typed the manuscript, and Drs. L. R. Hughes, M. Jarman, and R. G. Moran for helpful discussions.

Registry No. **1a**, 19181-53-4; **1b**, 18731-19-6; **1c**, 50440-82-9; **2a**, 13116-91-1; **2b**, 13116-92-2; **2c**, 131066-72-3; **3** (R = SMe), 91088-93-6; **3a**, 131066-74-5; **3b**, 131066-75-6; **3c**, 131066-73-4; **4**, 131066-76-7; **5a**, 131066-77-8; **5b**, 131066-78-9; **5c**, 131066-79-0; **6**, 131066-80-3; **7**, 76858-72-5; **8a**, 131078-76-7; **8b**, 131078-77-8; **8c**, 131066-81-4; **9a**, 131066-82-5; **9b**, 131066-83-6; **9c**, 131066-84-7; **10a**, 131066-85-8; **10b**, 131066-86-9; **10c**, 131066-87-0; **11**, 70280-71-6; **12**, 131078-75-6; **13**, 131066-88-1; **14**, 131066-89-2; TS, 9031-61-2.

Synthesis and Antitumor Activity of Structural Analogues of the Epipodophyllotoxins

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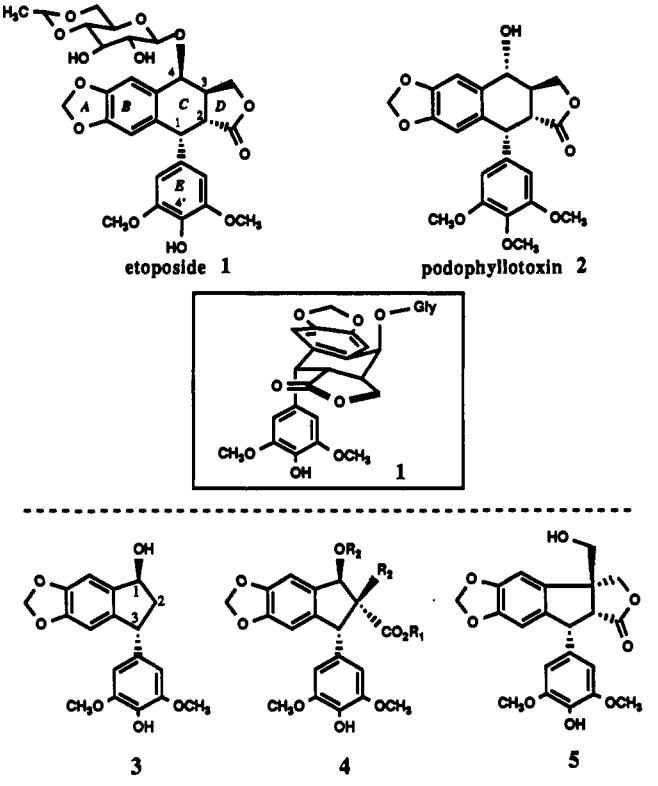
Several ring-contracted analogues of the antitumor agent etoposide have been prepared. The synthesis of the simple indanyl system **3** is described along with two bicyclic systems of general structure **4** prepared through a stereoselective allylation of the keto-ester **6**. A cis-fused lactone analogue **5**, which is isomeric with the etoposide aglycone, has been synthesized via a dialkylation of the indene-2-carboxylate anion. Regiochemical and stereochemical results of these alkylations are described. The cytotoxicity of these derivatives toward several tumor cell lines is described and generally follows the structure–activity relationships known for the agent podophyllotoxin (**2**).

Introduction

The antitumor agent etoposide **1** is a semisynthetic compound prepared in several steps from the naturally occurring lignan podophyllotoxin (**2**)[1a] (Scheme I). While both these molecules exhibit antitumor activity, podophyllotoxin has been shown to enact its effects via inhibition of cellular microtubule assembly mechanisms.[1b] In contrast to this activity, the potent antitumor activity of etoposide is believed to be due to inhibition of the enzyme topoisomerase-II and not due to any direct interaction with endogenous DNA.[2a] Although etoposide has seen much use in the clinic, problems such as myelosuppression, drug resistance, and poor bioavailability[2b] have encouraged further modifications. Recently, several amino analogues,[3a] ring C aromatic analogues,[3b] ring E desoxy analogues,[3c] and even a phosphorylated prodrug[3d] have been prepared and utilized in order to circumvent these problems.

At present, almost all reported antitumor agents in the podophyllotoxin series have been derived from the naturally occurring parent or incorporate its carbon skeleton and thus, the type and variation of analogues has been limited by the structure and stability of this parent. Besides some minor modifications on the two aromatic rings, little work has been done to affect changes in the gross structural skeleton.[4] In order to have greater freedom for modification of these molecules and access to new structural congeners, we felt a total synthetic effort was required. Therefore, we report here the synthesis of several sets of racemic analogues of **1** and **2** having modified ring systems, along with their in vitro antitumor activities against the A549, HT-29, and P388-D1 tumor cell lines.

Scheme I



Chemistry

Initially, we were interested in delineating which structural elements of etoposide were *minimally* required in order to produce the maximum desired activity. Upon investigation, the most striking structural aspect of etoposide is the trans diaxial placement[4] of the C4 glycoside and the C1 aryl substituents as shown in Scheme I. This

(1) (a) Keller-Juslen, C.; Kuhn, M.; von Wartburg, A.; Stahelin, H. *J. Med. Chem.* **1971**, *14*, 936. (b) Wilson, L.; Friedkin, M. *Biochemistry* **1967**, *6*, 3126.

(2) (a) Van Maanen, J. M. S.; Retel, J.; de Vries, J.; Pinedo, H. M. *J. Natl. Cancer Inst.* **1988**, *80*, 1526. Long, B. H. *NCI Monographs* **1987**, *No. 4*, 123. (b) Shah, J. C.; Chen, J. R.; Chow, D. *Pharm. Res.* **1989**, *6*, 408.

(3) (a) Nishimura, Y.; Saito, N.; Kondo, S.; Umezawa, H. *Chem. Lett.* **1987**, 799. Saito, H.; Nishimura, Y.; Kondo, S.; Komura, K.; Takeuchi, T.; *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2493. Lee, K.-H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H.-J.; Chen, C.-H. *J. Nat. Prod.* **1989**, *52*, 606. Lee, K.-H.; Beers, S. A.; Mori, M.; Wang, Z.-Q.; Kuo, Y.-A.; Li, L.; Liu, S.-Y.; Chang, J.-Y.; Hans, F.-S.; Cheng, Y.-C. *J. Med. Chem.* **1990**, *33*, 1364. (b) Beers, S. A.; Imakura, Y.; Dai, H.-J.; Li, D.-H.; Cheng, Y. C.; Lee, K.-H. *J. Nat. Prod.* **1988**, *51*, 901. (c) Saulnier, M. G.; Vyas, D. M.; Langley, D. R.; Doyle, T. W.; Rose, W. C.; Crosswell, A. R.; Long, B. H. *J. Med. Chem.* **1989**, *7*, 1420. (d) Senter, P. D.; Saulnier, M. G.; Schreiber, G. J.; Hirschberg, D. L.; Brown, J. P.; Hellstrom, I.; Hellstrom, K. E. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4842.

(4) Beard, A. R.; Drew, M. G. B.; Hilgard, P.; Hudson, B. D.; Mann, J.; Neidle, S.; Wong, L. F. T. *Anti-Cancer Drug Design* **1987**, *2*, 247. Yamamoto, D.; Ohishi, H.; Kozawa, M.; Inamori, Y.; Ishida, T.; Inoue, M. *Chem. Pharm. Bull.* **1988**, *36*, 3239.

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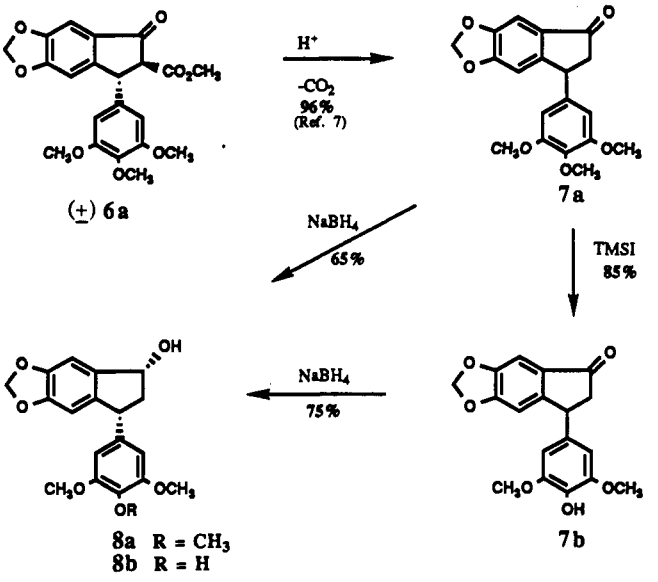
typically unstable conformation is "locked" in place due to the presence of the trans-fused lactone ring. Release of this strain through C2 epimerization has been found to occur in part upon in vivo administration of 1 in humans with the epimeric product exhibiting no antitumor activity.[5]

In order to circumvent this inactivation while still retaining structural similarity, we investigated three new systems (Scheme I): (1) The *trans*-3-arylindanol system **3**, where the lactone ring is omitted but the trans oxy and aryl substituents are retained in a 1,3-trans relationship on a smaller five-membered ring. This ring system, by virtue of its geometry, would allow for an arrangement of substituents similar to that in etoposide. If the lactone ring were necessary only to shape the molecule and not for its intrinsic electronic effects, these analogues should retain activity. (2) The indanol-2-carboxylate analogues (**4**), which in addition to retaining the above-mentioned structural elements also have the C2 carboxyl group in the desired cis stereochemistry relative to the C3 aryl substituent. Furthermore, to block the possible C2 epimerization in this series we have appended a carbon chain at C2 which can be incorporated into the structure of the target either as a spiro lactone ring (R_1–R_3 connection) or a tetrahydrofuran ring (R_2–R_3 connection). (3) The cis-fused lactone system (**5**) which is actually a constitutional isomer with the etoposide aglycone.

