Pergamon

Tetrahedron, Vol. 53, No. 46, pp. 15743-15760, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)10042-4

Preparation and Cytotoxicity of Podophyllotoxin Derivatives Lacking the Lactone Ring

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Abstract. Several cyclolignans lacking of the lactone moiety can easily be prepared from naturally occurring lignans such as podophyllotoxin and deoxypodophyllotoxin by simple chemical transformations. Their cytotoxicity has been studied in four tumoral cell lines. Most of the compounds show similar effects in all the neoplastic systems tested, except the aldehyde **9** (methyl 9-deoxy-9-oxo-α-apopicropodophyllate) and the hydrazones **16** and **17** which show a highly selective cytotoxicity towards HT-29 human colon carcinoma. Additionally, several molecular modeling studies have been done with aldehyde **9** and the corresponding saturated aldehyde **13** in comparison with podophyllotoxin. © 1997 Elsevier Science Ltd.

INTRODUCTION

The lignan family of natural products includes compounds with important antineoplastic and antiviral properties such as podophyllotoxin and two of its semisynthetic derivatives, etoposide and teniposide[1]. The latter are included in a wide variety of cancer chemotherapy protocols.[2] Due to these biological activities, lignans, and especially cyclolignans, have been the object of numerous studies which are compiled in several reviews.[3] The sources for new compounds could be plants, semisynthetic derivatization of natural products or total synthesis. All these studies are focused to prepare better and safer anticancer drugs.

The mechanism by which podophyllotoxin blocks cell division is related to its inhibition of microtubule assembly[4] in the mitotic apparatus; it is a competitive inhibitor of colchicine binding to tubulin. However, etoposide and teniposide were shown not to be inhibitors of microtubule assembly[5] which suggested that their antitumor properties were due to another mechanism of action, via their interaction with DNA. DNA topoisomerase II has been reported to be involved in the process of inducing DNA breakage and etoposide and

its analogues are believed to inhibit the strand-rejoining activity of this enzyme by stabilizing the Topo II-DNA complex in a cleavage state[6]. 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. Structural features that switch the activity of these analogues from being antimicrotubule to antitopoisomerase are demethylation at the C-4' position of ring E, the β configuration of the substituents at C-7 on ring C and the presence of a glycosidic moiety or similar at the C-7 position[7]. These substituents have been widely studied and although the antitopoisomerase activity varied greatly with the types of substitution at the 7-position, formulation of a useful SAR proved difficult. Recently a 3D-QSAR model has been developed[8] which can be used to design novel antitopoisomerase compounds. A new class of podophyllotoxin derivatives, namely podophenazines, has also been reported which retained or even improved the cytotoxic activity, but these were weak inhibitors of topoisomerase II *in vitro*.[9] The data revealed that such analogues exhibit a different, as yet unknown, mechanism of action.

The main deficiency of these compounds is their cytotoxicity[10] for normal cells and hence side effects derived from their lack of selectivity against tumoral cells. In this regard it is necessary to investigate and prepare new more potent and less toxic analogues, that is, with better therapeutic indices.

It is well accepted from structure-activity studies in this field that the *trans*-lactones are more potent as antineoplastics than the *cis*-lactones.[11] Not only is the configuration of the D ring an important factor for high cytotoxic activity, but also a quasi-axial arrangement of the E ring is necessary.[12] On this basis, studies on lignans have been addressed to modification of the lactone moiety and to preparation of analogues with heteroatoms at different positions of the cyclolignan skeleton.[13-15]

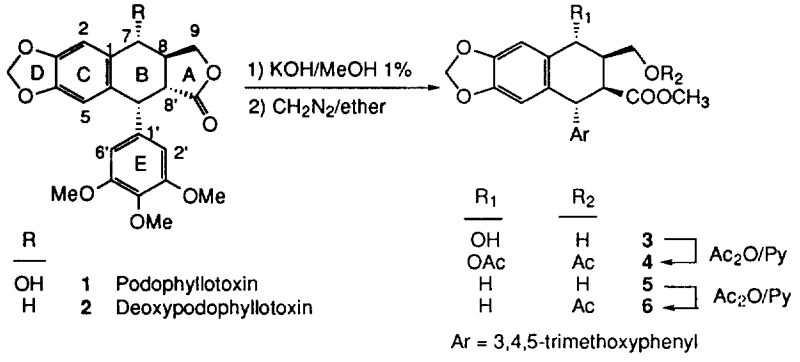
Our group has been working during the last few years on chemical transformations of podophyllotoxin and analogues and we have prepared a large number of cyclolignan derivatives some of which display potent antiviral and cytotoxic activities.[16] More recently we have reported several new cytotoxic agents with nitrogen atoms at C-7 or C-9[17] or at both C-7 and C-9.[18]

In this study we present the cytotoxicity of several semisynthetic derivatives obtained from podophyllotoxin (**1**) and deoxypodophyllotoxin (**2**). These naturally occurring lignans were isolated from *podophyllum* resin.[19] The podophyllotoxin derivatives obtained in this work presented either a carbonyl group at C-7 or C-9 or a heteroatom (nitrogen or sulfur) at these positions. This was done with the aim of studying the influence of variations in the molecular electrostatic potential produced by different heteroatoms in the activity of these compounds. They have been tested on cultures of different tumoral cell lines (P-388, A-549, HT-29 and MEL-28) and some of them have shown an interesting and selective cytotoxicity towards HT-29 human colon carcinoma.

CHEMISTRY

The lactone ring of cyclolignans **1** and **2** was opened by treatment of these compounds with potassium hydroxide. This method usually leads to the epimerization of the lactone at the C-8′ position.[20] The change of configuration occurs through the ring-opened hydroxyacid form and re-cyclisation upon acidification to pH 1-2. The cyclisation leads to the more stable *cis*-junction between the lactone and the tetralin ring, that is, compounds of the *picro* series.

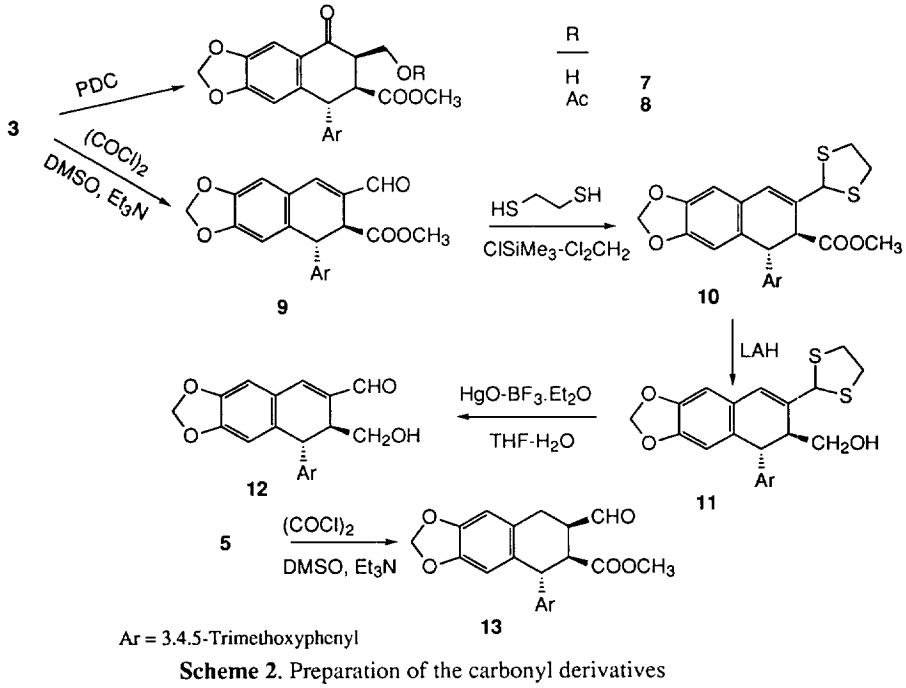
In order to isolate the hydroxyacids and to avoid lactonization, the base was neutralized with 2N HCl to pH 4-5 and the product treated with an ethereal solution of diazomethane to obtain the methyl esters **3** and **5**. Both of them, as expected, have the configuration at the C-8′ position inverted (scheme 1).



Scheme 1. Opening of the lactone ring

Preparation of carbonyl derivatives. (Scheme 2)

The oxidation of the hydroxyl groups of **3** could be performed by chromium reagents[21] or Swern oxidation.[22] Chromium reagents such as PDC, PCC and Jones′reagent lead to the oxidation of the benzylic hydroxyl group at C-7 to provide compound **7** as the only isolable product. The highest yield (55%) was obtained with PDC in dichloromethane. The oxidation of the C-7 hydroxyl group in podophyllotoxin and analogues is easily performed by different oxidants,[23] so the moderate yield in this oxidation could be due to the fact that the lactone ring is opened and the resulting oxidation product could undergo other degradation processes.



