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Articles

Conformational Analysis of Podophyllotoxin and Its Congeners. Structure–Activity Relationship in Microtubule Assembly

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Conformational analysis of podophyllotoxin and 11 congeners was carried out using 360-MHz ^{1}H NMR techniques in order to determine the effects of substituent modifications in the C and D rings of these compounds on their conformational properties. The results are used to explore structure–activity relationships for this series of congeners related to their ability to inhibit microtubule assembly. The derivatives studied include the antitumor compound VP-16-213 (4′-demethylepipodophyllotoxin ethylidene-β-D-glucoside); the cyclic ether, cyclic sulfide, and cyclic sulfone derivatives of podophyllotoxin and deoxypodophyllotoxin; epipodophyllotoxin; and picropodophyllotoxin. From an analysis of the Overhauser enhancement effects and the J couplings, the conformations of these derivatives were found to be identical, with the exception of picropodophyllotoxin. The relationship between the antimitotic activity of these compounds and the substituent modifications in their structures suggests that the C and D rings of the derivatives are involved in their interactions with tubulin. Specifically, their activities were shown to be sensitive to the configuration, size, and/or hydrophilic character of substituents at the 4 position in the C ring and to the steric features of substituents at the 12 position in the D ring. In the case of picropodophyllotoxin, we suggest that its reduced antimitotic activity may be due to two conformational forms of the drug: a minority conformation which is active and a majority conformation which is inactive.

The mechanism by which podophyllotoxin (Figure 1A) blocks cell division is related to its inhibition of microtubule assembly in the mitotic apparatus.[2a,b] The clinical application of podophyllotoxin in the treatment of cancer[3,4] has been limited by severe toxic side effects during the administration of the drug.[5,6] In an attempt to discover less toxic analogues, a variety of podophyllotoxin derivatives have been prepared. These include VP-16-213 (4′-demethylepipodophyllotoxin ethylidene-β-D-glucoside) and VM-26 (4′-demethylepipodophyllotoxin thenylidene-β-D-glucoside) (Figure 1B), both of which are effective in the treatment of a variety of leukemias and solid tumors,[7-10] and, more recently, derivatives which possess substituent modifications in the C and D rings, including the cyclic ether, cyclic sulfide, and cyclic sulfone derivatives of podophyllotoxin (Figure 1D) and deoxypodophyllotoxin (Figure 1E), respectively.

The antimitotic activity of many of these analogues have been determined by measurement of their cytotoxicity in different cell lines, their association constants for binding to tubulin, and their relative abilities to inhibit in vitro microtubule assembly.[11,12] Interestingly, VP-16-213 and VM-26 were shown not to be inhibitors of microtubule assembly, suggesting that their antitumor properties were due to another mechanism of action.[13] The results for the analogues of podophyllotoxin that possess substituent modifications in the C and D rings indicate a nearly three orders of magnitude difference in their inhibitory ability, and in certain cases the activity was greater than that of the parent compound (for example, 4′-demethyldeoxypodophyllotoxin).[12]

We herein report a 360-MHz high-resolution ^{1}H NMR study of podophyllotoxin and 11 congeners containing modifications in the C and D rings. This study was undertaken to assess the effect of substituent modifications in the C and D rings of podophyllotoxin on the conformational properties of these drugs. The NMR data clearly show that, with one exception, these analogues closely retain the conformation of the parent drug and that it is primarily the steric features of the substituents in the D ring of the derivatives which correlate with their antimitotic activities. The exception in this series of congeners is picropodophyllotoxin which exists in two conformations: one which resembles that of the parent compound, podophyllotoxin, and which is believed to be responsible for the observed activity of the drug and a second conformation which differs from the parent molecule and which is believed to be inactive.

