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Original article

Synthesis and antineoplastic activity of cyclolignan aldehydes

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Abstract – Several aldehydes related to methyl 9-deoxy-9-oxo-α-apopicropodophyllate, a selective antitumour agent against the HT-29 colon carcinoma, have been prepared and evaluated for their cytotoxic activities on four neoplastic cell lines (P-388, A-549, HT-29 and MEL-28). All of them lacked the lactone ring but maintained their cytotoxicity at, or under, the μM level. © 2000 Éditions scientifiques et médicales Elsevier SAS

cyclolignan aldehydes / non-lactonic derivatives / podophyllotoxin / selective cytotoxicity

1. Introduction

Two semi-synthetic derivatives of the cyclolignan Podophyllotoxin (**1**), etoposide and teniposide, are widely used anticancer drugs which show good clinical effects against several types of neoplasms, including testicular and small-cell lung cancers, lymphoma, leukaemia, Kaposi's sarcoma, etc. [1]. However, several limitations, such as myelosuppression, development of drug resistance and cytotoxicity towards normal cells still exist. In order to overcome the limitations of these compounds and to develop new compounds with better antitumour activity, numerous structural modifications have been performed on the cyclolignan skeleton [2–4]. In this sense, our group has been engaged in the design and synthesis of novel analogues of podophyllotoxin [5–10] and has also described new activities for these kind of compounds [11, 12].

Recently, we have reported the synthesis of several heterocycle-fused cyclolignans lacking the lactone moiety [5–7]. Most of these compounds showed similar effects in all the neoplastic systems tested, except the aldehyde: methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**2**) [6] (*figure 1*). This compound was tested at the National Cancer Institute (USA) against 60 different types of cancers and it showed a highly selective cytotoxicity towards colon carcinoma lines, with an IC_{50} in the range of *trans*-lactonic cyclolignans, which have always been the most potent compounds [8–10].

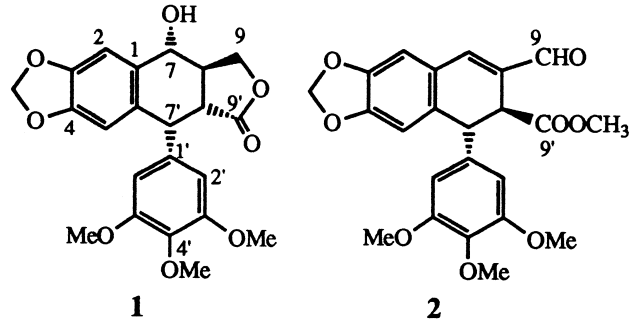

Figure 1. Structure of podophyllotoxin **1** and the selective aldehyde **2**.

With the aim of showing the influence of the aldehyde group on the antineoplastic selectivity and of establishing structure–activity relationships for these kinds of compounds, we have prepared and tested other aldehydes derived from podophyllotoxin with different configura-