Scheme 2. Preparation of the carbonyl derivatives

The Swern oxidation of diol-ester **3** led instead to the oxidation of the hydroxyl group at C-9 and the elimination of the hydroxyl group at C-7 to provide the unsaturated aldehyde **9**. In the same way, Swern oxidation of compound **5** afforded the aldehyde **13**.

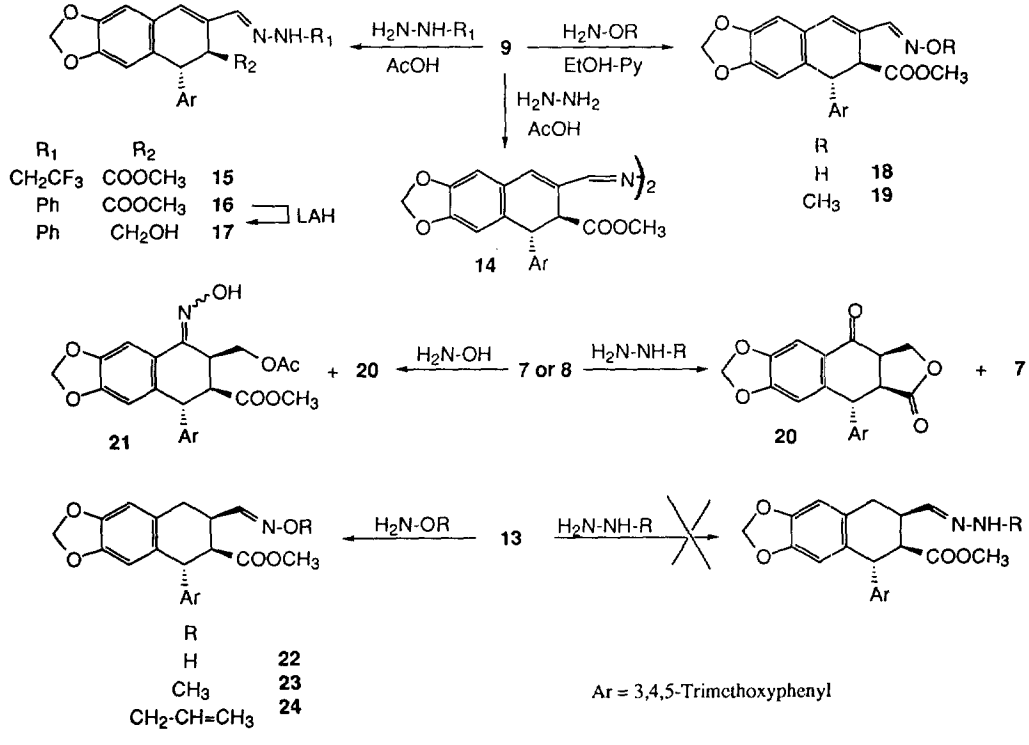
The synthesis of compound **12** was achieved by protection[24] of the aldehyde group in **9** as its dithiolane giving compound **10**. The reduction of the methyl ester with LAH led to **11** which after deprotection[25] with $HgO\text{-}BF_3.Et_2O$, produced the required hydroxyaldehyde **12** in an excellent yield.

In order to place nitrogen atoms at C-7 and C-9, the carbonyl compounds obtained were condensed with differently substituted hydrazines and hydroxylamines.

Reactions with hydrazines (Scheme 3).

Condensation of aldehyde **9** with hydrazine in acetic acid led to the azine **14**. When substituted hydrazines were used, the corresponding hydrazones **15** and **16** were obtained. Reduction of compound **16** with LAH yielded **17**.

Similar reaction of **7** with hydrazines did not lead to the expected hydrazones. The reaction products were a mixture of unreacted **7** and picropodophyllone (**20**).[26] The formation of **20** from **7** results from the facile lactonization under acidic conditions, already referred to. This ketolactone does not react with hydrazines under these conditions and was always a by-product in all reactions carried out with compound **7** or its acetylated derivative **8**.



Scheme 3. Formation of hydrazones and oximes

Reaction of the aldehyde **13** with various hydrazines appeared by TLC of the reaction mixture to give complete conversion to a new product, presumed to be the corresponding hydrazone, but in every case attempted purification by CC gave only the parent carbonyl compound.

Reactions with hydroxylamines (Scheme 3).

The condensation of compound **9** with hydroxylamines was done in ethanol and pyridine leading to the oximes **18** and **19** with variable yield. The oxime **21** was obtained by reaction of **8** with hydroxylamine in low yield due to the formation of the ketone **20**, resulting from a prior hydrolysis of the acetate and lactonization in the work up. Preparation of the oximes **22-24** was achieved by reaction of aldehyde **13** with the corresponding hydroxylamines in moderate yields.

BIOLOGICAL RESULTS.

The compounds thus prepared have been evaluated for their bioactivity against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma. The results obtained are shown in table 1.

Table 1.Antineoplastic Activity of Compounds **1-24**.(IC_{50} μM)

Compound	P-388	A-549	HT-29	MEL-28
1	0.012	0.012	0.029	
2	0.01	<0.006	0.006	
3	0.22	0.22	0.45	
4	1.10	1.10	1.10	1.10
5	0.058	0.058	0.12	0.058
6	0.21	0.21	0.21	0.21
7	5.63	5.63	5.63	5.63
8	0.20	1.00	1.00	1.00
9	0.23	0.12	0.012	.0.23
10	0.20	0.20	0.20	0.20
11	1.00	1.00	1.00	1.00
12	0.25	0.25	0.25	0.25
13	2.34	2.34	2.34	2.34
14	0.57	0.57	0.57	1.14
15	0.48	0.19	0.048	0.48
16	1.94	0.97	0.039	4.84
17	1.02	1.02	2.05	0.51
18	2.27	2.27	2.27	2.27
19	0.22	0.22	0.22	0.22
20	12.0	12.0	12.0	12.0
21	2.00	2.00	2.00	2.00
22	2.30	2.30	2.30	2.30
23	10.94	21.88	>21.88	>21.88
24	2.50	2.50	5.20	5.20

Opening of the lactone ring in the compound **1** gave **3** which is twenty times less potent in all the cell lines tested, whereas compound **5**, resulting from opening the lactone moiety of **2**, is less potent in A-549 and HT-29 than **2** but its potency on P-388 is the same order of magnitude. Acetylation of hydroxyl groups (**4** and **6** *versus* **3** and **5**) reduced the activity against all the cell lines. A carbonyl function at C-7 (**7** *versus* **3**) significantly reduced the activity. If the carbonyl group is at C-9 and the hydroxyl group at C-7 is eliminated (**9** *versus* **3**) no significant differences in cytotoxicity was observed against P-388 and A-549 but on HT-29, compound **9** is more potent than **3**, showing an IC_{50} comparable to compound **1**. Saturation of ring B of the cyclolignan skeleton (**13** *versus* **9**) significantly reduced the activity against all cell lines tested. Reduction of the methyl ester group of compound **9** led to compound **12** with no significant difference in cytotoxicity, except against HT-29, on which **12** lacked selectivity. Replacing the carbonyl group at C-9 by dithiolane (**10** *vs* **9**) removed the selectivity against HT-29 but did not significantly modify the activity against the other cell lines.

The hydrazone derivatives of aldehyde **9** (compounds **15** and **16**) are, in general, slightly less potent than the parent compound. The size of the substituent on the nitrogen seems important for selectivity. Compounds **15** and **16** have selective cytotoxicity against HT-29 whereas compound **14**, which is a dimeric structure, did not show that selectivity. Reduction of the methyl ester in compound **16** led to compound **17** which has also lost the selectivity against HT-29.

The formation of an oxime derivative significantly reduced the activity (**18** *vs* **9**) although if the oxime is substituted as in compound **19**, no differences in cytotoxicity were observed (**19** *vs* **9**) except for HT-29. In the same way, the oxime **23** is much less potent than the parent compound, the aldehyde **13**.

Compounds **9** and **16** have also been tested by the NCI against 60 different types of cancers and they were selective for colon cancer and breast cancer according to the results obtained.[27]

DISCUSSION

Several cyclolignan derivatives, lacking the lactone moiety, have been synthesized from the naturally occurring lignans podophyllotoxin (**1**) and deoxypodophyllotoxin (**2**) by simple chemical transformations. These derivatives include hydrazones and oximes in which nitrogen atoms have been introduced at different positions of the cyclolignan skeleton.