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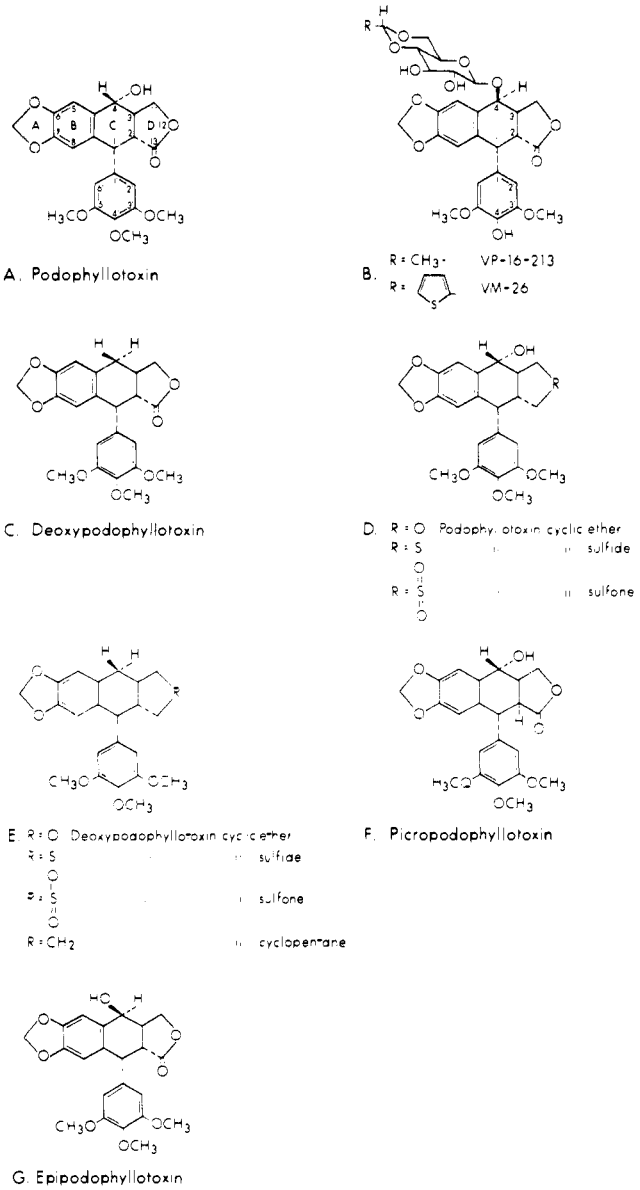

Figure 1. Structural formulas of podophyllotoxin and its derivatives.

Experimental Section

Materials. Podophyllotoxin, deoxypodophyllotoxin, and picropodophyllotoxin were provided by the National Cancer Institute, VP-16-213 was a gift from Dr. A. von Wartburg, Sandoz, Switzerland. Epipodophyllotoxin was provided by Dr. Francis Johnson, State University of New York at Stony Brook. Podophyllotoxin cyclic ether, cyclic sulfide, and cyclic sulfone; deoxypodophyllotoxin cyclic ether, cyclic sulfide, and cyclic sulfone; and cyclopentane were synthesized as described.[11] All compounds were examined for purity by thin-layer chromatography.

Methods. Proton NMR measurements were done on a WH 360-MHz Bruker spectrometer operating at approximately 86 kG. All samples were dissolved in deuteriochloroform to a final concentration of 5 mM or less. The 86-kG field was "locked" to the deuterium resonance signal of the solvent. Pulse techniques were used to accumulate the resonance signals. Typically, 100 free-induction decays were accumulated and then Fourier transformed on a Nicolet 1180 computer. Homonuclear decoupling experiments were done using approximately 10-dB attenuation of a nominal 200-mW radiofrequency source at 360 MHz. Nuclear Overhauser enhancement measurements were done using approximately 36-dB attenuation of the same radio-frequency source. The 90° pulse had a width of approximately 18 μs. Proton NMR spectra were also recorded on a JEOL PFT 100-MHz spectrometer operating in the pulsed Fourier transform mode, using a Nicolet 1080 computer for data collection and Fourier transformation.

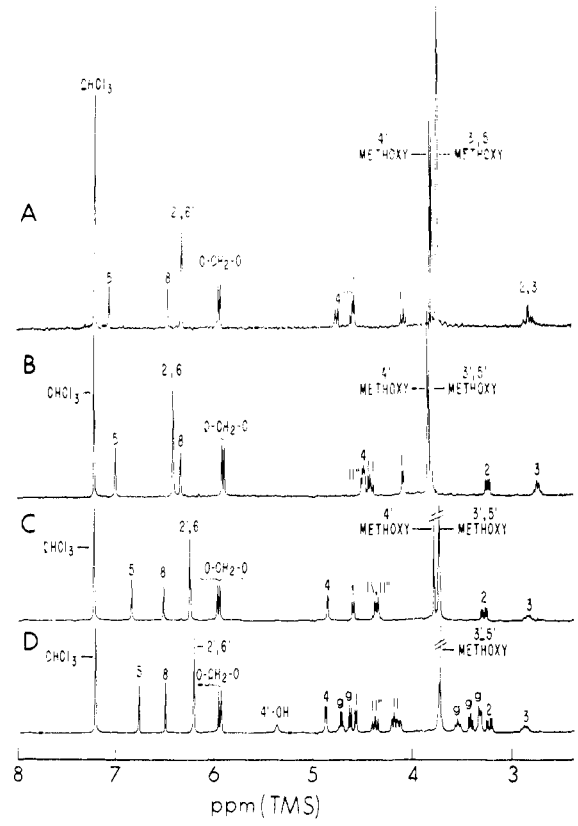

Figure 2. 360-MHz proton NMR spectra of: (A) podophyllotoxin, (B) picropodophyllotoxin, (C) epipodophyllotoxin, and (D) VP-16-213. Symbol g refers to resonances of the glucoside moiety.

