-ArH), 6.85 (s, 1 H, 1-ArH), 5.80 (d, J_{NH-6} = 7.2 Hz, 1 H, NH), 5.13 (ddd, J_{NH-6} = 7.2 Hz, J_{6-7} = 11.7 and 6.4 Hz, 1 H, 6β-H), 4.73 (t, J = 8.5 Hz, 1 H, 17α-H), 4.08 (qd, J = 7.1 Hz, J = 1.5 Hz, 2 H, OCH$_2$), 2.56 (m, 1 H), 2.28 (s, 3 H, 3-CH$_3$CO), 2.07 (s, 3 H, 17-CH$_3$CO), 1.95 (s, 3 H; 6-NCOCH$_3$), 2.36−1.26 (m, 17 H), 0.90 (s, 3 H, 18-CH$_3$); CIMS (isobutane) m/z (rel intensity) 472 (MH$^+$, 100). Anal. (C$_{27}$H$_{37}$NO$_6$) C, H, N. The above chromatography also gave compound **27** as a white foam (180 mg, 48%): IR (KBr, cm^{-1}) 3313, 2935, 1765, 1734, 1655, 1510; ^{1}H NMR (CDCl$_3$) δ 6.88 (s, 1 H, 4-ArH), 6.78 (s, 1 H, 1-ArH), 5.68 (d, J_{NH-6} = 8.5 Hz, 1 H, NH), 5.40 (ddd, J_{NH-6} = 8.5 Hz, J_{6-7} = 11.7 and 6.4 Hz, 1 H, 6α-H), 4.69 (t, J = 8.5 Hz, 1 H, 17α-H), 4.08 (qd, J = 7 Hz, J = 1.5 Hz, 2 H, OCH$_2$), 2.52 (m, 1 H), 2.30 (s, 3 H, 3-CH$_3$CO), 2.10 (s, 3 H, 17-CH$_3$CO), 2.07 (s, 6-NCOCH$_3$, 3 H), 2.36−1.26 (m, 17 H), 0.88 (s, 3 H, 18-CH$_3$); CIMS (isobutane) m/z (rel intensity) 472 (MH$^+$, 100). Anal. (C$_{27}$H$_{37}$NO$_6$) C, H, N.

B-Homo-6α-acetamido-2-ethoxy-3,17-β-estradiol (7). A solution of compound **26** (75 mg, 0.16 mmol) in methanol (14 mL) was deoxygenated by bubbling through it a slow stream of nitrogen for 30 min. A similarly deoxygenated 1 M solution of NaOH in water (1.6 mL, 16 mmol) was added and the mixture stirred at room temperature for 5 h. The reaction mixture was neutralized with acetic acid to pH = 6, and then the solvents were removed under reduced pressure. The residue was dissolved in a mixture of ethyl acetate (60 mL) and saturated sodium bicarbonate (50 mL). The organic layer was separated and washed with brine (2 × 30 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on a silica gel column using methylene chloride−acetone, 2:1 by volume, as the eluant gave the desired compound **7** (42 mg, 68%), which solidified from acetone/hexane/methylene chloride to afford a white solid: mp 196−198 °C; IR (KBr, cm^{-1}) 3328, 3313, 2937, 1650, 1512; ^{1}H NMR (CDCl$_3$) δ 6.84 (s, 1 H, 4-ArH), 6.77 (s, 1 H, 1-ArH), 5.73 (d, J_{NH-6} = 7.2 Hz, 1 H, NH), 5.57 (s, 1 H, OH), 5.08 (ddd, J_{NH-6} = 7.2 Hz, J_{6-7} = 11.7 and 6.4 Hz, 1 H, 6β-H), 4.08 (qd, J = 7 Hz, J = 1.5 Hz, 2 H, OCH$_2$), 3.75 (t, J = 8.5 Hz, 1 H, 17α-H), 2.50 (m, 1 H), 2.33 (m, 1 H), 1.95 (s, 3 H, 6-NCOCH$_3$), 2.18−1.26 (m, 17 H), 0.85 (s, 3 H, 18-CH$_3$); CIMS (isobutane) m/z

(rel intensity) 388 (MH$^+$, 100). Anal. (C$_{23}$H$_{33}$NO$_4$·^{2}/$_3$CH$_3$-COCH$_3$) C, H, N.