As can be deduced from the data shown in table 1, most of the compounds show similar responses for all the neoplastic systems tested, except the compounds **9**, **15** and **16** which were selective towards HT-29 human colon carcinoma. The IC_{50} of **9**, **15** and **16** for HT-29 is in the range of *trans*-lactonic cyclolignans such as **1** and **2**, placing them among the most potent compounds in this class.

Another feature that can be deduced from our study is the influence of some structural elements in the cytotoxicity. Acetylation of the free hydroxyl groups reduced the activity. Saturation of the double bond at C-7 led to a less cytotoxic compound. Reduction of the methyl esters to the corresponding alcohols also gave compounds less potent as antineoplastics.

The fact that these compounds, even lacking the lactone moiety, are as potent as lactonic cyclolignans on HT-29 could be justified through a spontaneous or enzyme-mediated hydrolysis of the hydrazones into the precursor aldehyde and this in turn could be transformed into more rigid structures, similar to the *trans*-lactones where the rings would be almost coplanar. This might be expected to make them more reactive and easily opened by nucleophilic attack by biomolecules.[18] In order to get more information about likely conformations

and reactivities, several molecular modeling studies have been done with aldehydes **9** and **13** that are discussed below.

MOLECULAR MODELING

After studying the biological results, the difference in activity between the aldehydes **9** and **13** appears surprising. From an electronic point of view, the saturated aldehyde **13** is expected to be more electrophilic than **9** and should therefore (based on the proposed mechanism of action[18]) be more potent. The differences in potency of these compounds cannot be explained by such electronic factors.

Several molecular modeling studies have been done with both aldehydes in comparison with the lead compound podophyllotoxin (**1**) with the aim of finding an explanation for the difference in potency. There are several metabolic events that could occur under the whole-cell conditions used in the assays. The range of possibilities is such that it would certainly be possible to separate why the unsaturated compound is more selective and more potent than the saturated compound. Until there is more evidence for the transformations involved, however, this would be total speculation.

These studies were based on the fact that these compounds are analogues of podophyllotoxin, a competitive inhibitor of colchicine binding to tubulin. Recent evaluation of the structural basis for tubulin polymerization inhibition has concluded that the three-dimensional conformation of the A, B, C and D rings is important for interaction with tubulin.[28] We have used the simplifying hypothesis that the site of action of all these compounds is largely occupied by **1**, and that therefore the degree of overlap of each compound with **1** could serve as a guide to expected activity. Some evidence in support of the hypothesis is provided by the reduced activity of the enantiomer at C-8' and the lack of activity against microtubule assembly of analogues substituted at C-7, such as etoposide,[11] and inactivity of ring A/B deleted analogues.[29] The degree of overlap would depend also on the conformer chosen for the compound being studied. For this reason we studied a range of conformers selected by hierarchical clustering methods and carried out alignments based on both the pair of aromatic rings and the tetracyclic ring system of podophyllotoxin (see experimental section). We show a few of the more interesting of these superpositions in figures 1-3.

For podophyllotoxin (**1**) only one conformation was found (except for alternative rotamers of the methoxy groups and ring-flips of the dioxolane). Aldehydes **9** and **13** have more flexibility and thus a large number of conformations that are local minima. These were selected on the basis of the distance between the aromatic rings and the disposition of the tricycle/tetracycle systems. Both parameters seem to be significant for the activity and the potency. The carbon C-9 has been identified as an important site for the interaction mechanism with biomolecules[18] and the carbonyl at C-9' is of some importance in the potency since its removal yielded less potent compounds, as has been discussed above.

Figure 1 shows the "best fit" of **13** to **1** based on rms separation of the carbons in the aromatic rings, and as can be seen, the methoxycarbonyl group appears separated from the position of the lactone of **1**. Figure 2 shows the lowest MOPAC energy conformation of **13** superimposed on **1** using the ABC ring and 8 and 8' substituent carbon atoms as matching points. In this case it is the aldehyde group which is displaced relative to the corresponding atom in **1**. Neither of these changes may be acceptable in the binding site, consistent with the known SAR of the CD ring fusion.

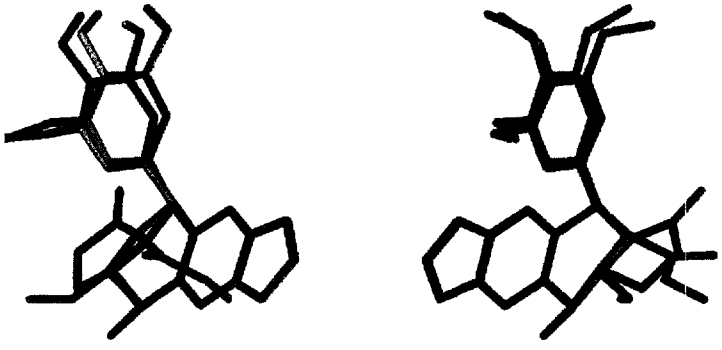


Figure 1: "Best Fit" based on aryl-aryl distances found for any conformer of **13** (magenta C carbons) to **1** (Black C carbons). Other atoms are coloured conventionally by atom type.

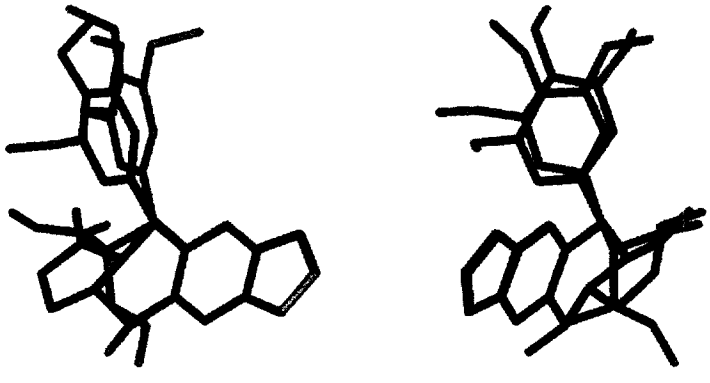


Figure 2: Optimal overlay based on the tetracycle of the lowest-energy conformer found of **13** (magenta C carbons) to **1** (Black C carbons). Other atoms are coloured conventionally by atom type.

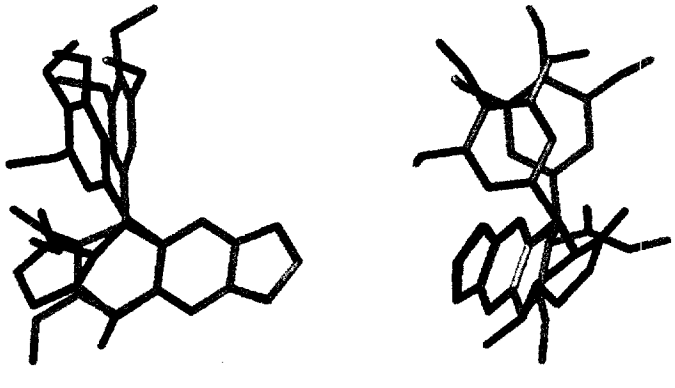


Figure 3: Optimal overlay based on the tetracycle of any conformer found of **9** (blue-green C carbons) to **1** (Black C carbons). Other atoms are coloured conventionally by atom type.

These results can be compared with those found for the unsaturated derivative. Aldehyde **9** showed flexibility similar to **13** and the "best fit" (based on aryl-aryl match) is also the lowest-energy conformer found. However, the disposition of the ester is similar to that seen in figure 1 for the saturated case. Given that the position of the ester is likely to be important, then an alternative conformation which is only slightly more strained (~1 Kcal/mol) could be considered a better choice as shown in figure 3.

As can be seen, the unsaturated species is much better able to place the trimethoxyphenyl, the ester and the aldehyde in positions similar to those seen in **1** than is the saturated system.

This study could explain why the IC_{50} of aldehyde **9** against HT-29 is more similar to that of **1**, while the saturated aldehyde **13** is much less potent. The selectivity against HT-29 human colon carcinoma is not explained by these studies, although it may be postulated that the active site of the enzyme in HT-29 could have small modifications such that certain flexibility is allowed regarding where the trimethoxyphenyl group is located relative to the ABC ring system.