Results

High-resolution proton nuclear magnetic resonance (NMR) spectra of podophyllotoxin and 11 derivatives were recorded in order to determine their relative stereochemical properties. The 360-MHz proton NMR spectra of podophyllotoxin, picropodophyllotoxin, epipodophyllotoxin, and VP-16-213 are shown in Figure 2. The chemical-shift assignments for the 2′, 6′, 5 and 8 aromatic protons of podophyllotoxin in deuteriochloroform are in agreement with those of Ayres and Lim.[14] The chemical-shift assignments for the methylenedioxy and methoxy protons of podophyllotoxin were made on the basis of their chemical-shift positions as well as their integrated intensities. Chemical-shift assignments (Table I) and coupling constants (Table II) for the aliphatic protons in rings C and D were determined using proton double-irradiation experiments and integrated intensity measurements. A characteristic feature of podophyllotoxin and all of its derivatives is that the 2′,6′ protons and the 3′,5′ methoxy protons, even at 360 MHz, give rise to single resonance peaks.

The spectrum of picropodophyllotoxin, which is the C-2 epimer of podophyllotoxin, clearly shows differences, with respect to podophyllotoxin, in the chemical-shift positions of the aromatic protons in rings B and E and the aliphatic protons in rings C and D (Table I). Where comparable, the data for podophyllotoxin and picropodophyllotoxin support that of Ayres et al.[15]

The ^{1}H NMR spectra of epipodophyllotoxin and VP-16-213 are very similar with respect to the chemical-shift positions of protons that are common to both structures and to the coupling constants of the aliphatic

Table I. Proton NMR Chemical-Shift Assignments for Podophyllotoxin and its Derivatives[a]

	5	8	2',6'	CH_2[b]	4''	4	1	11''	11	2	3	13''	13
podophyl	7.11	6.51	6.37	5.98		4.77	4.59	4.60	4.09	2.83	2.77		
podophyl cyclic ether	7.15	6.45	6.13	5.96		4.61	4.26	4.23	3.68	2.46	2.38	4.00	3.07
podophyl cyclic sulfide	7.11	6.40	6.16	5.94		4.58	4.18	broad		2.40	2.25	broad	
podophyl cyclic sulfone	7.05	6.39	6.11	5.95		4.60	4.18	3.61	3.03	2.86	2.64	3.20	2.52
deoxypodophyl	6.66	6.51	6.34	5.94	3.07	2.78	4.60	4.45	3.92	2.72[c]			
deoxypodophyl cyclic ether	6.63	6.45	6.09	5.91	3.01	2.55	4.25	4.06	3.43	2.30[c]		4.00	3.04
deoxypodophyl cyclopentane	6.60	6.41	6.12	5.90	3.02	2.45	4.14	broad		1.90	1.75	broad	
deoxypodophyl cyclic sulfide	6.61	6.42	6.12	5.90	3.20	2.65	4.23	broad		2.28[c]		broad	
deoxypodophyl cyclic sulfone	6.60	6.40	6.09	5.92	3.18	2.73	4.21	3.49	2.90	2.82	2.75	3.24	2.52
epipodophyl	6.84	6.56	6.23	6.01	4.88		4.62	4.41	4.35	3.29	2.83		
VP-16-213	6.80	6.53	6.25	5.98	4.90		4.60	4.40	4.20	3.24	2.86		
picropodophyl	7.05	6.38	6.45	5.94		4.51	4.11	4.53	4.44	3.24	2.74		

[a] Chemical-shift assignments for the 4, 11, and 13 protons refer to the protons at these positions which are above the plane of the ABCD rings of podophyllotoxin (Figure 1). Chemical-shift assignments for the 4'', 11'', and 13'' protons at these positions are below the plane of the ABCD rings. Chemical-shift assignments have been made relative to internal tetramethylsilane. Abbreviation used: podophyl, podophyllotoxin. [b] The methylenedioxy group. [c] Average position for H(2)-H(3).