B-Homo-6β-acetamido-2-ethoxy-3,17-β-estradiol (28). A solution of compound **27** (178 mg, 0.38 mmol) in deoxygenated methanol (14 mL) was treated as described for **7** to afford the desired compound **28** (98 mg, 67%), which was solidified from acetone/hexane/methylene chloride to afford a white solid: mp >162 °C (dec); IR (KBr, cm^{-1}) 3314, 2927, 1658, 1506; ^{1}H NMR (CDCl$_3$) δ 6.78 (s, 1 H, 4-ArH), 6.72 (s, 1 H, 1-ArH), 5.71 (d, J_{NH-6} = 8.8 Hz, 1 H, 6α-CNH), 5.61 (s, 1 H, OH), 5.38 (ddd, J_{NH-6} = 8.8 Hz, 1 H, J_{6-7} = 11.7 and 6.4 Hz, 1 H, 6αH), 4.11 (qd, J = 7.1 Hz, J = 1.5 Hz, 2 H, OCH$_2$), 3.75 (t, J = 8.5 Hz, 1 H, 17α-H), 2.48 (m, 1 H), 2.10 (s, 3 H, 6-NCOCH$_3$), 2.18–1.26 (m, 18 H), 0.83 (s, 3 H, 18-CH$_3$); CIMS (isobutane) m/z (rel intensity) 388 (MH$^+$, 100). Anal. (C$_{23}$H$_{33}$NO$_4$) C, H, N.

B-Homo-2-ethoxy-6-methoximino-3,17β-estradiol (29). To a solution of compound **16** (76 mg, 0.21 mmol) in pyridine (10 mL) was added methoxylamine hydrochloride (354 mg, 4.23 mmol) in one portion under nitrogen. The resulting mixture was heated at 100 °C for 3 h and then cooled to about 50 °C. The pyridine was removed under reduced pressure. The residue was dissolved in a mixture of ethyl acetate (50 mL) and water (50 mL). The ethyl acetate solution was separated and then washed with brine (2 × 30 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on silica gel, eluting with ethyl acetate−hexane, 3:5 by volume, gave compound **29** (78 mg, 94%), which was crystallized from ethyl acetate/hexane to afford white cystals: mp 181−183 °C; IR (KBr, cm^{-1}) 3511, 3318, 2972, 2897, 1618, 1576, 1508; ^{1}H NMR (CDCl$_3$) δ 7.03 (s, 1 H), 6.74 (s, 1 H), 5.53 (s, 1 H), 4.17 (q, J = 7 Hz, 2 H), 3.97 (s, 3 H), 3.78 (t, J = 8.5 Hz, 1 H), 2.58 (m, 2 H), 2.35 (m, 1 H), 1.94 (m, 3 H), 1.78−1.27 (m, 12 H), 0.88 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 374 (MH$^+$, 100). Anal. (C$_{22}$H$_{31}$NO$_4$) C, H, N.

B-Homo-3,17β-di-O-acetyl-2-ethoxy-6-methoximinoestradiol (30). Acetic anhydride (0.6 mL, 6.3 mmol) was added under argon at room temperature to a solution of compound **29** (46 mg, 0.12 mmol) in anhydrous pyridine (3 mL). The resulting mixture was stirred at room temperature for 20 h and poured into ice water (50 g). The compound was extracted with ethyl acetate (3 × 40 mL). The organic layers were washed with sodium bicarbonate (50 mL) and brine (50 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on a silica gel column, eluting with ethyl acetate−hexane, 1:5 by volume, yielded compound **30** (51 mg, 90%), which was dried under high vacuum at 40−50 °C as a stable white foam: IR (KBr, cm^{-1}) 2934, 1770, 1734, 1614, 1500; ^{1}H NMR (CDCl$_3$) δ 7.12 (s, 1 H), 6.81 (s, 1 H), 4.72 (t, J = 8.5 Hz, 1 H), 4.08 (q, J = 7 Hz, 2 H), 3.96 (s, 3 H), 2.57 (m, 2 H), 2.38 (s, 3 H), 2.35 (m, 1 H), 2.06 (s, 3 H), 2.2−1.2 (m, 15 H), 0.90 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 458 (MH$^+$, 100). Anal. (C$_{26}$H$_{35}$NO$_6$) C, H, N.