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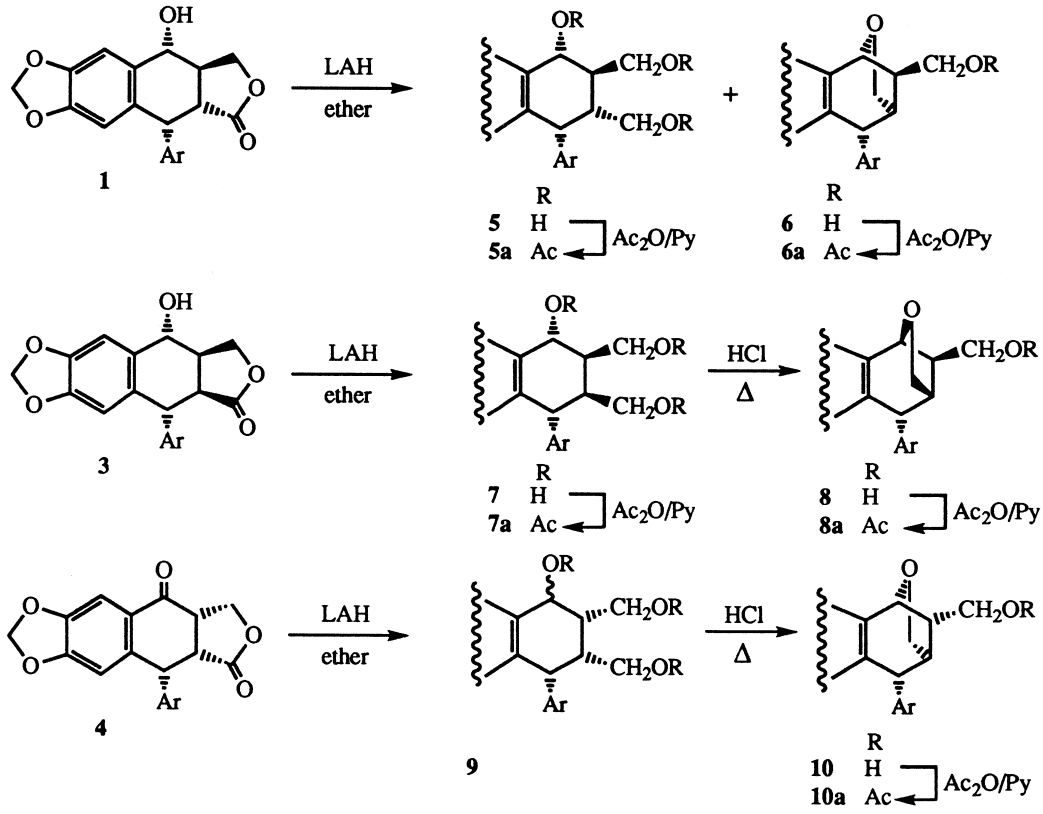

Figure 2. Reduction of the lactonic derivatives.

tions at the C-7, C-8 and C-8′ positions. Intermediates have also been tested on cultures of different tumour cell lines.

2. Chemistry

With this aim in mind we used, as starting materials, the *trans*-lactone podophyllotoxin (**1**) and *cis*-lactones picropodophyllin (**3**) and isopicropodophyllone (**4**). These were transformed into the corresponding triols **5**, **7** and **9** by reduction with $LiAlH_4$ which is known to maintain the stereochemistry at the centres cited above [13] (*figure 2*).

In the case of the reduction of **1**, the triol **5** was accompanied by the dehydration product neoanhydropodophyllol **6**. The formation of **6** can be explained by the presence of acid in the work up, because the triol **5** can be transformed quantitatively into the alcohol **6** by keeping it under $CHCl_3$-HCl reflux [13]. Reduction of **3** led only to the triol **7** which was transformed into neoanhydropicropodophyllol **8** when it was refluxed in chloroform solution acidified with a few drops of 2 N HCl. Reduction of **4** gave the triol **9** as a mixture of epimers at C-7. They were also transformed into the dehydration product **10** by refluxing them in acidified $CHCl_3$.

In order to obtain the lignan derivatives with an aldehyde group at C-9 and C-9′, Swern oxidation of the corresponding alcohols was performed [14] and the aldehydes **11**, **12** and **13** were obtained from the neoanhydropodophyllols **6**, **8** and **10** (*figure 3*).

The main reaction product of the Swern oxidation of triol **5** was the dialdehyde **14**, in which, not only did the oxidation of the hydroxyl groups at C-9 and C-9′ take place, but also the elimination of the benzylic hydroxyl group took place. Elimination to generate α,β-unsaturated carbonyl compounds is a common side reaction when carbonyl compounds with suitable leaving groups are generated in the presence of bases [15]. During oxidation, the epimerization of the C-8′ position, which is α to a carbonyl, also occurred in those cases where the stereochemistry of the parent alcohol was α.

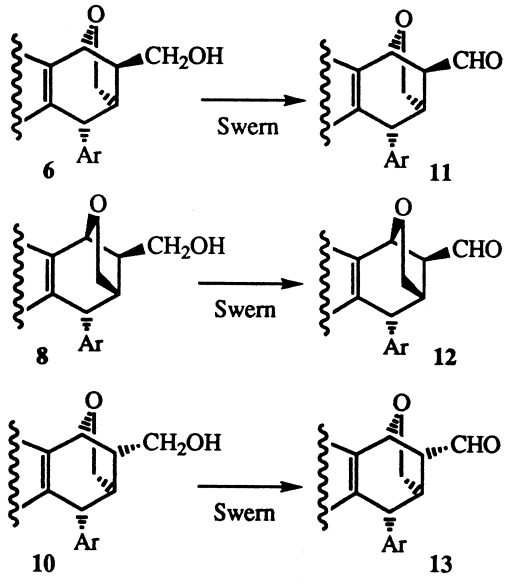

Figure 3. Swern oxidation of neoanhydropodophyllols.