Through investigation of computer-assisted molecular design we found that lactone **5** was a good structural match with the epipodophyllotoxins in terms of rings A, B, and C and the trans substituents at C1 and C4 of the parent structure.[6] The lactone oxygens are retained as in the parent for their potential electronic effects, and by virtue of this lactone being cis-fused, epimerization to the trans isomer would be highly disfavored. Although the structure of this ring is skewed relative to the plane of the tricyclic system, the C1, C2, and C3 substituents all show reasonable overlap to their respective groupings on the parent system **1** based on models. Since the glucoside moiety in **1** has been shown to be unimportant in terms of its in vitro topoisomerase-II activity, and in order to simplify the chemistry, several of our targets were initially planned as the non-glycosidated derivatives.

(a) Indan System. A previous synthetic approach to podophyllotoxin by Jung[8] described several easily accessible derivatives such as indanone **6** which served as our starting material (Scheme II). Decarboxylation of **6** afforded indanone **7a** which was reduced under standard conditions with sodium borohydride. Literature precedence[9] for reduction of such ketones suggested that the cis alcohol would be produced, and following NOE analysis of **8**, this stereochemical assignment was corraborated. Demethylation of the C-4′ methoxy substituent is believed to be imperative for activity versus the topo-II enzyme,[7] and literature methods have typically utilized HBr in polar solvents for that purpose with poor to moderate yields. We have found that treatment with trimethylsilyl iodide

Scheme II



(TMSI) smoothly affords the C4′ demethylated ketone **7b**. Subsequent reduction as before leads, in this case, to the diol **8b**. Investigation of the solvolysis reaction of alcohols **8a,b** was carried out in order to determine the stereochemical result of the reaction. In the case of the naturally occurring system, reaction of **2** with Lewis acids in the presence of a nucleophile afforded primarily the desired 1,4-trans product.[10] In our case, although **8a,b** underwent reaction in a smooth manner with the hydroxylic reactants shown in Scheme III, the isolated products consisted of cis–trans mixtures. The use of water or methanol as nucleophile led to a non-selective addition to the presumed indanyl cation intermediate, while cyclohexanol afforded higher selectivity with the desired trans isomer predominating. The generally poorer selectivity of the indanol series versus the parent series probably reflects the less sterically demanding structure of the cation formed from **8b**. When *tert*-butyl alcohol was used only the cis product was obtained due to the formation of the more stable *tert*-butyl cation. In order to obtain **11**, the trans alcohol **3** (as prepared above) was used as the nucleophilic component trapping the *tert*-butyl cation.

(b) Hydroxyindan-2-carboxylates. During the investigation of the chemistry of keto ester **6a** we found that alkylation with reagents such as allyl bromide, gave, as expected, the monoallyl product **12** (Scheme IV). The relative stereochemistry of the C2 carboxyl and C3 aryl substituents was shown to have the required cis relationship through NOE analysis. If excess base and bromide were used deprotenation at C3 presumedly occurs to yield the C2,C3-diallyl derivative **13**. The proposed cis stereochemistry of these two allyl groups is suggested by preliminary study of ^{1}H NMR data. From monoallyl product **12**, the key intermediate diol **14** was obtained through a sequence of reactions involving initial reduction to the indanol ester, oxidative cleavage of the olefin by OsO_4–$NaIO_4$ and subsequent reduction. Due to facile lactonization under these conditions, no better than a 1:1 mixture of easily separable diol–lactone (**14** and **16**, respectively) could be achieved in total 34% yield. This diol was treated with boron trifluoride etherate as in section a, affording ether **15a** in 94% yield via an intramolecular solvolysis reaction. After several attempts to remove the C4′-methyl

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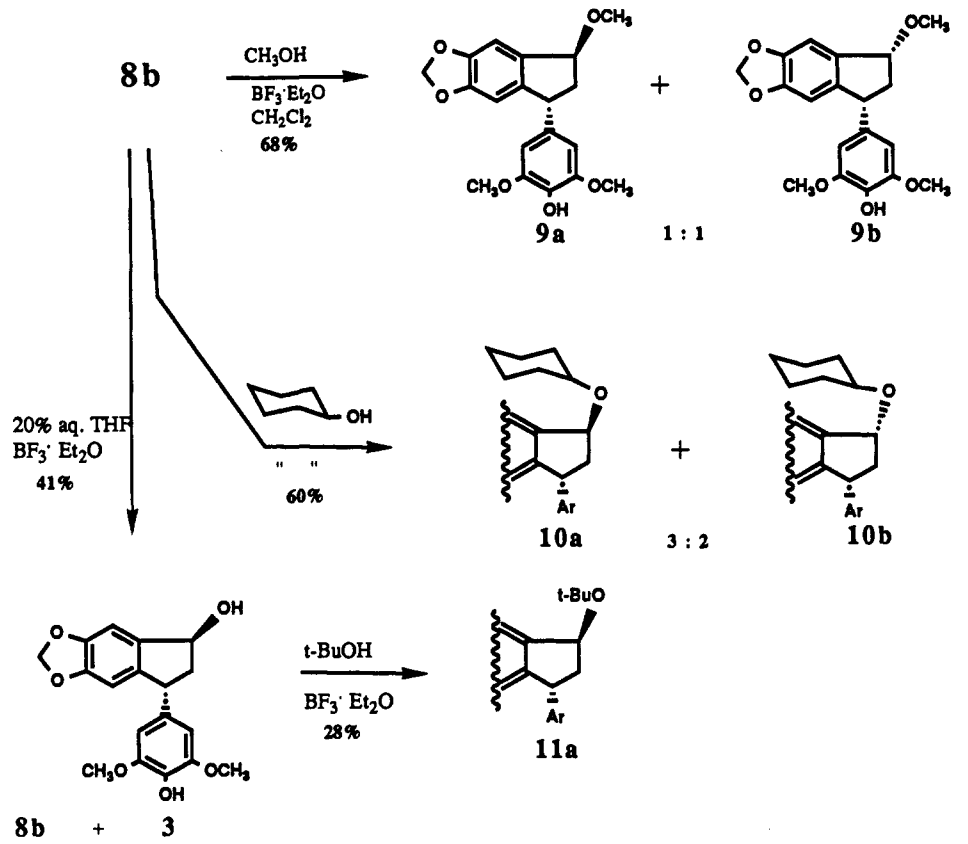
(9) Hanaya, K.; Kitamoto, S.; Kudo, H.; Mitsui, S. *Nippon Kagaku Kaishi* **1978**, *2*, 238; *Chem. Abstr.* **1978**, *88*, 151761s.

(10) Kuhn, M.; von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 948.

Scheme III



group, ether **15a** was finally demethylated with TMSI, albeit in 30% yield, to give the C4′-OH product **15b**. Toward our second target, diol **14** could also be lactonized to **16a** in 94% yield by treatment with diazabicycloundecene (DBU) (Scheme V). Demethylation of this lactone was carried out in an indirect manner since standard methods such as HBr or TMSI affected decomposition of the reactants. The C1 hydroxyl group of **16a** was "protected" by initial oxidation to ketone **17a**, and subsequent TMSI treatment afforded the demethylated ketone **17b** in total 90% yield. Hydride reduction of this ketone gave primarily the α-alcohol which, when finally treated with TMSI and hydrolyzed, gave the β-epimer **18**.