B-Homo-2-ethoxy-6-oximino-3,17β-estradiol (31). Under nitrogen at room temperature, compound **18** (200 mg, 0.58 mmol) was dissolved in anhydrous pyridine (10 mL), and hydroxylamine hydrochloride (807 mg, 11.6 mmol) was added. The reaction mixture was stirred for 20 h. The pyridine was removed under reduced pressure at 30−35 °C, and the residue was dissolved in a mixture of ethyl acetate (50 mL) and water (30 mL). The ethyl acetate layer was separated, washed with brine (2 × 20 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on silica gel, eluting with ethyl acetate−hexane, 5:4 by volume, yielded compound **31** (198 mg, 95%), which was recrystallized from ethyl acetate as white crystals: mp 226−228 °C; IR (KBr, cm^{-1}) 3452, 3160, 3039, 2932, 2897, 1722, 1599, 1501; ^{1}H NMR (CDCl$_3$ + DMSO-d_6) δ 9.73 (s, 1 H), 6.98 (s, 1 H), 6.73 (s, 1 H), 4.15 (q, J = 7.0 Hz, 2 H), 3.73 (t, J = 8.5 Hz, 1 H), 2.63−1.17 (m, 18 H), 0.86 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 360 (MH$^+$, 100). Anal. Calcd for (C$_{21}$H$_{29}$NO$_4$) C, H, N.

B-Homo-2-ethoxy-3,17β-estradiol-6-ene (32). A solution of methyllithium in THF/cumene (1.0 M, 2 mL, 2.0 mmol) was added dropwise to a stirred solution of compound **13** (75 mg, 0.15 mmol) in THF (10 mL) under argon at 0 °C. The reaction

mixture was allowed to warm to room temperature after 2 h and stirred for 20 h. The reaction mixture was poured into ice (50 g), acidified with 6 N HCl, and extracted with ethyl acetate (3 × 30 mL). The ethyl acetate extracts were washed with saturated sodium sulfate (30 mL) and brine (2 × 30 mL), combined, dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on a silica gel column, using ethyl acetate−hexane, 2:5 by volume, as the eluant, yielded compound **32** (26 mg, 54%), which was crystallized from ethyl acetate/hexane to afford white crystals: mp 167−168 °C; IR (KBr, cm^{-1}) 3503, 3231, 2930, 1603, 1510; ^{1}H NMR (CDCl$_3$) δ 6.82 (s, 1 H), 6.70 (s, 1 H), 6.54 (d, J = 10.1 Hz, 1 H), 6.06 (m, 1 H), 5.51 (s, 1 H), 4.15 (q, J = 7.0 Hz, 2 H), 3.74 (t, J = 8.7 Hz, 1 H), 2.21−1.12 (m, 16 H), 0.84 (s, 3 H, CH$_3$); CIMS (isobutane) m/z (rel intensity) 329 (MH$^+$, 100), 311 (52). Anal. (C$_{21}$H$_{28}$O$_3$·^{1}/$_4$H$_2$O) C, H.

B-Homo-2-ethoxy-7-oximino-3,17β-estradiol (33). Under nitrogen at room temperature, compound **12** (60 mg, 0.17 mmol) was dissolved in anhydrous pyridine (5 mL), and hydroxylamine hydrochloride (240 mg, 3.48 mmol) was added. The reaction mixture was stirred at room temperature for 40 h. The pyridine was removed under reduced pressure at 30−35 °C. The residue was dissolved in ethyl acetate (50 mL) and water (30 mL), and the ethyl acetate was washed with brine (2 × 20 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography of the residue on silica gel, eluting with ethyl acetate−hexane, 1.25:1 by volume, yielded compound **33** as off-white crystals (50.3 mg, 80.3%): mp 236−238 °C; IR (KBr, cm^{-1}) 3292 (br), 2930, 1737, 1592, 1510; ^{1}H NMR (CDCl$_3$ + DMSO-d_6) δ 9.88 (s, 1 H), 7.03 (s, 1 H), 6.77 (s, 1 H), 6.71 (s, 1 H), 4.12 (q, J = 7.0 Hz, 2 H), 3.86 (d, J = 20.1 Hz, 1 H), 3.68 (t, J = 8.5 Hz, 1 H), 3.43 (d, J = 19.8, 1 H), 2.20−1.10 (m, 16 H), 0.84 (s, 3 H); CIMS (isobutane) m/z (rel intensity) 360 (MH$^+$, 100). Anal. (C$_{21}$H$_{29}$NO$_4$·^{1}/$_6$H$_2$O) C, H, N.