Table II. Proton NMR Coupling-Constant Assignments for Podophyllotoxin and its Derivatives[a]

	1,2	2,3	3,4''	3,4	4,4''	3,11''	3,11	11,11''	13,13''	2,13''	2,13
podophyl	4.4			9.1		8	9	8.8			
podophyl cyclic ether	4.9			9.0		8.3	9.1	8.3	7.5	6.7	10.5
podophyl cyclic sulfide	5.0			9.1							
podophyl cyclic sulfone	5.6	13		9.9		8.4	9.8	13	11.2	6.6	12.6
deoxypodophyl	1.5					6.1[b] (2.7)	8.0[b] (5.5)	8.3			
deoxypodophyl cyclic ether	4.5		3.0	11	15	6.8	10	6.8	6.8	6.8	10.5
deoxypodophyl cyclopentane	6.3		4.5	11	16						
deoxypodophyl cyclic sulfide	6.4		3.8	9.8	15						
deoxypodophyl cyclic sulfone	6.4		3.2		15.4	6.5	10.9	13	5.8		13
epipodophyl	4.9	13.8	3			9.8	8				
VP-16-213	4.4	14.5	2.9			8.7	7.3	8			
picropodophyl	5.7	9.0		8.3		1.5	6.0	10.2			

[a] Abbreviation used: podophyl, podophyllotoxin. [b] Two possible sets of coupling constants were found to agree with the data. The upper set was chosen on the basis of its agreement with the same coupling constants determined for other derivatives.

protons in rings C and D. Small but significant differences in the chemical-shift positions do exist for proton 11 in ring D of these two compounds. No attempt was made to assign the resonances of VP-16-213 that are associated with the glucoside moiety.

The chemical-shift assignments for deoxypodophyllotoxin cyclic ether and the corresponding cyclopentane, cyclic sulfide, and cyclic sulfone analogues are listed in Table I. The absence of an hydroxyl group at the C-4 position in ring C in these analogues produces a substantial upfield chemical shift in the H-4 proton resonance relative to its position in podophyllotoxin. Removal of the carbonyl group in ring D of deoxypodophyllotoxin and replacement by a methylene group, as in the cyclopentane, cyclic ether, cyclic sulfide, and cyclic sulfone derivatives, give rise to upfield shifts in the position of the 2',6' resonance of the E ring due to removal of the deshielding effect of the carbonyl group in ring D. Interestingly, the 11, 11'', 13, and 13'' protons in the D ring of deoxypodophyllotoxin cyclic sulfide appear too broad to be resolvable. We feel that this may be due to either trace contamination with paramagnetic impurities, such as iron, that can bind to the sulfur or smearing out of the resonance due to "intermediate exchange rates"[16] between two conformational states of ring D which have different chemical-shift positions. This lack of resolvable resonances for these two methylene groups is also observed in the corresponding podophyllotoxin cyclic sulfide analogue. The coupling constants for the deoxypodophyllotoxin analogues shown in Table II were observed to be fairly similar to one another with the exception of the parent compound, deoxypodophyllotoxin, which was found to have an unusually small value for $J_{1,2}$.

The chemical-shift and coupling-constant data for podophyllotoxin cyclic ether, cyclic sulfide, and cyclic sulfone are given in Tables I and II, respectively.

The results of nuclear Overhauser enhancement (NOE) measurements for selected resonances of podophyllotoxin,

Table III. Proton NMR Nuclear Overhauser Enhancement Effects of Podophyllotoxin and its Derivatives[c]

	proton irrad	proton obsd[a]	% signal enhanc[b]
podophyl	H-2',6'	H-1	8.5
	H-8	H-1	5
	H-1	H-8	11.5
		H-2',6'	5
podophyl cyclic sulfone	H-2',6'	H-1	15
		H-3	14
		H-14	4.5
	H-8	H-1	11
	H-1	H-8	11
		H-2',6'	7
epipodophyl	H-2',6'	H-1	11
		H-2	3.5
		H-3	16
	H-8	H-1	6.1
	H-1	H-8	8.6
		H-2',6'	4.5
VP-16-213	H-2',6'	H-1	8.7
		H-3	10
	H-8	H-1	5
	H-1	H-8	12
picropodophyl	H-2',6'	H-1	14
		H-8	5
		H-2	8
		H-3	2
	H-8	H-1	2

[a] Only those resolved resonances with strong enhancements are listed. [b] The average deviations were between ±1 to ±2.5%. [c] Abbreviation used: podophyl, podophyllotoxin.

podophyllotoxin cyclic sulfone, epipodophyllotoxin, VP-16-213, and picropodophyllotoxin are given in Table III.