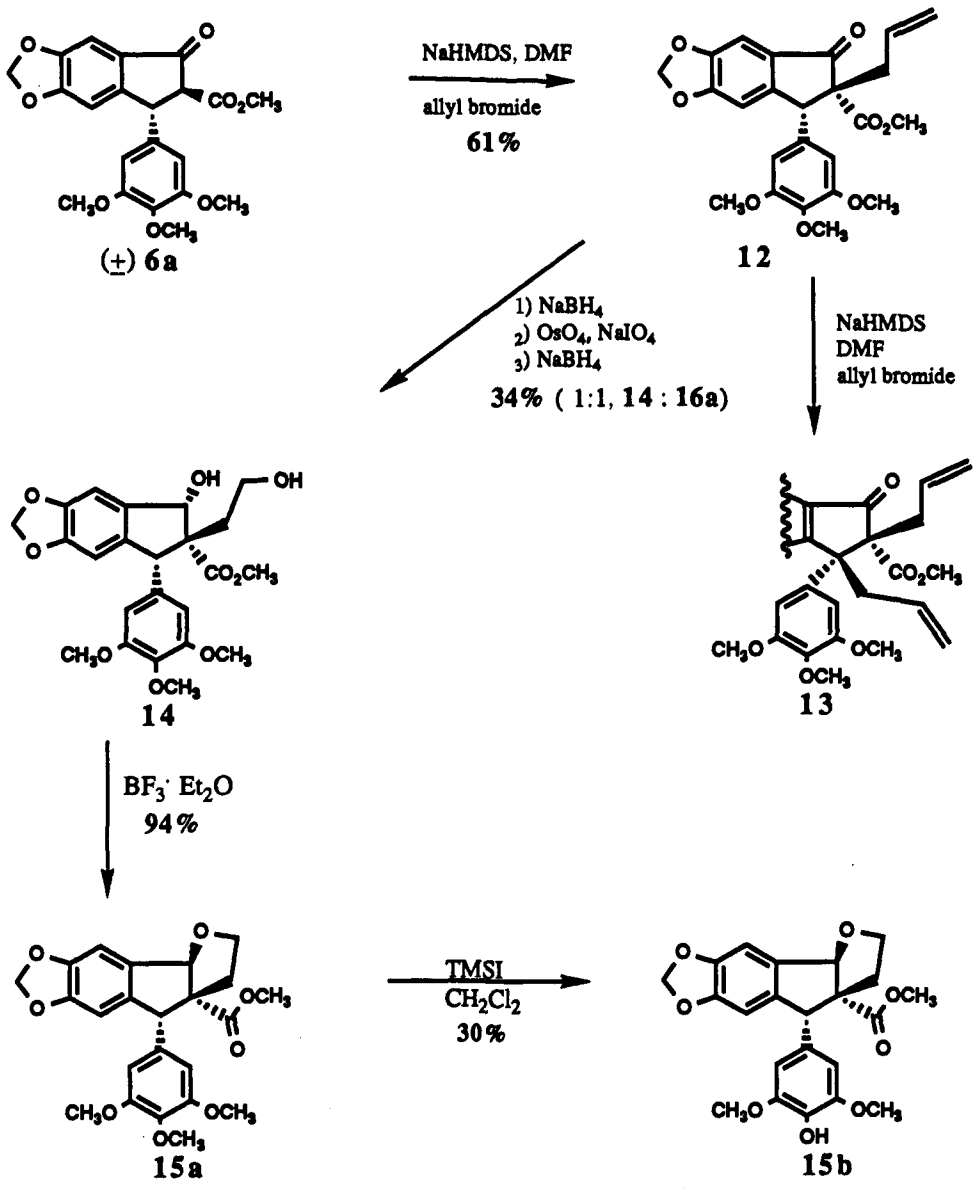
(c) Cis-Fused Lactone Analogue. Study of the structure of the target lactone **5** suggested that the requisite relative stereochemistry at C2–C3 could be obtained via cis hydrogenation of the olefin in indene **19** (Scheme VI). Lactonization of this product followed by demethylation of the C4′ position as before should lead to **5**. The synthesis of **19**, in turn, was envisioned as proceeding via geminal dialkylation of **20** with use of (benzyloxy)methyl chloride (BOMCl) as a formaldehyde equivalent. The latter reagent was chosen as electrophile since subsequent hydrogenolytic removal of the incipient benzyl groups could be carried out concomitant with the aforementioned cis hydrogenation.[11a] Previous to this work, no data was available regarding the regiochemical results of alkylations of such indene substrates. We realized that in order to produce indene **23**, alkylation of the initial anion of **20a** must occur at C1 and not at C3. Following deprotonation of this product, this anion could then undergo the second alkylation at C3 or C1 thus yielding the 1,3-dialkylated indene or the desired 1,1-dialkylated indene **20**, respectively. The preparation of **20a** itself proceeded in a straightforward manner in 73% yield from keto ester **6a** by standard reduction of the indanone carbonyl followed by elimination using toluenesulfonic acid. Initial studies of the alkylation of indene **20a** using methyl iodide as the model electrophile led to approximately 2:1 mixtures of 1,3- to 1,1-dialkylated product with some monoalkyl product.[11b] Variation of solvent, counterion, and base did not change the regioselectivity.

In spite of these results the olefin **20a** was treated with sodium hexamethyldisilazide as above, followed by 2.5 equiv of the electrophile (benzyloxy)methyl chloride (Scheme VII). This resulted in a 68% yield of products with the desired 1,1-dialkylated product **21** being favored 2:1 over the other isomers. These products could be separated at this stage or more conveniently hydrogenated directly, thereby reducing the C2–C3 olefin in a cis fashion, cleaving both benzyl protecting groups and effecting lactonization to the bicyclic product. Following chromatographic separation, lactone **22** was produced in 43% yield from **20a**. NOE analysis of the ^{1}H NMR gave clear evidence for the expected stereochemistry. Unfortunately all attempts to demethylate the C4′-methoxy substituent of **22** failed.

In order to circumvent this problem, the starting material keto ester **6a** was initially demethylated with TMSI to give keto ester **6b** (Scheme VI). Reduction and dehydration of **6b** as before smoothly afforded olefin **20b** in 56% total yield. Utilizing indene **20b** in the alkylation sequence had another advantage in that the penultimate phenolic (benzyloxy)methyl ether will be removed in the subsequent hydrogenation step without requiring an additional deprotection step.

(11) (a) Although racemic material was appropriate for our testing purposes, our synthetic plan was designed for the production of chiral product. Since all stereochemical centers are produced in this final step, an asymmetric hydrogenation or asymmetric lactonization of intermediate **23** could lead to a chiral synthesis of **5**. (b) Klein, L. L.; Yeung, C. M. Unpublished work.

Scheme IV



Alkylation of **20b** with BOMCl afforded a 70% yield of products which contained 57% of the desired 1,1-dialkylated 4′-[(benzyloxy)methyl]oxy]indan **23** (Scheme VII). Hydrogenation as before served to remove both the benzyl and (benyloxy)methyl protecting groups concomitant with reduction of the olefin, and following in situ lactonization, gave the desired racemic target **5** in 43% yield from **20b**. Spectral analysis and comparison to **22** supported the stereochemical structure shown.

Biological Results

All newly synthesized compounds were initially tested for DNA cleavage activity by the standard topoisomerase II-mediated DNA breakage assay described by Liu[12] and referenced to etoposide (1) and its equally active deglucosyl derivative as standards.[13] We found that none of our compounds showed measurable activity in this assay, and these results lead us to believe that the strained trans-fused lactone ring and/or the C1 hydroxymethyl group are conformationally sensitive.

We have observed, however, that several of these compounds exhibit in vitro antitumor activity. The antiproliferative effects of these agents were determined with human and murine tumor cell lines by using a colorimetric microtiter assay as modified from Mosmann[14] (see Experimental Section). Tumor cell lines obtained from the ATCC were the P388 mouse leukemia, A549 human lung carcinoma, and HT-29 human colon adenocarcinoma. Results of these assays are reported in Table I as the concentration of agent producing a 50% growth inhibition (IC_{50}) as determined by linear regression of dose–response curves. Standard reference antitumor agents etoposide (1), podophyllotoxin (2), and adriamycin were included in each experimental run for purposes of comparison.

Based upon the antitumor results listed in Table I several general correlations are observed (1) in any series having both the C4′ hydroxyl and C4′ methoxyl analogues, the latter derivative displayed greater antitumor activity; (2) in any series of C1–C3 cis and trans stereoisomers, the

(12) Liu, L. F.; Rowe, T. C.; Yang, L.; Tewey, K. M.; Chen, G. L. *J. Biol. Chem.* **1983**, *258*, 15365.