X-Ray Crystallographic Analysis of 7. A colorless plate of **7** hydrate, C$_{23}$H$_{35}$NO$_5$ (0.25 × 0.17 × 0.13 mm), was mounted on glass fibers in a random orientation. Preliminary examination and data collection were performed with Mo Kα radiation (Kα = −/71073 Å) on a Nonius Kappa CCD diffractometer. The cell constants and an orientation matrix for data collected were obtained from least-squares refinement using the setting angles of 4017 reflections in the ranges of 4 < θ < 27 °C. The data were collected at 293 ± 1 K. A total of 4017 reflections were collected, of which 2560 were unique. The structure was solved by direct methods using SIR97. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in refinement but restrained to ride on the atom to which they were bound, where the function minimized was $\sum w(|F_o|^2 - |F_c|^2)^2$. Refinement was performed on an AlphaServer 2100 using *SHELX-97.* Crystallographic drawings were done using programs ORTEP.

Tubulin Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[40] Determination of IC$_{50}$ values for inhibition of polymerization of purified tubulin was performed as described in detail elsewhere,[12] except that Beckman DU7400/7500 spectrophotometers equipped with "high performance" temperature controllers were used. Unlike the manual control possible with the previously used Gilford spectrophotometers, the polymerization assays required use of programs provided by MDB Analytical Associates, South Plainfield, NJ, since the Beckman instruments are microprocessor-controlled. The Beckman instruments were unable to maintain 0 °C, and the lower temperature in the assays fluctuated between 2 and 5 °C. Temperature changes were, however, more rapid than in the Gilford instruments with the jump from the lower temperature to 26 °C taking about 20 s and the reverse jump about 95 s. In most cases the Beckman instruments yield somewhat lower IC$_{50}$ values than were obtained previously with the Gilford instruments (cf. values for **1** and **8** with those previously reported). The reason for this is unknown. In brief, tubulin was preincubated at 26 °C with varying compound concentrations, reaction mixtures were chilled on ice, GTP (required for the polymerization reaction) was added, and polymerization

was followed at 26 °C by turbidimetry at 350 nm. The extent of polymerization after 20 min was determined. IC$_{50}$ values were determined graphically. All compounds were examined in at least two independent assays.

For examination of compounds that enhanced tubulin polymerization, the Gilford model 250 instruments, equipped with electronic temperature controllers, were used because of their superior temperature control. In addition, the Gilford instruments have more stable readings and are thus more reliable for rate comparisons. There was no drug−tubulin preincubation in these studies. Reaction mixtures (0.25 mL) contained 1.2 mg/mL (12 μM) tubulin, 4% (v/v) dimethyl sulfoxide, 0.8 M monosodium glutamate (pH 6.6), 0.4 mM GTP, and varying compound concentrations. Reaction mixtures were transferred to cuvettes held at 0 °C, baselines were established, and the temperature was jumped to 24 °C (about 50 s). The reaction was followed for about 25 min, and the temperature was reverse jumped to 0 °C (about 4 min). In these experiments the A_{350} in each sample was followed continuously for 5 s, and approximately three readings per minute were taken. Maximal rates were directly determined from the tracings. The EC$_{50}$ values were determined graphically, by direct comparison to a simultaneous reaction with 100 μM compound **16**.

Note Added in Proof: At the 91st Annual Meeting of the American Association for Cancer Research, April 1−5, 2000, a steroidal natural product was reported to have microtuble-stabilizing activity. Mooberry, S. L.; Hernandez, A. H.; Tien, G.; Hemscheidt, T. K. Discovery of a New Microtubule-Stabilizing Agent from a Tropical Plant. Abstract #3523.

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