Together with **14**, another two secondary compounds were also isolated from **5**: compound **15**, with the central ring of the cyclolignan skeleton aromatized, and compound **16** which had undergone deformylation. The formation of the latter can be explained through the intermediate dimethyl alkoxysulfonium salt, which reacts with the base to give the ylide **5′**. Then, by an intramolecular cyclic mechanism, the doubly benzylic hydrogen atom at C-7′ is abstracted instead of the proton α to the oxygen, the one normally removed in the Swern oxidation, to eliminate formaldehyde and dimethylsulfide (*figure 4*).

The dialdehyde **14** was also obtained from the aldehyde **2** (*figure 5*) by protection of the carbonyl group as its dithiolane to give compound **17**, followed by reduction of the methyl ester with LAH to give the alcohol **18**. Deprotection of **18** with $HgO\text{-}BF_3.Et_2O$ produced the hydroxyaldehyde **19** that led to the corresponding unsaturated dialdehyde **14** by Swern oxidation. Attempts to obtain the corresponding lactol from the hydroxyaldehyde **19** by treatment with PPTS were unsuccessful, leading instead to the methyl ether **20**.

Acetates and triacetates of the corresponding alcohols and triols were also prepared in order to assign unequivocally all the signals in the ^1H- and ^{13}C-NMR spectra.

3. Biological results and discussion

The prepared compounds were evaluated in vitro to establish their cytotoxicity against cell cultures of P-388 murine leukaemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human malignant melanoma [16]. The results obtained are shown in *table I*.

The tested derivatives showed cytotoxicity levels two or three orders lower than those of podophyllotoxin (**1**). However, some general observations can be made. It is worth noticing that several compounds (**4**, **7**, **8**, **8a** and **11**) are twice as potent against P-388 than the other tested lines.

Opening and reduction of the lactone ring led, in general, to much less cytotoxic compounds and the acetylation of the hydroxyl groups did not modify potency (**6**, **8**, **10** and **19** vs. **6a**, **8a**, **10a** and **19a**) except in the case of triol **7**, in which acetylation improves the potency significantly. Transformation of the triols into the dialdehyde **14** improved potency slightly or left it un-

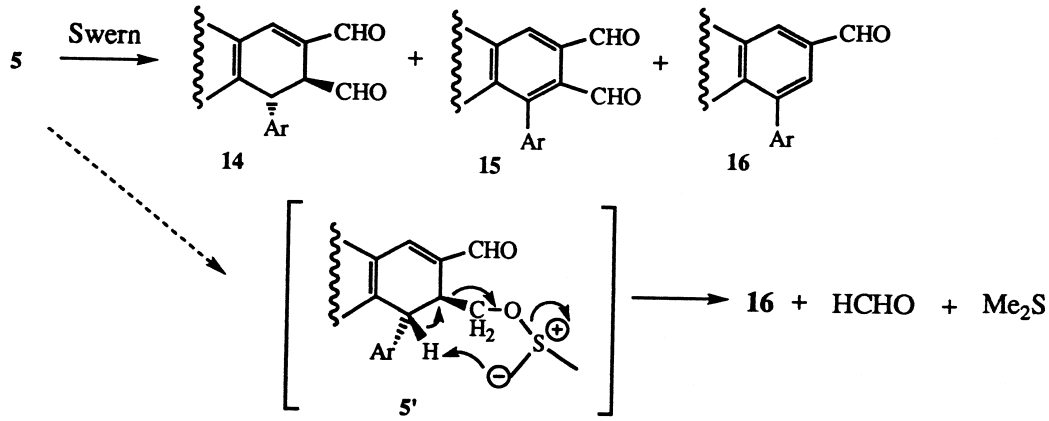

Figure 4. Swern oxidation of triol **5**.