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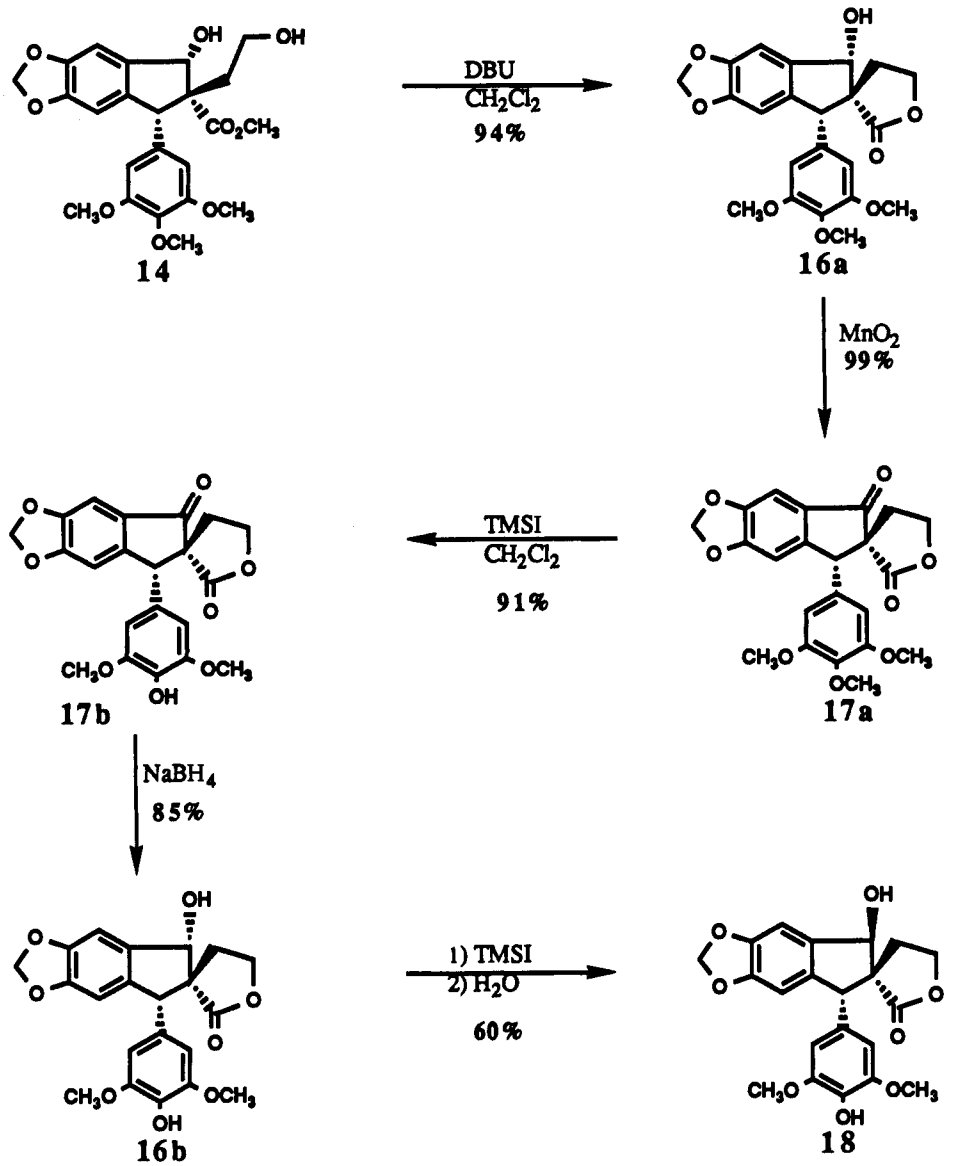
Scheme V



cis isomers showed greater activity; and (3) of the three general structural types studied, i.e. the simple indanes, the indanol-2-carboxylates, and the cis-fused lactones, the indance series exhibited greatest activity.

Of the derivatives listed in Table I, the most potent analogue was *cis*-3-(3′,4′,5′-trimethoxyphenyl)-5,6-(dioxymethylene)indanol (**8a**). Since the structural factors in etoposide such as the unsubstituted C4′ hydroxyl group and the trans stereochemistry between the C1 oxygen and the trioxyaromatic substituents have been shown to be critical for expression of the topo II mediate DNA breakage activity, and since these factors are contrary to those found in **8a**, it is not surprising that this derivative exhibits no breakage activity; however, since this compound was fairly potent in the in vitro cell culture assay, we suspect that its mode of action is similar to that of podophyllotoxin. Mode of action studies based on assays other than topo II have yet to be investigated for this new series.

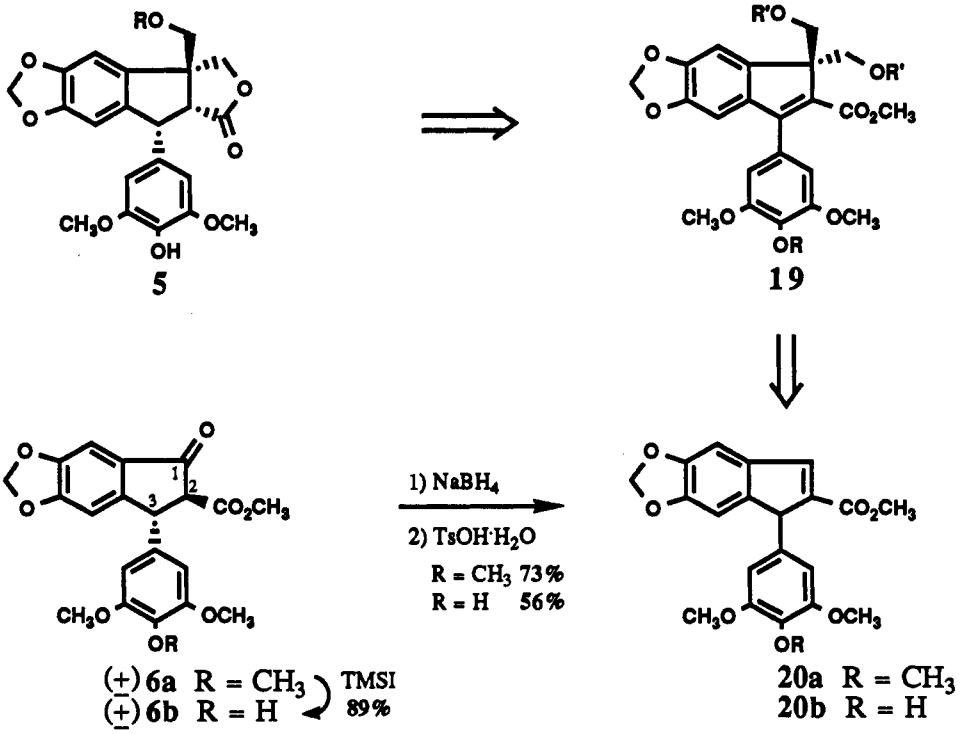
Experimental Section

^1H NMR spectra were recorded on a General Electric QE300 spectrometer with Me_4Si as an internal standard. Elemental analyses were performed by Abbott Laboratories, Abbott Park, IL 60064-3500. The high-resolution MS were obtained on a Kratos MS50 instrument at Abbott Laboratories. Analytical TLC (Silicagel, 0.25 mm) retention factors and elution solvents were listed for purification. E. Merck silica gel (230–400 mesh) obtained from VWR Scientific was used for column chromatography. Melting points were measured with a Fisher-Johns apparatus and are uncorrected. CH_2Cl_2 was distilled from P_2O_5 and THF was distilled from sodium and benzophenone. All other solvent were HPLC grade and were not purified prior to use.