EXPERIMENTAL

Molecular Modelling Studies .All computational work was carried out on a Silicon Graphics Indigo^2 Extreme workstation running IRIX 5.2 or 5.3. Quantum mechanical calculations were carried out using MOPAC 6.0 (J.J.P. Stewart, QCPE Program 455) using the PM3 Hamiltonian, eigenvector following geometry optimisation and PRECISE convergence criteria. All other computational studies were carried out within the Sybyl program (v. 6.04 to 6.2, Tripos Inc., St. Louis, Missouri).

Since to our knowledge there is no information available on the binding geometry of podophyllotoxin and analogues to the biological target, our approach sought to explore the possible conformational space of our analogues as widely as possible. The structures were built from fragments in the Sybyl fragment library or by modification of the structure of podophyllotoxin, which was built from these fragments. Each initially-drawn conformer was then minimised to default convergence using the Tripos forcefield to give the "initial conformer" of each structure. Conformers were generated using the Sybyl RANDOMSEARCH command. Thus, a set of bonds (the SEARCH_BOND set) was identified which at each iteration of the RANDOMSEARCH procedure were assigned random torsion values. The SEARCH_BOND set used in this study consisted of all single bonds between non-hydrogen atoms except bonds to terminal methyl groups. Each conformation thus generated was optimised using the Tripos forcefield, with a maximum of 1000 cycles of minimisation. After optimisation, each new structure generated that had a strain energy less than a cutoff value (set to 20 kcal/mol higher than the energy of the initial conformer) was compared with all those so far generated using the MATCH command, based on all non-hydrogen atoms. MATCH aligns molecules based on finding the set of atom-atom correspondences which leads to the smallest RMS fitting error. If the RMS error generated by one of these alignments was less than 0.2 Å, then the lower-energy conformer was retained in the database of conformers so far found and the other discarded. If no corresponding alignment was found in the database, then the new conformer was added to that database. The search was stopped if either more than 999 attempts had been made to find new conformers, or all those conformers that had been found were found at least 12 times each.

Compound	No. of Conformers found	No. of failed tries
1	35	777
9	270	568
13	275	570

In several cases, this process found large numbers of conformers. Two strategies were adopted to reduce these to a manageable number of representative conformers for the comparisons described in the results and discussion section. Firstly, all the conformers were superimposed one at a time on podophyllotoxin using the MATCH command. The atoms used in the matching process were the carbon atoms of the tetrahydronaphthyl and pendant phenyl ring, together with the carbon atoms of the lactone ring of podophyllotoxin, and all non-hydrogen atoms of the conformer to be matched. A fit was considered to have been found initially with a maximum RMS fitting error of 0.05 Å; if no fit was found at this level, the tolerance was increased in 0.05 Å steps until a fit was found. If several were found, the conformer of lowest energy was chosen as the representative "best fit" at the minimum tolerance specified.

Many of the conformers were observed to differ only in the disposition of the aromatic methoxy substituents. In order to ensure a range of conformers representing as widely as possible the conformational space explored in the RANDOMSEARCH, the remaining conformers were entered into a Sybyl molecular spreadsheet and distances were computed between the carbon atoms of the aromatic rings. The aromatic rings were chosen as their relative disposition was believed to be important to the biological activity of these molecules[12] and would also reflect changes in the ring-flip adopted by the tetrahydronaphthalene unit. Thus, a distance column was added to the table for the distance from each of the carbon atoms in the benzo ring of the tetrahydronaphthalene unit to the ortho, meta and para carbons of the pendant phenyl ring. Where two atoms were related by symmetry (i.e. the ortho and meta carbons of the pendant phenyl ring), the shorter distance was entered in the table. Thus, a total of 18 columns were added to the table. The distances in these columns were then analysed using Sybyl Hierarchical Clustering, using the AVERAGE mode of cluster grouping. The dendrograms derived from this calculation showed clustering of conformers based on the relative orientation of the two aromatic rings as described by the distances calculated. The dendrograms in each case showed either 5 or 6 distinct clusters, and the lowest-energy conformation in each cluster was picked as representative of that cluster. The conformations selected in this way were geometry optimised using MOPAC as described above. As a result of this, some conformers which had been found using the molecular mechanical methods to represent separate minima converged on a single minimum. All the distinct conformers modeled at this stage were superimposed on podophyllotoxin again using MATCH with either the carbons of the aromatic rings or the carbon atoms corresponding to the tetracyclic ring system of podophyllotoxin as the ternplate. Those considered in results and discussion were the lowest energy conformer (according to MOPAC) of those studied, the best aromatic-aromatic fit found and the best polycycle-polycycle fit found, with best fit defined as that with the smallest RMS error.

Chemistry. Melting points were determined by heating in an external silicone bath and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in chloroform solution and UV spectra on a Hitachi 100-60 spectrophotometer in ethanol solution. IR spectra were obtained on a Beckmann (Acculab VIII) spectrophotometer in chloroform solution. EIMS were run in a VG-TS-250 spectrometer working at 70 eV. NMR spectra were recorded at 200 MHz for ^{1}H and 50.3 for ^{13}C in deuterochloroform using TMS as internal reference, on a Bruker WP 200 SY. Chemical shift values are expressed in ppm followed by multiplicity and coupling constants (J) in Hz. Flash chromatography was performed on silica gel (Merck No 9385). Elemental analysis were carried out on a Perkin-Elmer 2400 CHN, Elemental Analyzer.

Methyl picropodophyllate (**3**) : 400 mg (0.97 mmol) of **1** were dissolved in 30 mL of 1% KOH/MeOH and stirred at room temperature for 30 min. After removing the methanol, water was added and the solution was neutralized by 2N HCl until pH 4-5 and extracted with EtOAc. The reaction product was treated with an ethereal solution of CH_2N_2 to afford 420 mg (97.5%) of **3**. $[\alpha]^{22}$ (λ): -92.5° (589), -97.5° (578), -111.4° (546), -197.0° (436), -322.5° (365) (c 0.64%, $CHCl_3$). UV λ_{max} (ε): 316 (23000), 290 (3700). IR: 3620, 3500, 1735, 1600, 1510, 1240, 1140, 1050 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.45 (m, H-8), 3.35 (dd J=8.0,3.7 hz, H-8'), 3.59-3.82 (m, H-9), 3.58 (s, OCH_3), 3.74 (s, $2OCH_3$), 3.79 (s, OCH_3), 4.23 (d, J=8.0 hz, H-7'), 4.83 (d J=5.2 hz, H-7), 5.86 (s, OCH_2O), 6.32 (s. H-5), 6.34 (s, H-2',6'), 6.80 (s, H-2). ^{13}C NMR ($CDCl_3$) δ 43.8, 45.5, 47.0, 51.9, 56.3, 60.8, 62.4, 69.5, 101.1, 106.7, 108.4, 109.5, 130.2, 130.8, 137.0, 140.2, 146.8, 147.8, 153.2, 174.9. Anal. calcd. for $C_{23}H_{26}O_9$: C, 61.88; H, 5.87; found: C, 61.82; H, 5.85.

Methyl diacetyl-picropodophyllate (**4**) : Acetylation of **3** (50 mg, 0.11 mmol) with acetic anhydre in pyridine afforded, after usual work-up, 52 mg (98.0%) of diacetate **4**. $[\alpha]^{22}$ (λ): -39.6° (589), -41.6° (578), -47.7° (546), -82.8° (436), -129.7° (365) (c 1.03%, $CHCl_3$). UV λ_{max} (ε): 210 (55000), 214 (14000), 291 (5300). IR: 1735, 1595, 1500, 1240, 1130 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.12 (s, 3H), 2.78 (m, 1H), 3.42 (dd J=10.3, 4.0 hz, 1H), 3.65 (s, 1H), 3.79 (m, 1H), 3.80 (s, 6H), 3.83 (s, 3H), 4.24 (dd J=11.5,5.9 hz, 1H), 4.31 (d J=10.3 hz, 1H), 5.90 (s, 2H), 5.99 (d J=3.5 hz, 1H), 6.36 (s, 1H), 6.39 (s, 2H), 6.82 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.7, 21.4, 39.8, 44.0, 46.4, 51.9, 56.2, 60.8, 61.9, 70.6, 101.3, 106.8, 109.5, 109.8, 124.8, 132.9, 137.3, 140.2, 146.7, 148.6, 153.3, 170.0, 170.4, 173.0. Anal. calcd. for $C_{27}H_{30}O_{11}$: C, 61.13; H, 5.70; found: C, 61.08; H, 5.74.