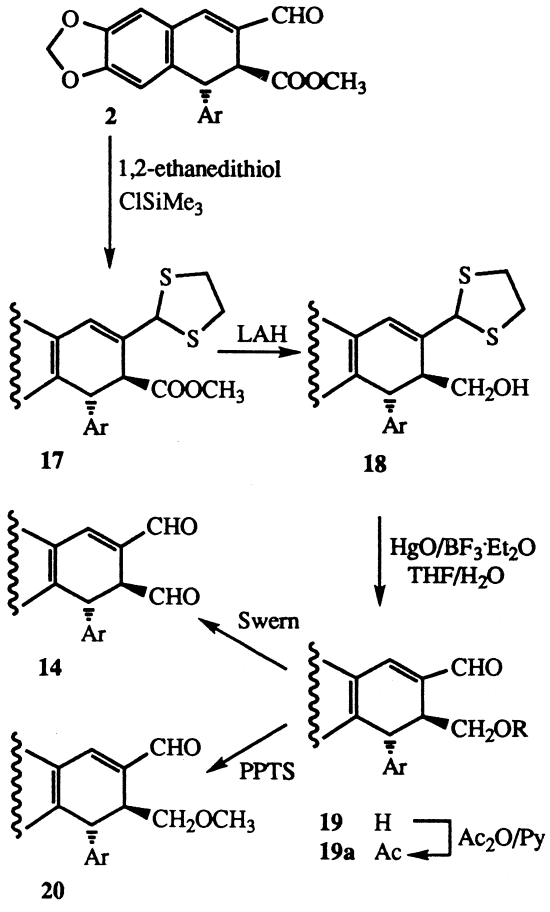

Figure 5. Synthesis of other derivatives from **2**.

changed (**14** vs. **5**, **7** and **9**) but selectivity of compound **2** against HT-29 was lost.

The formation of the alcohols with the methyleneoxy bridge (**6**, **8** and **10**) decreased the potency, which was partially recovered when these hydroxyl groups were transformed into the respective aldehydes (**11**, **12** and **13**), although the selectivity against HT-29 was lost again.

Even when the electrophilic aldehyde group at C-9 was present, compounds **11**, **13** and especially **12** showed disappointingly poor activity. While the stereochemistry of the substituents in **11** is formally the same as in **1**, the constraint imposed by the addition of the epoxy bridge on the ring conformation is such that the trimethoxyphenyl group is expected to adopt a pseudo-equatorial orientation and the aldehyde a pseudo-axial orientation, the reverse of their conformational distribution in **1**. In **13** the stereochemistry of the C ring is formally different from that in **1**, with the consequence that in the half-chair form,

Table I. Antineoplastic activity of cyclolignan derivatives (IC_{50} μM).

Compound	P-388	A-549	HT-29	MEL-28
1	0.012	0.012	0.029	
2	0.2	0.1	0.01	1.2
3	6.0	6.0	6.0	
4	6.0	12.1	12.1	12.1
5	1.2	1.2	1.2	
6	12.5	12.5	12.5	
6a	11.3	11.3	11.3	
7	23.9	47.9	23.9	
7a	4.6	4.6	7.4	
8	6.3	12.5	12.5	
8a	5.7	11.3	11.3	
9	0.2	0.2	0.2	0.2
10	0.2	0.2	0.2	0.2
10a	0.6	0.6	0.6	0.6
11	0.3	0.6	0.6	0.6
12	2.5	2.5	2.5	2.5
13	0.3	0.3	0.3	0.3
14	0.6	0.6	0.6	0.6
17	0.2	0.2	0.2	0.2
18	1.0	1.0	1.0	1.0
19	0.3	0.3	0.3	0.3
19a	0.2	0.2	0.2	0.2

the aldehyde and trimethoxyphenyl groups both display pseudo-equatorial dispositions. Hence, in either of these compounds the ideal relative disposition of these groups, presumably achieved by **1** is not obtainable, at least without significant distortion of ring C from its ground state conformation. Whilst **12** should dispose the C-9 and C-7′ substituents in near-ideal orientations, it also places the methyleneoxy bridge in areas of space which have hitherto not been thoroughly explored and which may not be available for ligands at the site of interaction of these compounds. Indeed, previous observations [7] on the *cis*-saturated aldehyde derived from **2** may also be interpreted as suggesting a lack of space in the active site for the β face of the C ring.