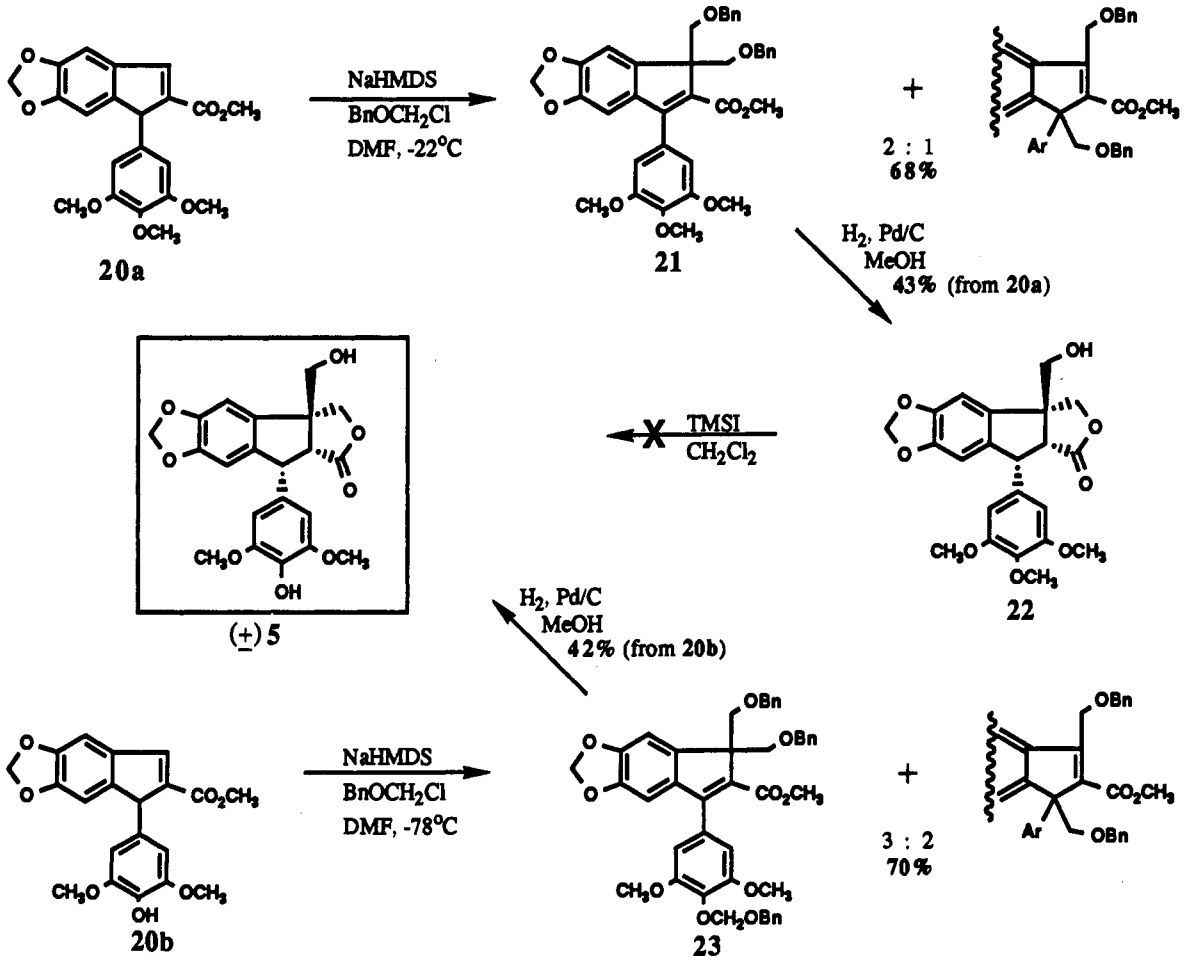
C4′-OH Indanone 7b. To a solution of indanone **7a**[8] (4.5 g, 13.2 mmol) in 90 mL of CH_2Cl_2 at room temperature was added TMSI (4.5 mL, 2.4 equiv). The brownish mixture was stirred for 6 h and quenched with 1 M $NaHCO_3$. The organic solvent was evaporated, and the resulting gummy material was purified by column chromatography on silica gel (200 g) with 2.5% MeOH/$CHCl_3$: yield, 3.69 g (85%); mp 200–204 °C (EtOAc/hexane); TLC (2.5% CH_3OH/CH_2Cl_2, R_f = 0.3); ^1H NMR ($CDCl_3$) δ 7.13 (1 H, s, H7), 6.62 (1 H, s, H4), 6.32 (2 H, s, H2′, 6′), 6.08 (2 H, AB q, OCH_2O), 5.45 (1 H, s, ArOH), 4.34 (1 H, dd, H3), 3.83 (6 H, s, OCH_3), 3.19 (1 H, dd, H2), 2.65 (1 H, dd, Hz); HRMS (EI/HRP) calcd for $C_{18}H_{16}O_6$ 328.0947, found 328.0948. Anal. ($C_{18}H_{16}O_6$) C, H; C: calcd, 65.83; found, 64.37.

Cis C4′-OH Indanol 8b. To a solution of **7b** (3.5 g, 10.7 mmol) in 100 mL of 95% MeOH at room temperature was added $NaBH_4$ (0.7 g, 1.73 equiv). After 0.5 h, the homogeneous mixture was quenched with 15 mL of 1 M H_3PO_4. The resulting solid obtained after solvent removal was purified by flash chromatography on silica gel (130 g) with 2.5% MeOH/CH_2Cl_2: yield, 2.65 g (75%) of **8b** as fine needles; mp 164–166 °C (30% EtOAc/hexane); ^1H

Scheme VI



Scheme VII



NMR ($CDCl_3$) δ 6.90 (1 H, s, H7), 6.45 (2 H, s, H2′, 6′), 6.40 (1 H, s, H4), 5.96 (1 H, d, J = 1.47 Hz, OCH_2O), 5.93 (1 H, d, J = 1.47 Hz, OCH_2O), 5.44 (1 H, s, ArOH), 5.17 (1 H, q, J = 7.35 Hz, H1), 4.02 (1 H, t, J = 8.09 Hz, H3), 3.85 (6 H, s, OCH_3), 3.00 (1 H, m, H2), 1.92 (1 H, M, H2). Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.43; H, 5.50. Found: C, 65.21; H, 5.45.

Trans C4′-OH Indanol 3. To a solution of indanol **8b** (100 mg, 0.3 mmol) in 2 mL of CH_2Cl_2, 2 mL of THF, and 0.5 mL of

Table I. In Vitro IC_{50} (μg/mL) Values for Indan Derivatives As Determined by MTT-Colorimetric Microtiter Assay[a]

compound	tumor cell lines		
	A549[b]	HT-29[c]	P388-D1[d]
adriamycin	0.053	0.11	0.017
etoposide (1)	1.03	1.7	0.063
podophyllotoxin (2)	0.0043	0.0043	0.0013
3	9.9	12.0	4.2
5	12.5	12.2	8.0
7a	2.9	2.9	1.1
7b	19.4	19.2	1.7
8a	0.038	0.032	0.014
8b	0.76	2.1	0.53
9a	46.9	41.5	8.1
9b	1.1	3.2	0.57
10a	9.7	8.6	6.6
10b	12.0	10.3	7.9
11a	31.3	28.8	7.1
15a	11.9	17.4	5.7
15b	>50.0	>50.0	43.0
16a	5.1	5.3	2.5
16b	>50.0	>50.0	>50.0
18	48.2	>50.0	16.8
22	1.2	1.2	0.59

[a] See Experimental Section for methods. [b] Human lung carcinoma. [c] Human colon adenocarcinoma. [d] Mouse leukemia.

H_2O at room temperature was added $BF_3{\cdot}OEt_2$ (0.15 mL, 4 equiv). After 1 h, the reaction was quenched with 1 M $NaHCO_3$ and extracted with EtOAc. The organic layer was evaporated, and the resulting solid was purified by flash chromatography on silica gel (30 g) with 10% Et_2O/CH_2Cl_2: yield, 15 mg (20%) of **3** with starting material recovery **8b** (24 mg, 24%); ^1H NMR ($CDCl_3$) δ 6.90 (1 H, s, H7), 6.46 (1 H, s, H4), 6.34 (2 H, s, H2′, 6′), 5.96 (1 H, d, J = 1.10 Hz, OCH_2O), 5.93 (1 H, d, J = 1.47, OCH_2O), 5.42 (1 H, s, ArOH), 5.29 (1 H, m, H1), 4.44 (1 H, t, H3), 3.84 (6 H, s, OCH_3), 2.52 (1 H, m, H2), 2.35 (1 H, m, H2), 1.68 (1 H, br s, OH). Anal. Calcd for $C_{18}H_{18}O_6$: C, 65.43; H, 5.50. Found: C, 65.28; H, 5.53.

Methyl Ether 9a. To a solution of indanol **8b** (100 mg, 0.3 mmol) and methanol (0.1 mL, 7.7 equiv) in 15 mL of CH_2Cl_2 at −25 °C was added $BF_3{\cdot}OEt_2$ (50 μL, 1.35 equiv). The reaction was stirred for 1 h and quenched with 1 M $NaHCO_3$. The residue obtained after solvent removal was purified by flash chromatography on silica gel (25 g) with 35% EtOAc/hexane, yield, 37 mg (35%) of trans **9a** with 35 mg (33%) of the cis isomer **9b**. **9a**: mp 113–115 °C; ^1H NMR ($CDCl_3$) δ 6.90 (1 H, s, H7), 6.46 (1 H, s, H4), 6.36 (2 H, s, H2′, 6′), 5.98 (1 H, d, J = 1.47 Hz, OCH_2O), 5.94 (1 H, d, J = 1.10 Hz, OCH_2O), 4.79 (1 H, dd, J = 1.47 and 6.25 Hz, H1), 4.41 (1 H, t, J = 7.71 Hz, H3), 3.85 (6 H, s, $ArOCH_3$), 3.40 (3 H, s, OCH_3), 2.62 (1 H, m, H2), 2.21 (1 H, m, H2); HRMS (EI/HRP), calcd for $C_{19}H_{20}O_6$ 344.1260, found 344.1252. Anal. ($C_{19}H_{20}O_6$) C, H; C: calcd, 66.25; found, 65.47. **9b**: mp 125–127 °C; ^1H NMR ($CDCl_3$) δ 6.89 (1 H, s, H7), 6.49 (2 H, s, H2′, 6′), 6.37 (1 H, s, H4), 5.98 (1 H, d, J = 1.47 Hz, OCH_2O), 5.96 (1 H, d, J = 1.10 Hz, OCH_2O), 4.79 (1 H, t, J = 6.25 Hz, H1), 4.01 (1 H, t, J = 7.72 Hz, H3), 3.86 (6 H, s, $ArOCH_3$), 3.49 (3 H, s, OCH_3), 2.93 (1 H, m, H2), 1.96 (1 H, m, H2).