Discussion

NMR Studies. CPK models of podophyllotoxin and the derivatives listed in Tables I–III suggest that their overall structures are similar, with the exception of picropodophyllotoxin. However, the models do not predict the rotational freedom of the E ring in these compounds, as found from the NMR results, nor do they allow determination of the precise angle of the E ring with respect to the C ring in the derivatives. They also do not provide unequivocal values of the bond angles in the C and D rings of these compounds. For these reasons, we have obtained empirical data on the conformational properties of podophyllotoxin and the derivatives listed in Tables I–III in order to assess the relationship between the structural features of these compounds and their antimitotic activities.

Gensler et al.[11] have reported that the circular dichroism and ORD curves for all of the compounds in Table I (no data are reported for epipodophyllotoxin, VP-16-213, and picropodophyllotoxin) were qualitatively similar, although the authors point out that these spectroscopic parameters are not sensitive functions of the detailed stereochemistry of the drugs. Ayres et al.[14,15] have reported limited chemical-shift assignments and coupling-constant data for podophyllotoxin, epipodophyllotoxin, deoxypodophyllotoxin, and picropodophyllotoxin using lower field (100 MHz) measurements and different solvents. On the other hand, measurements at 360 MHz have allowed us to make nearly complete chemical-shift and coupling-constant assignments for the above four compounds, as well as for the eight additional derivatives investigated. Gensler et al.[11] have published limited chemical-shift data for some of these latter compounds recorded at 60 MHz. The chemical-shift and coupling-constant data obtained at 360 MHz for these 12 compounds are given in Tables I and II, respectively.

In order to obtain information about the stereochemistry of these compounds, their vicinal coupling constants can be converted into corresponding dihedral angles using a modified form of the Karplus equation, which takes into account the effect of the electronegativity of substituents on coupling constants. Other effects, such as steric factors, influence vicinal coupling constants; however, these perturbations are difficult to account for quantitatively. They appear to be less important than substituent electronegativity effects based on correlations of the variations of vicinal coupling constants with the electronegativity of substituents for a given system (cf. ref 17).

Vorontsova and Bochkov[18] have reported a modified form of the Karplus equation which takes into account substituent electronegativity effects. The use of this modified equation gives an accuracy for calculating correct dihedral angles from vicinal coupling constants at ±18%, as compared to ±42% for the unmodified Karplus equation. Using this modified equation, values for the dihedral angles of podophyllotoxin and 11 derivatives are listed in Table IV.

With the exception of picropodophyllotoxin, the stereochemistry of the compounds listed in Table IV are very similar. The absolute magnitude of the dihedral angles may vary somewhat, depending on the constants chosen in the modified Karplus equation. Calibration of these constants would require standards with known bond angles in this series of compounds.[19] However, the relative magnitude of the dihedral angles calculated, after corrections for substituent effects have been made, is expected to be relatively accurate. Indeed, this is what is observed. The dihedral angles for $H_{1,2}$ for the compounds are roughly 50 ± 4°, with the exception, in this series, of deoxypodophyllotoxin, which has an unusually large value (68°). This may be due to torsional effects which are absent in the other derivatives. The dihedral angles found for other vicinal proton pairs in the C and D rings of these compounds also agree well with each other. The data, therefore, strongly suggest that, with the exception of picropodophyllotoxin, the conformation of the drugs listed in Table IV is essentially the same.

Because of the conformational flexibility of the "cis" C/D ring junction in picropodophyllotoxin, the observed coupling constants for picropodophyllotoxin are actually average values between two possible conformations of the molecule: one exists in which the E ring is nearly perpendicular (equitorial) to the ABCD ring system; the second conformation exists in which the E ring flips down into a quasiaxial position, similar to that found for podophyllotoxin and the other compounds listed in Table I. The calculated average dihedral angle of 130° for the $H_{1,2}$ of picropodophyllotoxin is consistent with the molecule existing primarily in the equitorial conformation in deuterated chloroform. The NOE data (below) also support this assignment. Ayres and co-workers[15] have estimated that 65% of picropodophyllotoxin in deuterated dimethyl sulfoxide exists in the equitorial conformation. They attribute the tendency for picropodophyllotoxin to assume a greater percentage of the equitorial conformation to be due to unfavorable 1,4 aryl–hydroxyl interactions in the boat conformation (quasiaxial).