Methyl deoxypicropodophyllate (**5**) : A solution of **2** (200 mg, 0.50 mmol) in 25 mL of 1% KOH/MeOH was stirred for 30 min at room temperature. Following the procedure described before, 198 mg (91.1%) of **5** was obtained. $[\alpha]^{22}$ (λ): -66.0° (589), -69.3° (578), -83.3° (546), -136.7° (436) (c 0.15%, $CHCl_3$). UV λ_{max} (ε): 207 (23000), 294 (2400). IR: 3600-3400, 1770, 1600, 1510, 1230, 1140, 1045 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.40 (m, 1H), 2.73 (dd J=16.7, 7.5 hz, 1H), 2.91 (dd J=16.7,5.3 hz, 1H), 3.05 (dd J=6.4,3.6 hz, 1H), 3.50-3.80 (m, 2H), 3.62 (s, 3H), 3.77 (s, 6H), 3.81 (s, 3H), 4.33 (d J=6.4 hz, 1H), 5.86 (s, 2H), 6.27 (s, 2H), 6.36 (s, 1H), 6.58 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 30.7, 35.9, 46.4, 49.6, 51.7, 56.3, 60.7, 63.6, 100.7, 106.8, 108.3, 109.6, 128.3, 129.5, 137.2, 141.0, 146.2, 146.6, 153.2, 174.3. Anal. calcd. for $C_{23}H_{26}O_8$: C, 64.18; H, 6.09; found: C, 64.07; H, 6.12.

Methyl acetyl-deoxypicropodophyllate (**6**): Acetylation of **5** in the usual way, afforded the appropriate acetate **6** (95.3%). $[\alpha]^{22}$ (λ): -63.7° (589), -66.3° (578), -77.5° (546), -132.5° (436) (c 0.08%, $CHCl_3$). UV λ_{max} (ε): 209 (20900), 294 (2300). IR: 1740, 1600, 1500, 1230, 1140 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.00 (6H, s), 2.53 (1H, m), 2.77 (1H, dd J=16.9,6.8 hz), 2.98 (1H, dd J=16.9,5.1 hz), 3.02 (1H, dd J=6.8,3.4 hz), 3.64 (3H, s), 3.77 (6H, s), 3.81 (3H, s), 4.04 (1H, dd J=11.1,7.8 hz), 4.21 (1H, dd J=11.1,6.2 hz), 4.35 (1H, d J=6.8 hz), 5.86 (1H, s), 5.88 (1H, s), 6.26 (2H, s), 6.34 (1H, s), 6.57 (1H, s). ^{13}C NMR ($CDCl_3$) δ 20.7, 31.1, 33.1, 45.8, 49.6, 51.6, 56.4, 60.8, 65.2, 100.8, 106.6, 108.3, 109.8, 127.6, 129.5, 137.2, 140.8, 146.4, 146.5, 153.2, 170.6, 173.1. Anal. calcd. for $C_{25}H_{28}O_9$: C, 63.55; H, 5.97; found: C, 63.45; H, 5.84.

Methyl picropodophyllonate (**7**): Pyridinium dichromate (PDC) (400 mg, 1.06 mmol) was added to a solution of **3** (300 mg, 0.67 mmol) in dry CH_2Cl_2 (20 mL) and stirred at room temperature for 24 h. The excess of PDC was removed by filtration followed by flash chromatography of the residue to give 165 mg (55.2 %) of **7**. mp: 76-78°C (H/CH_2Cl_2). $[\alpha]^{22}$ (λ): -50.4° (589), -53.6° (578), -64.9° (546), -174.5 (546) (c 0.67%, $CHCl_3$). UV λ_{max} (ε): 234 (13800), 275 (6300), 316 (5200). MS m/z (rel. abond. %): 444 (4) (M^+), 426 (23), 412 (85), 367 (100), 355 (14), 297 (8), 168 (5), 139 (4). IR: 3500, 1740, 1680, 1600, 1510, 1260, 1045. ^{1}H NMR ($CDCl_3$) δ 2.95 (m, H-8), 3.28 (dd, J=3.8,2.8 hz, H-8'), 3.58 (dd, J=11.2,4.4 hz, H-9a), 3.61 (s, $COOCH_3$), 3.74 (s, $2OCH_3$), 3.80 (s, OCH_3), 4.24 (dd, J=11.2,7.4 hz, H-9b), 4.59 (d, J=2.8 hz, H-7'), 6.00 (1H, s, OCH_2O), 6.03 (1H, s, OCH_2O), 6.22 (s, H-2',6'), 6.50 (s, H-5), 7.51 (s, H-2). ^{13}C NMR ($CDCl_3$) δ 45.9 (C-8), 47.8 (C-7'), 50.9 (C-8'), 52.1 ($COOCH_3$), 56.5 ($2OCH_3$), 60.7 (OCH_3), 62.1 (C-9), 101.9 (OCH_2O), 105.7 (C-2), 106.6 (C-2',6'), 109.3 (C-5), 127.8 (C-1), 137.1 (C-6), 137.8 (C-1'), 137.9 (C-4'), 147.7 (C-3), 152.8 (C-4), 153.7 (C-3',5'), 172.7 (C-9'), 188.8 (C-7). Anal. calcd. for $C_{23}H_{24}O_9$: C, 62.16; H, 5.41; found: C, 61.82; H, 5.19.

Methyl acetyl-picropodophyllonate (**8**): Acetylation of **7** yielded acetate **8** (98.3%). $[\alpha]^{22}$ (λ): -72.4° (589), -77.2° (578), -92.5° (546) (c 0.92%, $CHCl_3$). UV λ_{max} (ε): 210 (26000), 235 (19000), 278 (5500), 320 (4700). IR: 1745, 1625, 1600, 1510, 1250, 1140, 1010 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.99-3.07 (m, 1H), 3.41 (dd, J=4.7,3.4 hz, 1H), 3.65 (s, 3H), 3.77 (s, 6H), 3.84 (s, 3H), 4.30 (dd, J=11.6, 8.5 hz, 1H), 4.64 (d, J=3.4 hz, 1H), 4.71 (dd, J=11.6,5.6 hz, 1H), 6.03 (d, J=1.1 hz, 1H), 6.06 (d, J=1.1 hz, 1H), 6.25 (s, 2H), 6.52 (s, 1H), 7.55 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.7, 43.6, 47.4, 50.6, 52.1, 56.5, 60.8, 62.4, 101.9, 106.0, 106.4, 109.2, 127.6, 137.0, 137.6, 138.0, 148.0, 152.7, 153.7, 170.5, 172.2, 192.7. Anal. calcd. for $C_{25}H_{26}O_{10}$: C, 61.72; H, 5.39; found: C, 61.82; H, 5.37.

Methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**9**): To a precooled (-55°C) and stirred solution of oxalyl chloride (0.24 mL, 2.7 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise 0.4 mL (5.6 mmol) of DMSO in dry CH_2Cl_2 (2 mL). After 5 min at -55°C, a solution of 400 mg (0.90 mmol) of **3** in 3 mL of dry CH_2Cl_2 was slowly added. The mixture was stirred at the same temperature for 30 min, then triethylamine (1.27 mL, 9.10 mmol) was added dropwise. The mixture was warmed to 0°C over 1h, quenched with water and extracted with CH_2Cl_2. The reaction product gave 276 mg (72.2%) of **9** after flash chromatography. mp: 72-74°C (CH_2Cl_2) $[\alpha]^{22}$ (λ): -160.9° (589), -171.1° (578), -210.7° (546), -699.8° (436) (c 0.45%, $CHCl_3$). UV λ_{max} (ε): 215 (34500), 250 (30000), 358 (26300). MS m/z (rel. abond. %): 426 (59) (M^+), 367 (100), 339 (31), 324 (28), 277 (4), 183 (7). IR: 2950, 1740, 1680, 1600, 1510 1230, 1140 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.60 (s, $COOCH_3$), 3.70 (s,$2OCH_3$), 3.75 (s, OCH_3), 3.98 (d, J=3.1 hz, H-8'), 4.60 (d, J=3.1 hz, H-7'), 5.96 (s, OCH_2O, 1H), 5.98 (s, OCH_2O, 1H), 6.18 (s, H-2',6'), 6.65 (s, H-5), 6.86 (s, H-7), 7.34 (s, H-2), 9.57 (s, H-9). ^{13}C NMR ($CDCl_3$) δ 44.5 (C-8'), 46.4 (C-7'), 52.4 ($COOCH_3$), 56.3 ($2OCH_3$), 60.7 (OCH_3), 101.8 (OCH_2O), 105.4 (C-2',6'), 108.9 (C-2), 110.1 (C-5), 125.2 (C-8), 133.3 (C-6), 133.9 (C-1), 137.3 (C-1'), 137.8 (C-4'), 145.3 (C-7), 147.5 (C-3), 150.5 (C-4), 153.4 (C-3',5'), 172.0 (C-9'), 191.0 (C-9). Anal. calcd. for $C_{23}H_{22}O_8$: C, 64.79; H, 5.16; found: C, 64.72; H, 4.87.