From these results it may be concluded that, in general, aldehydes at C-9 are more potent than alcohols at this position. This is consistent with previous suggestions [5] that an electrophilic group at this position is critical for the possible interaction with the biomolecules. The modifications made in the structural vicinity of the aldehyde function of **2** did not significantly modify cytotoxic potency against the tested cell lines, but the selectivity of the compound against HT-29 cells was lost. These findings indicate that the selectivity of these compounds is affected not only by the presence of the aldehyde function at C-9, but also by other aspects such as the absence of the Δ^7 double bond in all the substances tested in this

work. The degree of oxidation at C-9′ could also be important for this selectivity, although further studies will be necessary to elucidate the mechanism of action at the molecular level.

4. Experimental protocols

4.1. 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 (δ) 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 analyses were carried out on a Perkin-Elmer 2400 CHN Elemental Analyzer.

4.1.1. Isolation and preparation of compounds **1–8**

Podophyllotoxin **1** was isolated from *Podophyllum emodi* resin and transformed into the derivatives **2–8** by described procedures [7, 13, 17].

4.1.2. Isopicropodophyllol **9**

Isopicropodophyllone **4** (180 mg, 0.44 mmol) in dry ether (15 mL) was slowly added to a suspension of $LiAlH_4$ (220 mg, 5.79 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 168 mg (92%) of **9**, after flash chromatography (CH_2Cl_2/MeOH, 95:5). IR: 3 600, 1 590, 1 505, 1 484, 1 234, 1 126, 1 039, 875 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.1.3. Neoanhydroisopicropodophyllol **10**

A few drops of 2 N HCl were added to a solution of the triol **9** (95 mg, 0.23 mmol) in $CHCl_3$ and heated under reflux for 1 h. After washing with water, drying over Na_2SO_4 and evaporation of the solvent, 90 mg (98%) of **10** was obtained. $[\alpha]^{22}$ (λ): –75.2° (589), –79.1° (578), –91.8° (546), –163.4° (436) (c = 0.16%). UV λ_{max} (ε): 214 (18 800), 292 (2 900). IR: 3 400, 1 589, 1 505, 1 484, 1 461, 1 231, 1 127, 1 039, 935, 874 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

Acetylation of alcohol **10**: to a solution of **10** (30 mg) in pyridine (1 mL), acetic anhydride (0.5 mL) was added. The reaction mixture was kept at room temperature for 12 h. Then ice was added and the mixture was extracted with EtOAc. The organic layer was washed successively with 2 N HCl, aq. sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the organic solvent was evaporated to yield 31 mg (93%) of acetate **10a**. $[\alpha]^{22}$ (λ): –52.8° (589), –57.1° (578), –66.7° (546), –123.3° (436) (c = 0.21%). UV λ_{max} (ε): 214 (21 500), 290 (3 800). IR: 1 740, 1 589, 1 505, 1 485, 1 231, 1 126, 1 038 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.1.4. Swern oxidations

4.1.4.1. Neoanhydropodophyllal **11**

To a pre-cooled (–55 °C) and stirred solution of oxalyl chloride (1.2 mL, 2 M) in dry CH_2Cl_2 (3 mL) was added dropwise DMSO (0.34 mL) in dry CH_2Cl_2 (3 mL). After 5 min at –55 °C, a solution of 320 mg (0.8 mmol) of the alcohol **6** in 5 mL of dry CH_2Cl_2 was slowly added. The reaction mixture was kept at the same temperature for 30 min, then triethylamine (1.1 mL) was added. The mixture was warmed to 0 °C over 1 h, quenched with water and extracted with CH_2Cl_2. The reaction product gave, after flash chromatography (CH_2Cl_2/EtOAc, 8:2 as eluent), 252 mg (79%) of the aldehyde **11**. M.p. 73–75 °C (Hex/CH_2Cl_2). Anal. calcd. for $C_{22}H_{22}O_7$: C, 66.33; H, 5.53; found: C, 66.04; H, 5.52. MS m/z: 398 (M^+), 380, 339, 324, 198, 173, 115. $[\alpha]^{22}$ (λ): 0°. UV λ_{max} (ε): 208 (21 300), 238 (8 700), 335 (6 500). IR: 1 730, 1 600, 1 510, 1 495, 1 470, 1 240, 1 140, 1 050, 950 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

In the same way as described for **11** and after column chromatography of the reaction product, the following aldehydes were obtained using the corresponding starting materials.