Cyclohexyl Ether 10a. **10a** was prepared according to the procedure outlined in the synthesis of **9a**, yield, 30 mg (24%) of trans **10a** and 45 mg (36%) of the cis isomer **10b** starting with 100 mg of **8b**. **10a** (amorphous solid): ^1H NMR ($CDCl_3$) δ 6.85 (1 H, s, H7), 6.45 (1 H, s, H4), 6.34 (1 H, s, H2′, 6′), 5.96 (1 H, d, J = 1.47 Hz, OCH_2O), 5.92 (1 H, d, J = 1.47 Hz, OCH_2O), 5.03 (1 H, dd, J = 2.57 and 6.25 Hz, H1), 4.43 (1 H, t, H3), 3.84 (6 H, s, OCH_3), 3.42 (1 H, m, $OCHR_2$), 2.55 (1 H, m, H2), 2.22 (1 H, m, H2), 1.15–2.0 (10 H, m, cyclohexyl); HRMS (EI/HRP), calcd for $C_{24}H_{28}O_6$ 412.1886, found 412.1883. Anal. ($C_{24}H_{28}O_6$) C, H; C: calcd, 69.87; found, 68.66. **10b**: ^1H NMR ($CDCl_3$) δ 6.86 (1 H, s, H7), 6.49 (2 H, s, H2′, 6′), 6.36 (1 H, s, H4), 5.91 (2 H, AB q, OCH_2O), 4.95 (1 H, t, J = 6,62 Hz, H1), 3.96 (1 H, t, H3), 3.85 (6 H, s, OCH_3), 3.52 (1 H, m, $OCHR_2$), 2.92 (1 H, m, H2), 1.95 (1 H, m, H2), 1.2–2.1 (10 H, m, cyclohexyl). Anal. ($C_{24}H_{28}O_6$) C, H; C: calcd, 69.87; found, 68.90.

***tert*-Butyl Ether 11a.** **11a** was prepared according to the procedure outlined in the synthesis of **9a**: yield, 33 mg (28%) starting with 100 mg of **3**; mp 131–134 °C; ^1H NMR ($CDCl_3$) δ 6.78 (1 H, s, H7), 6.44 (1 H, s, H4), 6.30 (2 H, s, H2′, 6′), 5.95 (1 H, d, OCH_2O), 5.91 (1 H, d, OCH_2O), 5.10 (1 H, dd, H1), 4.41 (1 H, dd, H3), 3.83 (6 H, s, OCH_3), 2.5 (1 H, m, H2), 2.32 (1 H, m, H2), 1.30 (9 H, s, *tert*-butyl); HRMS (EI/HRP), calcd for $C_{22}H_{28}O_6$ 386.1729, found 386.1731. Anal. ($C_{22}H_{28}O_6$) C, H; C: calcd, 68.36; found, 66.85.

Allyl Keto Ester 12. To a solution of keto ester **6a**[7] (4.5 g, 11.3 mmol) in 100 mL of DMF at room temperature was added portionwise 1 M NaHMDS/THF (10 mL, 1.8 equiv). The reaction was stirred for 15 min and allyl bromide (1.5 mL, 1.5 equiv) was added. After 0.5 h, the reaction mixture was heated at 50 °C for 1 h and 40 °C for 16 h. The mixture was quenched with 10 mL of H_2O and the oily residue obtained after solvent removal was taken up into CH_2Cl_2. The organic layer was washed with 1 M H_3PO_4, and H_2O. The residue obtained after solvent evaporation was purified by flash chromatography on silica gel (100 g) with 50% EtOAc/hexane: yield, 2.75 g (61%) of **3**; mp 133–135 °C (20% EtOAc/hexane) with starting material recovery **6a** (0.44 g, 10%); ^1H NMR ($CDCl_3$) δ 7.20 (1 H, s, H7), 6.71 (1 H, s, H4), 6.29 (2 H, s, H2′, 6′), 6.13 (1 H, d, J = 1.11 Hz, OCH_2O), 6.10 (1 H, d, J = 1.11 Hz, OCH_2O), 5.6–5.75 (1 H, m, CH=C), 5.62–5.70 (2 H, m, C=CH_2), 4.47 (1 H, s, H3), 3.83 (3 H, s, C4′OCH_3), 3.79 (6 H, s, $ArOCH_3$), 3.22 (3 H, s, OCH_3), 2.80–3.00 (2 H, m, CH_2C=C). Anal. Calcd for $C_{24}H_{24}O_8$: C, 65.43; H, 5.50. Found: C, 65.51, H, 5.60.

Diallyl Keto Ester 13. **13** was prepared by following the procedure outlined in the synthesis of **12**. Instead of quenching with H_2O, the reaction was treated with another portion of 1 M NaHMDS (10 mL, 0.9 equiv) and allyl bromide (1 mL, 1 equiv) and heated at 60 °C for 2 h before workup: yield, 1.7 g (34%) of **13** as an amber oil with recovery of **12** (1.2 g, 24%); ^1H NMR ($CDCl_3$) δ 6.87 (1 H, s, H7), 6.82 (1 H, s, H4), 6.30 (2 H, s, H2′, 6′), 6.0–6.14 (1 H, s, CH=C), 5.97 (1 H, d, J = 1.47 Hz, OCH_2O), 5.96 (1 H, d, J = 1.47 Hz, OCH_2O), 5.62–5.78 (1 H, m, CH=C), 5.24–5.45 (2 H, m, C=CH_2), 4.87–5.0 (2 H, m, C=CH_2), 4.62 (2 H, m, CH_2C=C), 3.81 (3 H, s, C4′OCH_3), 3.76 (6 H, s, $ArOCH_3$), 3.69 (3 H, S, OCH_3), 3.0–3.2 (2 H, m, CH_2C=C); MS (DCI) m/z 481 (MH^+, 100), 441 (10), 423 (20). (C2 and C3 relative stereochemistry unproven.)

Diol Ester 14. (i) To a solution of **12** (4.0 g, 9 mmol) in 200 mL of 95% MeOH at room temperature was added $NaBH_4$ (0.68 g, 2 equiv). The reaction was stirred for 1.25 h and quenched with 12 mL of 1 M H_3PO_4. The residue obtained after solvent removal was partition between H_2O and CH_2Cl_2. The organic layer was evaporated, and the residual solid was purified by flash chromatography on silica gel (100 g) with 10% Et_2O/CH_2Cl_2: yield, 3.2 g. (ii) To a solution of the above indanol (3.2 g, 7.2 mmol) in 100 mL of 10% H_2O/THF at room temperature was added O_8O_4 (0.2 g, 0.1 equiv). The reaction was stirred for 20 min, and a solution of $NaIO_4$ (4.6 g, 3 equiv) in 50 mL of H_2O was added. After 1 h, the reaction was quenched with 1 L of saturated NH_4Cl solution and extracted with CH_2Cl_2. The oily residue obtained after solvent evaporation was purified by flash chromatography on silica gel (75 g) with 10–30% Et_2O/CH_2Cl_2: yield, 3.2 g. (iii) To a solution of the aldehyde (3.2 g, 7.2 mmol) in 50 mL of 95% MeOH at room temperature was added $NaBH_4$ (0.54 g, 2 equiv). The reaction was stirred for 1 h and quenched with 1 M H_3PO_4 to pH = 7. The residue obtained after solvent removal was partitioned between H_2O and CH_2Cl_2. The organic layer was evaporated, and the residue was purified by column chromatography on silica gel (100 g) with 10–50% Et_2O/CH_2Cl_2: overall yield, 0.67 g (16.5%) of **14**, mp 75–79 °C, and 0.62 mg (16.5%) of **16a**; ^1H NMR (C_6D_6) δ 7.16 (1 H, s, H7), 6.55 (1 H, s, H3), 6.36 (2 H, s, H2′,6′), 5.38 (2 H, s, OCH_2O), 5.15 (1 H, d, J = 10.35 Hz, H1), 4.26 (1 H, d, J = 10.64 Hz, C1-OH), 3.84 (3 H, s, CO_2CH_3), 3.79 (2 H, m, CH_2OH), 3.75 (1 H, s, H3), 3.39 (6 H, s, OCH_3), 3.01 (1 H, br s, OH), 2.88 (3 H, s, OCH_3), 2.46 (1 H, m, CCH_2), 1.79 (1 H, m, CCH_2); HRMS (EI/HRP), calcd for $C_{23}H_{26}O_9$ 446.1577, found 446.1583. Anal. ($C_{23}H_{26}O_9$) C, H; C: calcd, 61.86; found, 61.29.

Cyclic Ether 15a. To a solution of diol **14** (150 mg, 0.3 mmol) in 10 mL of CH_2Cl_2 at −35 °C was added $BF_3{\cdot}OEt_2$ (60 μL, 1.45 equiv). The reaction was stirred for 0.5 h and quenched with 1 M $NaHCO_3$. The residue obtained after solvent removal was purified by flash chromatography on silica gel (30 g) with 2%

MeOH/$CHCl_3$: yield, 135 mg (94%); mp 156–160 °C; TLC (2.5% CH_3OH/CH_2Cl_2; R_f = 0.4); ^1H NMR ($CDCl_3$) δ 6.88 (1 H, dd, *J* = 0.47 Hz, H7), 6.44 (1 H, s, H4), 6.18 (2 H, s, H2′,6′), 5.96 (1 H, d, *J* = 1.19 Hz, OCH_2O), 5.95 (1 H, d, *J* = 1.19 Hz, OCH_2O), 5.91 (1 H, s, H1), 4.39 (1 H, s, H3), 3.67–3.95 (2 H, m, CH_2OR), 3.79 (3 H, s, $ArOCH_3$), 3.76 (6 H, s, $ArOCH_3$), 3.23 (3 H, s, CO_2CH_3), 2.59–2.68 (1 H, m, CCH_2), 2.1–2.2 (1 H, m, CCH_2); HRMS (EI/HRP) calcd for $C_{23}H_{24}O_8$ 428.1471, found 428.1471. Anal. ($C_{23}H_{24}O_8$) C, H; C: calcd, 64.46; found, 63.94.