Nuclear Overhauser enhancement measurements provide an independent method of probing molecular conformation.[20] The results of the NOE experiments (Table

Table IV. Dihedral Angles (H-C-C-H), in Degrees, Calculated for Podophyllotoxin and Its Derivatives Using a Modified Karplus Equation[a-b]

	1,2	2,3	3,4''	3,4	3,11''	3,11	2,13''	2,13
podophyl	54			152	39	146		
podophyl cyclic ether	52			152	38	147	131	27
podophyl cyclic sulfide	52			152				
podophyl cyclic sulfone	49	159		156	40	147	133	21
deoxypodophyl	68				47	140		
deoxypodophyl cyclic ether	53		60	149	44	150	131	27
deoxypodophyl cyclopentane	46		55	149				
deoxypodophyl cyclic sulfide	46		57	156				
deoxypodophyl cyclic sulfone	46		59		47	150	130	21
epipodophyl	54	164	60		31	141		
VP-16-213	52	169	60		35	138		
picropodophyl	128	37		147	68	133		

[a] $J = 0.77(X) + \cos^2\theta[20.1 - 3.4(X)] - 1.74$, where $X = \Sigma_1^4 (X_n - X_H)$, and X_H and X_n are the electronegatives of hydrogen and the substituents R_1 to R_4 in the system $R_1R_2CHCHR_3R_4$, respectively. Electronegativity values (E_R) for substituents (R): R = H, E_R = 2.2; R = CH_2R, E_R = 2.5; R = CHR_2, E_R = 2.5; R = COO, E_R = 2.55; R = S, E_R = 2.58; R = phenyl, E_R = 2.75; R = OR, E_R = 3.4; R = OH, E_R = 3.43. [The electronegativity values 2.2, 2.5, 2.5, and 2.58 are Pauling electronegatives; the values 2.55, 2.75, and 3.43 are from Cavanaugh and Daily; and the value 3.4 is estimated from the values in J. R. Cavanauh and B. P. Daily, *J. Chem. Phys.*, 34, 1099 (1961)]. [b] Abbreviation used: podophyl, podophyllotoxin.

III) are consistent with the geometry of the molecules predicted from the coupling constant data in Tables II and IV. Although the results were not quantitatively the same, the qualitative results for podophyllotoxin, podophyllotoxin cyclic sulfone, epipodophyllotoxin, and VP-16-213 clearly show a consistently different pattern for resonance enhancement effects involving the H-2',6' and H-1, -2, and -3 protons as compared to the NOE effects of picropodophyllotoxin. For example, irradiation at the 2',6' resonance position in picropodophyllotoxin produced enhanced signal intensity in the H-1 and -2 resonances, which is consistent with the E ring existing predominantly in the equitorial conformation (see above), while similar experiments with other compounds listed in Table III demonstrated enhancement effects at the H-1 and -3 protons, which is consistant with the E ring of these compounds existing in a quasiaxial conformation. (Enhancement effects at the H-3 of podophyllotoxin could not be determined because of lack of resolution of this resonance.) These data support the conclusion that, with the exception of picropodophyllotoxin, the stereochemistries of the fused ring system of podophyllotoxin and its derivatives are very similar to one another. The large NOE of ca. 9–15% observed for the H-1 and -3 resonances when the H-2',6' resonances were saturated indicates (1) an E-ring orientation in which the H-2',6' protons are in close proximity to the H-1 and -3 protons and (2) that the orientation of the E ring, relative to the ABCD fused ring structure, is basically unchanged in all the derivatives, excluding picropodophyllotoxin. However, the 2',6' proton resonances and 3',5' methoxy protons in *all* derivatives are also observed as single resonance peaks. The NMR data thus suggest that the equivalence of the 2' and 6' protons and the equivalence of the 3' and 5' methoxy protons arise from a rapid exchange of the E ring between two conformers. These two conformers are presumed to differ by a 180° rotation about the C-1',1 bond, and, aside from a relabeling of atoms, are indistinguishable. These two conformers maintain proximity between the H-2',6' and H-1,3 protons.

An interesting observation regarding VP-16-213 and epipodophyllotoxin is that only the H-11 resonance of the former is significantly different in its chemical-shift position from that of the latter. All of the other resonances that are common to the two compounds have nearly the same chemical-shift positions as well as coupling constants. It appears that the β-glucoside moiety of VP-16-213 preferentially deshields the H-11 proton, which indicates its proximate location is over this area of the molecule in solution, at least part of the time.

Table V. Inhibition of in Vitro Microtubule Assembly by Podophyllotoxin Derivatives Modified in the C and D Rings[12,c]

	ID_{50}, μM[a]
podophyl	0.6
podophyl cyclic ether	1.0
podophyl cyclic sulfide	10.0
podophyl cyclic sulfone	>>100[b]
deoxypodophyl	0.5
deoxypodophyl cyclic ether	0.8
deoxypodophyl cyclopentane	5.0
deoxypodophyl cyclic sulfide	10.0
deoxypodophyl cyclic sulfone	>>100[b]
epipodophyl	5
VP-16-213	>>100[b]
picropodophyl	30

[a] ID_{50} represents the concentration of drug necessary to inhibit chicken brain microtubule assembly by 50% during a 30-min incubation period at 37 °C. Values represent the average of triplicate experiments in which the standard deviation is <15%. [b] No inhibition of microtubule assembly observed at 100 μM. [c] Abbreviation used podophyl, podophyllotoxin.