4.1.4.2. Neoanhydroepipicropodophyllal **12**

From alcohol **8** (79%). M.p. 92–94 °C (MeOH/CH_2Cl_2). Anal. calcd. for $C_{22}H_{22}O_7$: C, 66.33; H, 5.53; found: C, 66.12; H, 5.51. $[\alpha]^{22}$ (λ): +73.2° (589), +77.8° (578), +91.0° (546), +172.3°(436) (c = 0.08%). UV λ_{max} (ε): 208 (22 100), 271 (9 100), 313 (7 200). IR: 1 730, 1 600, 1 510, 1 490, 1 470, 1 220, 1 140, 1 050, 950 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.1.4.3. Neoanhydroisopicropodophyllal **13**

From alcohol **10** (75%). $[\alpha]^{22}$ (λ): –71.3° (589), –75.8° (578), –87.6° (546), –164.3° (436) (c = 0.09%). UV λ_{max} (ε): 219 (19 700), 290 (6 100). IR: 1 723, 1 589, 1 505, 1 485, 1 231, 1 126, 1 039, 935, 875 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

Table II. ^{1}H-NMR data of compounds **9**–**20**.

H	**9**	**10**	**10a**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**19a**	**20**
2	6.82 *s*	6.67 *s*	6.70 *s*	6.74 *s*	6.72 *s*	6.75 *s*	6.87 *s*	7.32 *s*	7.32 *s*	6.71 *s*	6.66 *s*	7.28 *s*	7.31 *s*	7.29 *s*
5	6.45 *s*	6.58 *s*	6.57 *s*	6.54 *s*	6.48 *s*	6.60 *s*	6.74 *s*	6.98 *s*	6.26 *s*	6.57 *s*	6.59 *s*	6.70 *s*	6.71 *s*	6.68 *s*
7	4.75 *d* (2.9)	4.69 *s*	4.68 *s*	5.00 *d* (5.2)	5.08 *s*	5.14 *s*	7.47 *s*	8.25 *s*	8.16 *s*	6.68 *s*	6.63 *s*	6.87 *s*	6.88 *s*	6.72 *s*
8	2.35 *m*	2.60 *m*	2.59 *m*	3.28 *m*	3.05 *m*	3.11 *m*								
9	3.40–3.70 *m*	3.70 *m*	4.13 *m*	9.66 *d* (1.1)	9.66 *s*	9.81 *s*	9.71 *s*	9.99 *s*	10.12 *s*	5.34 *s*	5.23 *s*	9.54 *s*	9.56 *s*	9.57 *s*
O–CH_2–O	5.85 *s*	5.92 *d* (1.1)	5.91 *s*	5.92 *d* (1.8)	5.87 *d* (1.1)	5.93 *d* (1.5)	6.02 *d* (1.8)	6.13 *s*	6.10 *s*	5.90 *s*	5.92 *s*	6.01 *s*	6.03 *s*	6.03 *s*
	5.86 *s*	5.89 *d* (1.1)	5.95 *s*	5.96 *d* (1.8)	5.93 *d* (1.1)	5.96 *d* (1.5)	6.03 *d* (1.8)							
2′,6′	6.57 *s*	6.41 *s*	6.40 *s*	6.36 *s*	6.16 *s*	6.42 *s*	6.18 *s*	6.58 *s*	6.65 *s*	6.26 *s*	6.24 *s*	6.18 *s*	6.15 *s*	6.19 *s*
7′	4.12 *d* (6.2)	4.48 *d* (3.3)	4.50 *d* (3.6)	4.57 *d* (4.5)	4.18 *s*	4.52 *d* (3.3)	4.71 *d* (2.0)			4.36 *d* (1.6)	4.21 *s*	4.31 *s*	4.12 *s*	4.30 *s*
8′	2.11 *m*	2.50 *m*	2.59 *m*	3.04 *c* (4.5)	3.05 *m*	3.04 *m*	3.99 *d* (2.0)		7.76 *d* (1.8)	3.03 *m*	2.80 *m*	3.25 *m*	3.32 *m*	3.32 *m*
9′	3.40–3.70 *m*	3.90 *m*	3.90 *m*	3.60–3.90 *m*	3.75 *m*	3.60 *m*	9.51 *s*	10.6 *s*			3.43 *dd* (10.3; 8.4)	3.40 *m*	3.80 *m*	3.60–3.90 *m*
					4.00 *m*						3.85 *m*	3.80 *m*	4.00 *m*	
CH_3O-3′,5′	3.76 *s*	3.80 *s*	3.81 *s*	3.80 *s*	3.73 *s*	3.81 *s*	3.72 *s*	3.86 *s*	3.88 *s*	3.74 *s*	3.74 *s*	3.72 *s*	3.73 *s*	3.74 *s*
CH_3O-4′	3.81 *s*	3.84 *s*	3.83 *s*	3.84 *s*	3.79 *s*	3.85 *s*	3.76 *s*	3.97 *s*	3.95 *s*	3.77 *s*	3.77 *s*	3.76 *s*	3.76 *s*	3.78 *s*
$COCH_3$			2.11 *s*										2.00 *s*	
$COOCH_3$										3.63 *s*				
CH_2–CH_2										2.97–3.17 *m*	3.03–3.24 *m*			
CH_3														3.49 *s*