Ethylenethioacetal of methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**10**): 0.15 mL (1.30 mmol) of 1,2-ethanedithiol and 0.3 mL of $SiMe_3Cl$ were added to 100 mg (0.23 mmol) of **9** in 3 mL of CH_2Cl_2 and

stirred at room temperature for 20 h under Argon. Then the reaction mixture was treated with satd. aq. NaOH and extracted with EtOAc. After removing the solvent 112 mg (95.1%) of **10** was obtained. mp: 82-84°C (CH_2Cl_2). $[\alpha]^{22}$ (λ): -96.1° (589), -102.6° (578), -122.2° (546), -302.2° (436) (c 0.23%, $CHCl_3$). UV λ_{max} (ε): 238 (15300), 311 (8400). IR: 1735, 1600, 1510, 1230 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.97-3.17 (m, 5H), 3.63 (s, 3H), 3.74 (s, 6H), 3.77 (s, 3H), 4.36 (d, J=1.6 hz. 1H), 5.34 (s, 1H), 5.90 (s, 2H), 6.26 (s, 2H), 6.57 (s, 1H), 6.68 (s, 1H), 6.71 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 38.5, 39.3, 47.6, 49.3, 52.3, 56.4, 58.1, 60.8, 101.2, 105.6, 107.4, 109.4, 127.0, 127.4, 129.2, 130.6, 136.8, 138.3, 146.9, 147.6, 153.1, 172.9. Anal. calcd. for $C_{25}H_{26}O_7S_2$: C, 59.74; H, 5.21; found: C, 59.62; H, 5.24.

Ethylenethioacetal of 9-deoxy-9-oxo-α-apopicropodophyllol (**11**): A solution of 40 mg (0.08 mmol) of **10** in dry ether (3 mL) was slowly added to a suspension of 50 mg (1.3 mmol) of LAH in dry ether. The reaction mixture was stirred at r.t. under Argon for 3 h. Then, wet EtOAc was added, filtered, dried and evaporated to afford 37 mg (98.0%) of **11**. $[\alpha]^{22}$ (λ): -16.2° (589), -17.7° (578), -23.1° (546), -71.5° (436) (c 0.13%, $CHCl_3$). UV λ_{max} (ε): 214 (23200), 313 (8200). IR: 3700-3200, 1600, 1510, 1230, 1040 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.81 (m, 1H), 3.03-3.24 (m, 4H), 3.43 (dd, J=10.3,8.4 hz, 1H), 3.74 (s, 9H), 3.77 (s, 3H), 3.80-3.94 (m, 1H), 4.21 (s, 1H), 5.23 (s, 1H), 5.92 (s, 2H), 6.24 (s, 2H), 6.59 (s, 1H), 6.63 (s, 1H), 6.66 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 38.7, 39.4, 46.1, 47.5, 56.4, 58.0, 60.6, 64.2, 101.0, 105.9, 107.1, 110.3, 126.5, 127.2, 129.7, 133.7, 137.3, 139.3, 146.7, 147.5, 153.0. Anal. calcd. for $C_{24}H_{26}O_6S_2$: C, 60.74; H, 5.52; found: C, 60.62; H, 5.50.

9-deoxy-9-oxo-α-apopicropodophyllol (**12**): To a solution of 40 mg (0.19 mmol) of HgO and 0.25 mL of $BF_3{\cdot}Et_2O$ in 5 mL of THF/H_2O (85:15), 40 mg (0.08 mmol) of **11** were added. The reaction mixture was stirred at room temperature under Argon for 3 h. Then diluted with CH_2Cl_2 and the precipitate discarded. The solution yielded 30 mg (95.0%) of **12** after evaporation. $[\alpha]^{22}$ (λ): -85.1° (589), -90.2° (578), -113.0° (546), -431.6° (436) (c 0.22%, $CHCl_3$). UV λ_{max} (ε): 215 (16500), 245 (14300), 356 (8200). IR: 3600-3100, 2860, 1670, 1600, 1510, 1240, 1045 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.26 (m, 1H), 3.38 (dd J=10.3,8.4, 1H), 3.64 (dd, J=10.3,5.4 hz, 1H), 3.72 (s, 6H), 3.76 (s, 3H), 4.31 (s, 1H), 6.01 (s, 2H), 6.18 (s, 2H), 6.70 (s, 1H), 6.87 (s, 1H), 7.28 (s, 1H), 9.54 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 42.5, 45.1, 56.3, 60.7, 63.5, 101.7, 105.4, 108.7, 110.9, 125.2, 134.3, 136.0, 137.5, 139.3, 146.2, 147.3, 150.5, 153.3, 192.6. Anal. calcd. for $C_{22}H_{22}O_7$: C, 66.32; H, 5.57, found: C, 66.10; H, 5.58.

Methyl 9-deoxy-9-oxo-deoxypicropodophyllate (**13**): Following the same procedure described for aldehyde **9**, 82 mg (54.9%) of **13** were obtained from 150 mg (0.35 mmol) of **5** after flash chromatography. $[\alpha]^{22}$ (λ): -68.9° (589), -71.5° (578), -83.3° (546), -151.9° (436) (c 0.27%, $CHCl_3$). UV λ_{max} (ε): 213 (42500), 294 (6300). MS m/z (rel. abond. %): 428 (100) (M^+), 410 (13), 367 (15), 351 (22), 339 (71), 283 (16), 252 (21), 173 (10), 149 (16), 135 (5). IR: 2840, 1735, 1600, 1510, 1230 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.80 (td, J=8.4,3.4 hz, 1H), 3.16 (d, J=8.4 hz, 2H), 3.40 (dd, J=3.4,3.2 hz, 1H), 3.62 (s, 3H), 3.74 (s, 6H), 3.79 (s, 3H), 4.65 (d, J=3.2 hz, 1H), 5.88 (s, 1H), 5.91 (s, 1H), 6.22 (s, 2H), 6.44 (s, 1H), 6.65 (s, 1H), 9.74 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 26.6, 43.8, 46.4, 48.1, 52.0, 56.3, 60.7, 100.9, 106.6, 108.3, 109.8, 127.5, 128.6, 138.3, 140.3, 146.6, 147.0, 153.4, 172.1, 201.9. Anal. calcd. for $C_{23}H_{24}O_8$: C, 64.49; H, 5.61, found: C, 64.22; H, 5.40.

Azine of methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**14**): Hydrazine hydrochloride (30 mg, 0.44 mmol) was added to a solution of **9** (50 mg, 0.12 mmol) in 5 mL of glacial acetic acid and stirred at room temperature for 4 h. Then water was added and 51 mg (98.0%) of **14** precipitated and were collected. $[\alpha]^{22}$ (λ): -166.0° (589), -178.0° (578), -218.0° (546) (c 0.05%, $CHCl_3$). UV λ_{max} (ε): 210 (19600), 260 (7000), 403 (13800). MS m/z (rel. abond. %): 848 (18) (M^+), 821 (7), 789 (10), 762 (7), 681 (12), 425 (25), 366 (100), 312 (8), 226 (11), 168 (8). HRMS (EI) calcd for $C_{46}H_{44}N_2O_{14}$ 848.2792, found m/z: 848.2787. IR: 3600-3000, 1730, 1600, 1510, 1270 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.64 (s, 6H), 3.71 (s, 12H), 3.75 (s, 6H), 4.20 (d, J=2.8 hz, 2H), 4.52 (d, J=2.8 hz, 2H), 5.95 (s, 4H), 6.26 (s, 4H), 6.63 (s, 2H), 6.77 (s, 2H), 6.92 (s, 2H), 8.16 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 46.6, 46.9, 52.3, 56.3, 60.7, 101.4, 105.4, 108.1, 109.9, 126.7, 129.9, 132.0, 137.0, 137.5, 138.1, 147.2, 148.6, 153.3, 161.6, 172.7.