Structure–Activity Correlations. As an index of the antimitototic activities of the podophyllotoxin derivatives investigated in this study, their ID_{50} values for the inhibition of the in vitro polymerization of chicken brain microtubules are given in Table V.[12] The relative antimitotic activities of these compounds are also in general accord with their association constants for binding to mouse brain tubulin,[21] as determined from competitive binding studies against radioactive colchicine. However, the association constants of the drugs do not always directly correlate with their relative concentrations necessary to inhibit antimitotic effects. Indeed, Margolis and

Wilson[22] and Sternlicht and Ringel[23] have shown that colchicine possesses substantial antimitotic activity at substoichiometric concentrations of the drug. Since colchicine and podophyllotoxin appear to bind to the same site in tubulin and act by similar mechanisms, there is reason to believe that a strict linear relation between the affinities of podophyllotoxin congeners for tubulin and their antimitotic activities will not hold true. Despite these differences, however, the relative antimitotic activities of these derivatives and their affinity for tubulin do, in general, correlate with each other, though not in a simple manner. What is clear is that the antimitotic activity of these compounds is sensitive to chemical modifications of podophyllotoxin, as observed in Table V.

In order to develop a structure–activity relationship for these derivatives of podophyllotoxin, the effect of the substituents on the conformation of the molecules must be determined. The NMR results indicate that the dihedral angles in rings C and D of the compounds listed in Table IV, with the exception of picropodophyllotoxin, are very similar to each other. The E ring of these compounds was found to undergo rapid exchange between two conformations that are at right angles with respect to the rest of the molecule. The orientation of the E ring with respect to the C ring in these compounds can be described as quasiaxial, as was found in the crystal structure of 2′-bromopodophyllotoxin.[24] Although the NMR data for these compounds were obtained using deuteriochloroform as solvent, we feel that these compounds are sufficiently rigid so that the above conclusions regarding their structures also hold true for aqueous media. Since the NMR data indicate essentially no change in the conformation of these derivatives, their varying antimitotic activities must be due to the nature of the different substituents in these compounds.

It can be seen that changes in the configuration, size, and chemical nature of substituents in the C-ring of podophyllotoxin markedly affect the activity of the analogues (Table V). While deoxypodophyllotoxin exhibits nearly the same ID_{50} as podophyllotoxin, epipodophyllotoxin, the 4-hydroxyl epimer of podophyllotoxin, is an order of magnitude less potent than podophyllotoxin. Further substitution of epipodophyllotoxin with ethylidene- and thenylideneglucoside moieties to give VP-16-213 and VM-26, respectively, results in inactive congeners.[12] One possible explanation for the decreasing activity of these compounds is the increasing hydrophilic nature of the C-4 substituents. This could decrease the affinity of these derivatives for tubulin by increasing their solubility in water. The second possibility is that the tubulin binding site is sensitive to the size and configuration of substituents at the C-4 position of podophyllotoxin. It is interesting to note that the NMR results indicate that one of the preferred rotamer conformations for the ethylideneglucoside moiety of VP-16-213 is over the H-11 proton of the D ring. Thus, the tubulin binding site appears to be sensitive to the size, configuration, and possibly the hydrophilic nature of substituents at the C-4 position of podophyllotoxin derivatives.

Substitutions in the D ring of podophyllotoxin and deoxypodophyllotoxin have the same parallel effects in decreasing the activity of these two parent compounds (Table V). As previously noted,[11,12] the lactone group in the D ring of these two parent compounds is not required for activity, since substitution of the carbonyl group by a methylene group to give the cyclic ether analogues shows only a twofold loss in activity. However, substitutions at position 12 in the D ring of derivatives containing a methylene group at position 13 show significant losses in activity, depending on the nature of the substituents incorporated. Since the data in this paper indicate that the conformation of these derivatives is the same, the difference in their activities must be due to other effects. The electronic properties of the substituents at the 12 position of the derivatives, as indexed by their Hammett σ substituent constants (meta),[25] do not correlate with the relative antimitotic activities of the congeners. The parameter which provides the best structure–activity correlation is the size of the substituents at position 12. The van der Waals radii of oxygen, sulfur, and the sulfone group increase in this order, while the antimitotic activity of the corresponding derivatives of both podophyllotoxin and deoxypodophyllotoxin decreases in this order. The van der Waals radii for the methylene and ketone group of the cyclopentane and cyclopentanone derivatives 12 also correlate with their inhibitory activities. These results suggest that the podophyllotoxin binding site on tubulin has strict steric requirements in the region where the 12 position of the D ring of these compounds interacts with tubulin.