Table III. ^{13}C-NMR data of compounds **9–20**.

C	9	10	10a	11	12	13	14	15	17	18	19	19a	20
1	128.7	129.7	129.5	129.9	132.3	129.7	134.4	132.3*	130.6	133.7	136.0	134.5	133.1
2	106.5	107.5	107.5	107.6	107.8	107.5	109.0	103.8	107.4	107.1	108.7	108.8	108.7
3	145.0	146.0	146.1	146.5	146.8	146.4	147.6	145.7	146.9	146.7	147.3	147.8	147.0
4	146.5	147.5	147.6	148.4	148.2	148.0	151.0	150.3	147.6	147.5	150.5	150.5	151.0
5	109.1	110.1	110.0	110.2	110.9	110.6	110.4	105.7	109.4	110.3	110.9	110.8	110.9
6	133.3	134.3	133.8	132.6	129.3	132.6	130.9	132.3*	129.2	129.7	134.3	133.7	131.2
7	77.2	78.2	78.2	76.4	76.8	76.9	147.0	128.4	127.4	126.5	146.2	146.0	146.7
8	50.0	51.0	52.7	43.9	43.9	52.1	124.7	130.8*	127.0	127.2	125.2	125.2	124.6
9	61.3	62.3	65.8	198.9	200.3	200.5	191.0	192.7	58.1	58.0	192.6	191.5	192.6
O-CH_2-O	100.0	101.0	101.0	101.2	101.2	101.3	101.9	102.2	101.2	101.0	101.7	101.8	101.7
1′	137.9	138.9	138.5	138.2	139.6	138.0	137.9	130.1*	138.3	139.3	139.3	139.1	139.2
2′,6′	105.5	106.5	106.5	107.3	106.7	106.1	105.3	107.8	105.6	105.9	105.4	105.3	105.5
3′,5′	152.1	153.1	153.1	153.5	153.5	153.3	153.5	153.4	153.1	153.0	153.3	153.3	153.3
4′	135.6	136.6	136.8	–	137.6	137.0	138.6	138.1	136.8	137.3	135.4	138.0	136.0
7′	51.0	53.0	47.7	48.0	52.9	47.4	52.1	132.2*	49.3	47.5	45.1	45.6	45.2
8′	43.2	44.2	44.4	57.7	53.4	59.4	42.5	130.8*	47.6	46.1	42.5	38.2	42.5
9′	65.0	66.0	63.8	69.0	70.8	66.3	197.0	193.7	172.9	64.2	63.5	64.6	63.8
CH_3O-3′,5′	55.2	56.2	56.2	56.5	56.4	56.2	56.4	56.3	56.4	56.4	56.3	56.3	56.4
CH_3O-4′	59.8	60.8	60.9	60.9	60.7	60.8	60.7	61.1	60.8	60.6	60.7	60.7	60.7
$\underline{C}OCH_3$			171.0									170.8	
$CO\underline{C}H_3$			20.9									20.7	
$COO\underline{C}H_3$									52.3				
CH_2–CH_2									38.4	38.7			
									39.3	39.4			
CH_3O-9′													56.4

* Exchangeable assignments.