C4′-OH Cyclic Ether 15b. To a solution of **15a** (220 mg, 0.5 mmol) in 10 mL of CH_2Cl_2 at room temperature was added TMSI (0.2 mL, 2.7 equiv). The reaction mixture was stirred for 1 h and quenched with 1 M $NaHCO_3$. The organic layer was evaporated, and the oily residue was purified by column chromatography on silica gel (20 g) with 10% Et_2O/CH_2Cl_2: yield, 64 mg (30%) as a white foam; mp 145–148 °C; ^1H NMR ($CDCl_3$) δ 6.90 (1 H, s, H7), 6.45 (1 H, s, H4), 6.19 (2 H, s, H2′,6′), 5.98 (1 H, d, *J* = 1.47 Hz, OCH_2O), 5.96 (1 H, d, *J* = 1.10 Hz, OCH_2O), 5.93 (1 H, s, H1), 5.42 (1 H, s, ArOH), 4.41 (1 H, s, H3), 3.67–3.97 (2 H, m, CH_2OR), 3.80 (6 H, s, $ArOCH_3$), 3.77 (3 H, s, CO_2CH_3), 2.58–2.68 (1 H, m, CCH_2), 2.1–2.2 (1 H, m, CCH_2). Anal. Calcd for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.78; H, 5.43.

Spirolactone Indanol 16a. To a solution of diol **14** (300 mg, 0.7 mmol) in 10 mL of CH_2Cl_2 at room temperature was added DBU (0.1 mL, 1 equiv). The reaction was stirred for 2 h and quenched with saturated NH_4Cl solution. The residue obtained after solvent removal was purified by flash chromatography on silica gel (30 g) with 2.5% $MeOH/CH_2Cl_2$: yield, 260 mg (94%); mp 248–251 °C; ^1H NMR ($CDCl_3$) δ 6.92 (1 H, s, H7), 6.53 (2 H, s, H2′,6′), 6.39 (1 H, s, H4), 6.00 (1 H, d, *J* = 1.47 Hz, OCH_2O), 5.96 (1 H, d, *J* = 1.10 Hz, OCH_2O), 5.07 (1 H, d, *J* = 11.91 Hz, H1), 4.18 (1 H, s, H3), 4.16 (1 H, m, CH_2OCO), 3.86 (3 H, s, OCH_3), 3.18 (6 H, s, OCH_3), 3.68–3.74 (1 H, m, CH_2OCO), 2.84–2.92 (1 H, m, CCH_2), 2.74 (1 H, d, *J* = 11.91 Hz, OH) 2.52–2.57 (1 H, m, CCH_2); MS (DCI) *m/z* 432 (MNH_4^+, 100), 414 (M^+, 10), 397 (80). Anal. ($C_{22}H_{22}O_8$) C, H.

C4′-OH Spirolactone Indanone 17b. To a solution of indanol **16a** (0.32 mg, 0.8 mmol) in 10 mL of CH_2Cl_2 was added MnO_2 (1 g). The reaction was stirred for 1 h at room temperature and filtered through Celite. The white solid (320 mg) obtained after solvent evaporation was dissolved in 4 mL of CH_2Cl_2 and treated with TMSI (0.27 mL, 2.4 equiv). The reaction was stirred for 7 h at room temperature and quenched with 1 M $NaHCO_3$. The residue obtained after solvent removal was purified by flash chromatography on silica gel (30 g) with 20% Et_2O/CH_2Cl_2: yield, 280 mg (91%); amorphous solid; TLC (2.5% CH_3OH/CH_2Cl_2; R_f = 0.3) ^1H NMR ($CDCl_3$) δ 7.21 (1 H, s, H7), 6.67 (1 H, d, *J* = 0.84 Hz, H4), 6.53 (2 H, s, H2′, 6′), 6.13 (1 H, d, *J* = 1.10 Hz, OCH_2O), 6.11 (1 H, d, *J* = 1.1 Hz, OCH_2O), 5.56 (1 H, s, ArOH), 4.38–4.45 (1 H, m, CH_2OCO), 4.38 (1 H, s, H3), 4.04–4.12 (1 H, m, CH_2OCO), 3.85 (6 H, s, OCH_3), 3.12–3.22 (1 H, m, CCH_2), 2.42–2.52 (1 H, m, CCH_2). Anal. ($C_{21}H_{18}O_8$). C: calcd, 63.80; found, 62.83; H: calcd, 4.56; found, 5.09.

Cis C4′-OH Spirolactone Indanol 16b. To a solution of **17b** (190 mg, 0.5 mmol) in 30 mL of 95% MeOH at room temperature was added $NaBH_4$ (40 mg, 2.1 equiv). The suspension was stirred for 1.5 h and filtered. The filtered solid was washed with Et_2O, H_2O, and cold MeOH: yield, 162 mg (85%); mp 291–295 °C; ^1H NMR (DMSO) δ 8.36 (1 H, br s, ArOH), 6.81 (1 H, s, H7), 6.58 (2 H, s, H2′,6′), 6.38 (1 H, s, H4), 6.05 (1 H, d, *J* = 0.74 Hz, OCH_2O), 6.04 (1 H, br s, H1), 5.96 (1 H, d, OCH_2O), 5.95 (1 H, br s, OH), 4.21 (1 H, s, H3), 4.05–4.14 (1 H, dd, *J* = 7.36 and 15.81 Hz, CH_2OCO), 3.67–3.74 (1 H, dd, CH_2OCO), 3.69 (6 H, s, OCH_3), 2.59 (2 H, br t, *J* = 7.35 Hz, CCH_2); MS (DCI) *m/z* 418 (MNH_4^+, 100), 383 (40), 268 (10). Anal. ($C_{21}H_{20}O_8$) C, H.

Trans C4′-OH Spirolactone Indanol 18. To a suspension of indanol **16b** (66 mg, 0.16 mmol) in 8 mL of CH_2Cl_2 at room temperature was added TMSI (47 μL, 2 equiv). The reaction was stirred for 45 min and quenched with 1 M $NaHCO_3$. Stirring was continued for an additional 6 h. The organic layer was evaporated, and the resulting solid was purified by flash chromatography on silica gel (10 g) with 2.5% $MeOH/CH_2Cl_2$: yield, 27 mg (60%); mp 228–232 °C; starting material recovery 20 mg (30%); ^1H NMR (DMSO) δ 8.31 (1 H, br s, ArOH), 6.91 (1 H, s, H7), 6.44 (1 H, s, H4), 6.32 (2 H, s, H2′, 6′), 6.04 (1 H, br s, OCH_2O), 6.00 (1 H, br s, OCH_2O), 5.72 (1 H, br s, H1), 5.02 (1 H, br d, *J* = 4.41 Hz, OH), 4.49 (1 H, s, H3), 4.17–4.27 (1 H, m, CH_2OCO), 4.02–4.10 (1 H, m, CH_2OCO), 3.64 (6 H, s, OCH_3), 2.65–2.75 (1 H, m, CCH_2), 2.1–2.2 (1 H, m, CCH_2). Anal. Calcd for $C_{21}H_{20}O_8$: C, 62.98; H, 5.04. Found: C, 62.70; H, 5.05.

Demethylation of Indanone 6a. To a solution of keto ester **6a**[8] (0.2 g, 0.5 mmol) in 10 mL of CH_2Cl_2 at 0 °C was added TMSI (0.36 mL, 5 equiv) and following addition, the ice bath removed. After 5 h, the mixture was recooled to 0 °C and quenched with saturated NH_4Cl and H_2O, in succession. The solvent was evaporated giving white solids which were sufficiently pure for further use: yield of **6b**, 0.17 g (89%); mp 203–207 °C; TLC (ether; R_f = 0.4); ^1H NMR ($CDCl_3$) δ 6.62 (1 H, s, H7), 6.32 (2 H, s, H2′,6′), 6.09 (2 H, AB q, OCH_2O), 5.48 (1 H, s, H4), 4.76 (1 H, d, H3), 3.84 (6 H, s, OCH_3), 3.81 (3 H, s, CO_2CH_3), 3.68 (1 H, d, H2); MS (DCI) *m/z* 387 (MH^+, 100). Anal. ($C_{20}H_{18}O_8$) C, H; C: calcd, 62.16; found, 60.63.