2,2,2-Trifluoroethylhydrazone of methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**15**): 0.10 mL (0.8 mmol) of 2,2,2-trifluoroethylhydrazine was added to a solution of 100 mg (0.23 mmol) of **9** in 5 mL of glacial acetic acid and stirred at room temperature for 72 h. After addition of water, the unreacted aldehyde (20 mg) precipitated and was filtered off. The filtrate was treated with satd. aq. $NaHCO_3$ and extracted with EtOAc. After removing the solvent, 73 mg (75.8%) of **15** was obtained. mp: 148-150°C (H/CH_2Cl_2). $[\alpha]^{22}$ (λ): -90.4° (589), -95.8° (578), -117.1° (546), -324.2° (436) (c 0.24%, $CHCl_3$). UV λ_{max} (ε): 213 (16400), 337 (13600). MS m/z (rel. abond. %): 522 (32) (M^+), 490 (68), 463 (51), 426 (24), 367 (50), 33 (17), 324 (16), 245 (5), 207 (6), 168 (31), 119 (100), 91 (19). HRMS (EI) calcd for $C_{25}H_{25}N_2O_7F_3$ 522.1614, found m/z: 522.1610. IR: 1730, 1670, 1600, 1510, 1230 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.60 (s, 3H), 3.60-4.80 (m, 2H), 3.72 (s, 6H), 3.76 (s, 3H), 4.12 (d J=2.5 hz, 1H), 4.46 (d J=2.5 hz, 1H), 5.90 (s, 1H), 5.91 (s, 1H), 6.28 (s, 2H), 6.54 (s, 1H), 6.62 (s, 1H), 6.68 (s, 1H), 7.43 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 46.4, 46.7, 52.0, 56.2, 60.6, 101.2, 105.4, 107.4, 109.8, 127.1, 129.8, 130.0, 130.6, 137.1, 138.6, 141.3, 147.1, 147.8, 153.2, 172.9.

Phenylhydrazone of methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**16**): 0.1 mL (1 mmol) of phenylhydrazine was added to a solution of 200 mg (0.47 mmol) of **9** in 5 mL of glacial acetic acid and stirred at room temperature for 48 h. Then water was added and 212 mg (87.5%) of **16** precipitated and were collected. mp: 172-174°C (CH_2Cl_2) $[\alpha]^{22}$ (λ): -309.1° (589), -332.6° (578), -416.1° (546) (c 0.15%, $CHCl_3$). UV λ_{max} (ε): 209 (28600), 377 (24100). MS m/z (rel. abond. %): 515 (12) (M^+), 441 (3), 428 (5), 415 (6), 391 (4), 212 (5), 196 (5), 168 (10), 151 (7), 124 (7), 94 (100). IR: 1740, 1610, 1600, 1510, 1260 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.66 (s, $COOCH_3$), 3.75 (s, $2OCH_3$), 3.78 (s, OCH_3), 4.33 (d J=3.3 hz, H-8'), 4.49 (d J=3.3 hz, H-7'), 5.92 (s, OCH_2O, 1H), 5.93 (s, OCH_2O, 1H), 6.39 (s, H-2',6'), 6.52 (s, H-5), 6.65 (s, H-7), 6.68 (s, H-2), 6.81 (t J=7.5 hz, H-4"), 6.91 (d J=7.5 hz, H-2",6"), 7.18 (t J=7.5 hz, H-3",5"), 7.39 (s, H-9). ^{13}C NMR ($CDCl_3$) δ 46.8 (C-8'), 47.0 (C-7'), 52.2 ($COOCH_3$), 56.3 ($2OCH_3$), 60.7 (OCH_3), 101.2 (OCH_2O), 105.2 (C-2',6'), 107.4 (C-2), 109.7 (C-5), 112.8 (C-2",6"), 120.0 (C-4"), 127.3 (C-8), 129.2 (C-3",5"), 130.5 (C-1,6), 137.0 (C-4'), 138.5 (C-1'), 138.7 (C-7), 144.7 (C-9), 147.0 (C-3), 147.5 (C-4), 153.2 (C-3',5'), 173.4 (C-9'). Anal. calcd. for $C_{29}H_{28}O_7N_2$: C, 67.44; H, 5.43; N, 5.43; found: C, 67.12; H, 5.34; N, 4.97.

Phenylhydrazone of 9-deoxy-9-oxo-α-apopicropodophyllol (**17**): 100 mg (0.19 mmol) of **16** in dry ether (5 mL) was slowly added to a suspension of LAH (130 mg, 3.40 mmol) in dry ether. The reaction mixture was stirred at room temperature under Argon for 3 h. Then, wet EtOAc was added, filtered, dried and evaporated to afford 92 mg (97.3 %) of **17**. mp: 96-98°C (CH_2Cl_2). $[\alpha]^{22}$ (λ): -206.5° (589), -220.0° (578), -276.7° (546) (c 0.22%, $CHCl_3$). UV λ_{max} (ε): 230 (16000), 377 (14700).. IR: 3400, 1610, 1600, 1510, 1260 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.30 (m, 1H), 3.60 (m, 2H), 3.70 (s, 6H), 3.75 (s, 3H), 4.20 (bs, 1H), 5.92 (s, 2H), 6.34 (s, 2H), 6.42 (s, 1H), 6.64 (s, 1H), 6.69 (s, 1H), 6.79 (t, 1H), 6.96 (d, 2H), 7.17 (t, 2H), 7.42 (s,1H).^{13}C NMR ($CDCl_3$) δ 44.0, 45.3, 56.3, 60.7, 64.6, 101.1, 105.4, 107.2, 110.5, 112.8, 120.1, 127.2, 129.3, 130.0, 133.4, 137.1, 140.0, 140.2, 144.6, 146.7, 147.6, 153.1. Anal. calcd. for $C_{28}H_{28}O_6N_2$: C, 68.85; H, 5.74; N, 5.74; found: C, 68.79; H, 6.01; N, 5.37.

Oxime of methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**18**): 0.1 mL of pyridine and 35 mg (0.5 mmol) of hydroxylamine hydrochloride were added to a solution of 100 mg (0.23 mmol) of **9** in 15 mL of ethanol. The reaction mixture was stirred at 95 °C for 72 h. Then, it was concentrated under vacuum and extracted with EtOAc. The reaction product was purified by flash chromatography to provide 42 mg (40.6%) of **18**. $[\alpha]^{22}$ (λ): -150.0° (589), -159.1° (578), -192.7° (546), -513.6° (436) (c 0.11%, $CHCl_3$). UV λ_{max} (ε): 204 (32500), 327 (13200). MS m/z (rel. abond. %): 441 (20) (M^+), 423 (29), 392 (39), 364 (100), 349 (23), 333 (15), 252 (7), 191 (4), 153 (5). IR: 3500-3100, 1740, 1600, 1510, 1230 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.64 (s, 3H), 3.72 (s, 6H), 3.77 (s, 3H), 4.00 (d J=2.4 hz, 1H), 4.48 (d J=2.4 hz, 1H), 5.94 (s, 1H), 5.95 (s, 1H), 6.27 (s, 2H), 6.64 (s, 1H), 6.72 (s, 1H), 6.74 (s, 1H), 7.87 (s, 2H), 8.24 (bs,OH). ^{13}C NMR ($CDCl_3$) δ 46.5, 52.3, 56.3, 60.7, 101.3, 105.3, 107.8, 109.6, 126.4, 126.8, 131.1, 133.2, 137.6, 138.2, 147.1, 148.3, 151.4, 153.3, 172.5. Anal. calcd. for $C_{23}H_{23}O_8N$: C, 62.59; H, 5.22; N, 3.17; found: C, 62.37; H, 5.04; N, 3.07.

O-Methyloxime of methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**19**): The same procedure was used as that for compound **18**. Aldehyde **9** (100 mg, 0.23 mmol) was used as starting material and after 48 h of stirring at 95°C with *O*-methylhydroxylamine hydrochloride (53 mg, 0.6 mmol) and pyridine (0.1 mL), 87 mg (81.5%) of **19** were obtained. mp: 138-141°C (CH_2Cl_2). $[\alpha]^{22}$ (λ): -163.5° (589), -173.9° (578), -210.8° (546), -580.0° (436) (c 0.32%, $CHCl_3$). UV λ_{max} (ε): 241 (15000), 339 (13700). MS m/z (rel. abond. %): 455 (63) (M^+), 424 (20), 396 (52), 392 (100), 364 (45), 350 (19), 334 (13), 198 (9), 167 (8), 149 (10). IR: 1735, 1600, 1510, 1240 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.62 (s, 3H), 3.72 (s, 6H), 3.76 (s, 3H), 3.80 (s, 3H), 4.06 (d J=2.7 hz, 1H), 4.48 (d J=2.7 hz, 1H) 5.90 (s, 1H), 5.91 (s, 1H), 6.29 (s, 2H), 6.62 (s, 1H), 6.65 (s, 1H), 6.69 (s, 1H), 7.79 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 46.3, 46.7, 52.2, 56.2, 60.7, 61.9, 101.3, 105.3, 107.8, 109.7, 126.6, 127.1, 131.1, 132.6, 137.5, 138.2, 147.1, 148.2, 149.6, 153.2, 172.5. Anal. calcd. for $C_{24}H_{25}O_8N$: C, 63.30; H, 5.49; N, 3.08; found: C, 63.69; H, 5.54; N, 2.89.