In contrast to the above results, the loss of activity of picropodophyllotoxin relative to podophyllotoxin has previously been attributed to differences in the conformation of the two molecules (cf. ref 11). Gensler and Gatsonis[26] have suggested that the small residual activity of picropodophyllotoxin could be due to a small amount of podophyllotoxin in the sample that could arise from epimerization of the C-2 proton. However, in all cases reported, the interconversion of the two epimers is a base-catalyzed reaction.[25] It therefore seems unlikely that, under the conditions of the antimitotic assay (i.e., neutral pH at 37 °C for approximately 30 min[12]), epimerization of picropodophyllotoxin to form even small amounts of podophyllotoxin could occur.

On the other hand, picropodophyllotoxin is a conformationally flexible molecule, and we suggest that the reduced activity of the drug may be due to the presence of two conformations of the molecule, only one of which is active and exists as the minority species in solution. As discussed above, picropodophyllotoxin can exist with the E ring in an equitorial or a quasiaxial conformation. Both our results and those of Ayres and co-workers[15] indicate that the equitorial conformation exists predominantly in organic solvents. Ayres also points out that increasing solvation of the 4-hydroxyl of picropodophyllotoxin would be expected to increase the amount of the equitorial conformation, since unfavorable 1,4 axial-hydroxyl interactions would occur in the quasiaxial conformation. Therefore, we assume that the predominant conformation of picropodophyllotoxin is equitorial in aqueous solution in which the antimitotic assays are performed.

It has been previously observed by Loike et al.[12] that dehydropodophyllotoxin, in which the C ring is aromatic and the E ring is perpendicular to the ABCD ring system, is totally inactive toward inhibiting microtubule assembly. Since picropodophyllotoxin has greatly reduced antimitotic activity and dehydropodophyllotoxin possesses no detectible activity, we make the assumption that positioning the E ring perpendicular to the rest of the molecule results in an inactive conformation. The residual activity of picropodophyllotoxin, we believe, is due to the minority quasiaxial conformation of the drug. This conformation results in puckering of the D ring, but places the E ring in the same orientation as that found in podophyllotoxin and its biologically active derivatives. We suggest, therefore, that this conformational form, although present

in only small amounts in aqueous solutions of picropodophyllotoxin, is the form responsible for the inhibition of microtubule assembly by this drug.

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A Model for Thyroid Hormone–Receptor Interactions

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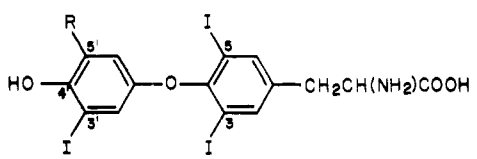
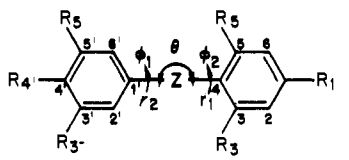
Theoretical electronic structure calculations on the thyroid hormones and analogues, as well as model hormone–receptor interactions, have been carried out. These studies (a) support the concept that the 4′-OH group is a H-bond donor to the in vivo nuclear receptor and suggest that at the receptor this OH group is trans to the 3′ (distal) substituent; (b) indicate that there is an important intramolecular interaction between 3′ and 4′ substituents, and those 3′ substituents that most favor both 4′ OH orientation trans to the 3′ group and a more acidic OH group substantially increase binding and biological activity; and (c) support the concept that there is a direct correlation between the conformational free energy of the aromatic rings and biological activity.

The two thyroid hormones, thyroxine (T_4: I, R = I) and



I, R = H or I

triiodothyronine (T_3: I, R = H), elicit a multitude of biological responses and are essential for normal growth and development.[1,2] A number of hypotheses[2] have been proposed to relate the various structural features of the thyroid hormones and their analogues (II) to the expression of their biological effects.



II

Biological activities measured in whole animals are influenced by metabolism, distribution, and the sequence of events between binding and the expression of their biological effect. Thus, an important recent advance in the study of the thyroid hormones and their analogues has been the development of suitable in vitro assays which appear to correlate well with in vivo thyromimetic activity.

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