Trimethoxy Indene-3-carboxylate 20a. To a 0 °C solution of ketone **6a** (0.5 g, 1.25 mmol) in 30 mL of a 2:1 mixture of CH_3OH/CH_2Cl_2 was added solid $NaBH_4$ (70 mg, 1.84 mmol) in several portions. After 20 min the reaction was quenched with saturated NH_4Cl and solvents were evaporated. The residue was partitioned between ethyl acetate and saturated NaCl. The organic layer was separated, dried over Na_2SO_4, and evaporated to give a brown foam. This was dissolved directly in 20 mL of CH_2Cl_2 along with several crystals of $TsOH·H_2O$ and refluxed for 0.5 h. The mixture was washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4 and solvents evaporated. The residue was triturated in cold CH_3OH and filtered, and the collected solid dried, giving **20a**: yield 0.35 g (73%); mp 172–174 °C; TLC (ether; R_f = 0.6) ^1H NMR ($CDCl_3$) δ 7.69 (1 H, d, H1), 6.96 (1 H, s, H7), 6.72 (1 H, s, H4), 6.29 (2 H, s, H2′,6′), 5.9 (2 H, Ab q, OCH_2O), 4.66 (1 H, d, H3), 3.81 (3 H, s, OCH_3), 3.79 (6 H, s, OCH_3), 3.72 (3 H, s, CO_2CH_3); MS (DCI) *m/z* 402 (MNH_4^+, 80), 385 (MH^+, 100) (20–30% of C2–C3 olefin present from NMR). Anal. ($C_{21}H_{20}O_7$) C, H; C: calcd, 65.60; found, 65.00.

3,5-Dimethoxy-4-hydroxy Indene-3-carboxylate 20b. Similar to preparation of **20a**, **20b** was prepared by using **6b** (1 g, 2.6 mmol), $NaBH_4$ (0.1 g, 1 equiv) in 60 mL of the solvent mixture. After similar workup, and reflux with catalytic $TsOH·H_2O$, **20b** was obtained: yield 0.54 g, 56%; mp 196–200 °C; TLC (ether; R_f = 0.7) ^1H NMR (DMSO-d_6) δ 8.2 (1 H, s, OH), 7.75 (1 H, d, H1), 7.16 (1 H, s, 7), 6.81 (1 H, s, H4), 6.3 (2 H, s, H2′,6′), 6.03 (2 H, AB q, OCH_2O), 4.72 (1 H, d, H3), 3.37 (9 H, s, OCH_3 × 2, CO_2CH_3); MS (DCI) *m/z* 388 (MNH_4^+, 80), 371 (MH^+, 100) (20–25% of C2–C3 olefin present from NMR). Anal. ($C_{20}H_{18}O_7$) C, H; C: calcd, 64.84; found, 62.40.

Trimethoxy Lactone 22. To a solution of indene **20a** (0.31 g, 0.8 mmol) stirred under N_2 at −22 °C is added 1 M NaHMDS (2.26 mL, 2.8 equiv) dropwise causing a yellow coloration. After 0.5 h at −22 °C, (benzyloxy)methyl chloride (BOMCl) (0.45 mL, 4 equiv) was added dropwise and these mixture allowed to warm to room temperature overnight. The mixture was quenched and worked up as in the methylation reactions above. Purification was carried out via chromatotron (silica gel) with 3% ether in CH_2Cl_2 used to separate **21** and the 1,3 dialkylated product obtained in a 2:1 ratio, respectively, in 68% yield. However, in most cases the crude reaction products were directly hydrogenated as follows. The crude alkylation products were dissolved in CH_3OH (50 mL) with 20% Pd on charcoal (0.6 g) and treated with H_2 (4 atm) for 20 h. After filtering of the catalyst and evaporation of the solvents, two components were obtained. Purification via chromatotron using ether afforded the major component (lower R_f), **22**, as a clear oil: yield from **20a** 0.15 g, 43%; ^1H NMR ($CDCl_3$) δ 6.75 (1 H, s, H7), 6.51 (1 H, s, H4), 6.3 (2 H, s, H2′, 6′), 5.99 (2 H, AB q, OCH_2O), 4.74 (1 H, d, H3), 4.38 (2 H, AB q, CH_2OCO), 3.88 (2 H, br d, CH_2OH), 3.86 (3 H, s, C4′OCH_3), 3.8 (6 H, s, OCH_3 × 2), 3.59 (1 H, d, H2), 1.9 (1 H, br t, OH); HRMS (FAB/HRP), calcd for $C_{22}H_{22}O_8$ 414.1315, found 141.1310. Anal. ($C_{22}H_{22}O_8$) C, H. C: calcd, 63.74; found, 62.34.

Lactone 5. To a solution of indene **20b** (0.1 g, 0.27 mmol) stirred under N_2 at −60 °C is added 1 M NaHMDS (1.08 mL, 4 equiv) dropwise. After 0.5 h, BOMCl (0.113 mL, 3 equiv) was added dropwise, and the mixture was allowed to warm to 0 °C overnight. The reaction was worked up as above to give **23** and isomers in total 70% yield. This mixtures was directly hydro-

genated as for **22**, affording a mixture of components: yield 76.5 mg, from which **5** was isolated via chromatotron with use of ethyl acetate/hexane (4:1); total yield of **5** from **20b**, 43.5 mg, 42%; mp 219–220 °C; ^{1}H NMR ($CDCl_3$) δ 6.75 (1 H, s, H7), 6.5 (1 H, s, H4), 6.31 (2 H, s, H2′, 6′), 6.00 (2 H, AB q, OCH_2O), 5.48 (1 H, s, ArOH), 4.74 (1 H, d, H3), 4.37 (2 H, AB q, CH_2OCO), 3.87 (2 H, br d, CH_2OH), 3.82 (6 H, s, $OCH_3 \times 2$), 3.57 (1 H, d, H2), 1.81 (1 H, br t, OH); HRMS (FAB/HRP), calcd for $C_{21}H_{20}O_8$ 400.1156, found 400.1162. Anal. ($C_{21}H_{20}O_8$) C, H; C: calcd, 62.98; found, 61.04.

Biological Assay. Cells were grown in RPMI 1640 supplemented with 10% fetal calf serum. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted first with Earle's Balanced Salt Solution, followed by culture medium, to twice the highest concentration of compound to be tested. From this concentrated stock, 2-fold serial dilutions were prepared in 96-well microtiter trays. Each concentration was tested in triplicate and compared to triplicate drug-free controls. A 100-μL aliquot of cells (2.5×10^3 cells) was added to the wells of the microtiter plate containing 100 μL of growth medium with or without test drugs. Plates were incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. After 72 h, 20 μL of 5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added, and cells were incubated for 90 min to allow reduction of the formasan by the surviving cells. Following washing and solubilization by DMSO, absorbance of each well was measured spectrophotometrically at 570 nm. The IC_{50} is determined as the concentration of compound tested required to reduce the absorbance to 50% of non-drug-treated control values.

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Supplementary Material Available: Microanalysis and mass spectra for several compounds mentioned in the text (31 pages). Ordering information is given on any current masthead page.

Relationships between the Structure of Taxol Analogues and Their Antimitotic Activity§

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A variety of synthetic analogues of taxol, a naturally occurring antitumor diterpene, were examined for their potency to inhibit microtubule disassembly. For some of the compounds, the in vitro cytotoxic properties showed a good correlation with the tubulin assay. This structure–activity relationship study shows that inhibition of microtubule disassembly is quite sensitive to the configuration at C-2′ and C-3′. A correlation between the conformation of the side chain at C-13 and the activity is suggested. Of all the compounds examined, one of the most potent in inhibiting microtubule disassembly and in inhibiting murine P388 leukemic cells, *N*-debenzoyl-*N*-*tert*-(butoxycarbonyl)-10-deacetyltaxol, named taxotere, was selected for evaluation as a potential anticancer agent.

Several antitumor drugs prevent the formation of the mitotic spindle during cell division by interfering with the tubulin–microtubules system. Among the different classes of natural "mitotic spindle poisons", the anticancer diterpene taxol[1] promotes the assembly of microtubules and inhibits the disassembly process of microtubules to tubulin[2,3] in contrast to the vinblastine and colchicine type compounds which prevent microtubule assembly. Among natural substances, relationships between structure and microtubule assembly in vitro have been reported mostly for vinblastine,[4] colchicine,[5] maytansine,[6] podophyllotoxin,[7] and steganacine.[8] A good correlation between the inhibition of tubulin assembly and cytotoxicity has been shown for some of these compounds. In the vinblastine series, a new hemisynthetic "Vinca alkaloid", 5′-noranhydrovinblastine or Navelbine[9] was selected by using this in vitro assay as a possible useful chemotherapeutic agent and this compound is now used in clinics.[10]

In the taxol series, investigation of the structure–activity relationships has been limited because of the poor availability of taxol (1) from natural sources (only 50–150 mg/kg of dried trunk bark can be isolated from several species of yew (genus Taxus, family Taxaceae)[1,11]). However, some closely related taxol congeners, mostly

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