Oxime of methyl acetyl-picropodophyllonate (**21**): Following the same procedure described before and after 70 h of stirring at 95 °C, 80 mg (80.0%) of **20** and 16 mg (15.5%) of **21** were obtained from 100 mg (0.21 mmol) of **8**. $[\alpha]^{22}$ (λ): -71.7° (589), -75.2° (578), -88.1° (546) (c 0.64%, $CHCl_3$). UV λ_{max} (ε): 212 (30000), 269 (10000), 312 (6400). IR: 3570, 3300, 1745, 1600, 1510, 1240 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.98 (s, 3H), 3.17-3.30 (m, 2H), 3.65 (s, 3H), 3.80 (s, 6H), 3.84 (s,3H), 4.04 (dd J=10.6,5.5 hz, 1H), 4.35-

4.60 (m, 2H), 5.93 (s, 2H), 6.26 (s, 1H), 6.35 (s, 2H), 7.33 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.7, 33.6, 43.9, 49.4, 51.8, 56.4, 60.8, 61.3, 101.3, 105.1, 107.1, 109.2, 133.5, 134.9, 137.9, 139.1, 145.5, 149.8, 153.4, 153.8, 171.9. Anal. calcd. for $C_{25}H_{27}O_{10}N$: C, 59.88; H, 5.43; N, 2.79; found: C, 59.80; H, 5.39; N, 2.77.

Oxime of methyl 9-deoxy-9-oxo-deoxypicropodophyllate (**22**): To a solution of 60 mg (0.14 mmol) of **13** in 10 mL of EtOH and 0.1 mL of pyridine, 25 mg (0.36 mmol) of hydroxylamine hydrochloride were added. The reaction mixture was stirred at 95 °C for 20 h; then it was concentrated under vacumm and extracted with EtOAc. Flash chromatography of the reaction product with $H/CH_2Cl_2/EtOAc$ (4:3:3) yielded 20 mg (32.2%) of **22**. $[\alpha]^{22}$ (λ): -64.0 (589), -67.4° (578), -77.0° (546) (c 0.81%, $CHCl_3$). UV λ_{max} (ε): 213 (27000), 291 (4100), 326 (940). IR: 3580, 3340, 1735, 1595, 1505, 1240 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.85-3.17 (m, 3H), 3.62 (s, 3H), 3.77 (s, 6H), 3.82 (s, 3H), 4.40 (d J=7.1 hz, 1H), 5.87 (s, 1H), 5.89 (s, 1H), 6.29 (s, 2H), 6.34 (s, 1H), 6.58 (s, 1H), 7.53 (d J=5.3 hz, 1H). ^{13}C NMR ($CDCl_3$) δ 31.6, 34.5, 45.6, 51.0, 51.7, 56.4, 60.8, 100.8, 106.8, 108.2, 109.7, 127.3, 129.4, 137.4, 140.3, 146.6, 152.2, 153.3, 172.7. Anal. calcd. for $C_{23}H_{25}O_8N$: C, 62.30; H, 5.68; N, 3.16; found: C, 62.35; H, 5.70; N, 3.14.

O-Methyloxime of methyl 9-deoxy-9-oxo-deoxypicropodophyllate (**23**): The same procedure and the same scale was used as that for compound **22**. Aldehyde **13** (100 mg, 0.23 mmol) and *O*-methylhydroxylamine hydrochloride (53 mg, 0.6 mmol) were used as starting compounds. 26 mg (24.4%) of **23** were obtained after flash chromatography with $H/CH_2Cl_2/EtOAc$ (45:45:10). $[\alpha]^{22}$ (λ): -69.8° (589), -73.6° (578), -84.1° (546), -146.2° (436) (c 1.18%, $CHCl_3$). UV λ_{max} (ε): 215 (30000), 295 (4800), 326 (610), 334 (430). MS m/z (rel. abond. %): 457 (78) (M^+), 426 (94), 409 (94), 394 (26), 366 (41), 351 (100), 339 (44), 324 (24), 252 (9), 174 (23). IR: 1740, 1600, 1510, 1240 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.90-3.20 (m, 3H), 3.64 (s, 3H), 3.78 (s, 6H), 3.79 (s,3H), 3.83 (s, 3H), 4.41 (d J=6.4 hz, 1H), 5.88 (s, 1H), 5.89 (s, 1H), 6.28 (s, 2H), 6.36 (s, 1H), 6.59 (s, 1H), 7.48 (d J=5.5 hz, 1H). ^{13}C NMR ($CDCl_3$) δ 31.7, 34.4, 45.9, 51.0, 51.6, 56.4, 60.8, 61.3, 100.8, 106.9, 108.2, 109.7, 127.4, 129.4, 137.5, 140.4, 146.5, 146.6, 150.7, 151.0, 153.3, 172.7. Anal. calcd. for $C_{24}H_{27}O_8N$: C, 63.01; H, 5.95; N, 3.06; found: C, 62.95; H, 5.80; N, 3.01.

O-Allyloxime of methyl 9-deoxy-9-oxo-deoxypicropodophyllate (**24**): Following the same procedure described above and after 7 h of stirring at 95 °C, 20 mg (30.8%) of **24** were obtained from 65 mg (0.15 mmol) of **13** and 35 mg (0.32 mmol) of *O*-allylhydroxylamine hydrochloride. $[\alpha]^{22}$ (λ): -69.2° (589), -72.8° (578), -83.0° (546), -147.4° (436) (c 0.70%, $CHCl_3$). UV λ_{max} (ε): 214 (33000), 295 (5400), 326 (780), 339 (620). MS m/z (rel. abond. %): 483 (36) (M^+), 426 (53), 409 (57), 394 (23), 366 (40), 351 (100), 339 (80), 324 (27), 252 (20), 174 (28). IR: 1735, 1595, 1505, 1230, 1010, 945 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.90-3.20 (m, 3H), 3.63 (s, 3H), 3.77 (s, 6H), 3.82 (s,3H), 4.41 (d J=6.8 hz, 1H), 4.49 (d J=5.8 hz, 2H), 5.06-5.30 (m, 2H), 5.75-6.02 (m, 1H), 5.87 (d J=1.5 hz, 1H), 5.89 (d J=1.5 hz, 1H), 6.28 (s, 2H), 6.35 (s, 1H), 6.58 (s, 1H), 7.52 (d J=5.9 hz, 1H). ^{13}C NMR ($CDCl_3$) δ 31.9, 34.6, 45.8, 51.0, 51.7, 56.4, 60.7, 74.5, 100.8, 106.6, 108.2, 109.7, 117.3, 127.4, 129.4, 134.2, 137.5, 140.5, 146.6, 150.9, 151.6, 153.3, 172.7. Anal. calcd. for $C_{26}H_{29}O_8N$: C, 64.50; H, 6.05; N, 2.90; found: C, 64.38; H, 6.08; N, 2.85.

Bioactivity. A screening procedure[30] was used to assess the antitumoural activity against the following cell lines: P-388 (lymphoid neoplasma from DBA/2 mouse), A-549 (human lung carcinoma), HT-29 (human colon carcinoma) and MEL-28 (human melanoma).

Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of 1×10^4 (P-388), 2×10^4 (A-549, HT-29 and MEL-28) cells/well, respectively, in 1 ml aliquots of MEM 10FCS medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After four days at 37°C, under a 10 % CO_2, 98 % humid atmosphere, P-388 cells were observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls, whereas A-549, HT-29 and MEL-28 were stained with crystal violet before examination.

Acknowledgements. Financial support for this work came from Spanish DGICYT (PB 93/616) and Junta de Castilla y León (Consejería de Educación y Cultura, SA-35/94 and SA 57/96). We thank Dr. B. Macias (University of Salamanca) for the elemental analyses and Prof. A. F. Barrero (University of Granada, Spain) for facilitating the realization of HRMS for compound **14** and **15**. One of us (P.A.G.) gratefully acknowledge a Grant from Foundation Ramón Areces .

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(*Received in UK* 28 *July* 1997; *revised* 9 *September* 1997; *accepted* 11 *September* 1997)