4.1.4.4. α -Apopicropophyllal **14**

According to the same procedure described above and after column chromatography of the reaction product of Swern oxidaton of triol **5**, the following compounds were eluted with a mixture of CH_2Cl_2/EtOAc, 96:4: aldehyde **16** (11%) (MS m/z: 366 (M^+), 351, 323, ^{1}H-NMR, *table II*), dialdehyde **15** (25%, MS m/z: 394 (M^+), 363, 379, ^{1}H-NMR, *table II*. ^{13}C-NMR, *table III*) and dialdehyde **14** (41%). (λ): –87.5° (589), –93.1° (578), –117.3° (546) (c = 0.10%). UV λ_{max} (ε): 208 (16 200), 275 (8 300), 350 (7 900). IR: 1 600, 1 510, 1 490, 1 470, 1 240, 1 135, 1 045, 945, 870 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.1.5. Transformations on aldehyde **2**

4.1.5.1. Ethylene thioacetal of methyl 9-deoxy-9-oxo-α-apopicropodophyllate **17**

To a solution of 100 mg (0.23 mmol) of **2** in 3 mL of dry CH_2Cl_2, was added 0.15 mL of 1,2-ethanedithiol and 0.3 mL of $ClSiMe_2$. The reaction mixture was stirred at room temperature under argon for 20 h. Then CH_2Cl_2 was added, washed with NaOH 4% and brine, dried and evaporated to afford 112 mg (97%) of **17**. M.p. 82–84 °C (CH_2Cl_2). $[\alpha]^{22}$ (λ): –96.1° (589), –102.6° (578), –122.2° (546), –302.2° (436) (c = 0.23%). UV λ_{max} (ε): 238 (15 300), 311 (8 400). IR: 1 735, 1 600, 1 510, 1 490, 1 470, 1 230, 1 145, 1 050, 1 010 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.1.5.2. Ethylene thioacetal of 9-deoxy-9-oxo-α-apopicropodophyllol **18**

A solution of compound **17** (40 mg, 0.08 mmol) in dry ether (3 mL) was slowly added to a suspension of LAH (50 mg, 1.32 mmol) in dry ether. Following the procedure described above and after flash chromatography (CH_2Cl_2/EtOAc, 9:1), 37 mg (98%) of **18** were obtained. $[\alpha]^{22}$ (λ): –16.2° (589), –17.7° (578), –23.1° (546), –71.5° (436) (c = 0.13%). UV λ_{max} (ε): 214 (23 200), 313 (8 200). IR: 3 500, 1 600, 1 510, 1 490, 1 470, 1 230, 1 130, 1 040, 940 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.1.5.3. 9-Deoxy-9-oxo-α-apopicropodophyllol **19**

To a solution of 40 mg of HgO and 0.25 mL of BF_3.Et_2O in 5 mL of THF/H_2O, 85:15 was added 40 mg (0.08 mmol) of **17**. The reaction mixture was stirred at room temperature under argon for 3 h. After addition of water, the precipitate was filtered. The filtrate was ex-

tracted with EtOAc. After removing the solvent and flash chromatography (CH_2Cl_2/EtOAc, 8:2) 30 mg (94%) of **19** was obtained. $[\alpha]^{22}$ (λ): –85.1° (589), –90.2° (578), –113.0° (546), –431.6° (436) (c = 0.22%). UV λ_{max} (ε): 215 (16 500), 245 (14 300), 356 (8 200). IR: 3 450, 1 670, 1 600, 1 510, 1 490, 1 240, 1 135, 1 045, 940 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

Acetylation of **19**, following the above procedure, yielded acetate **19a** (95%). $[\alpha]^{22}$ (λ): –87.2° (589), –92.7° (578), –116.0° (546) (c = 0.19%). UV λ_{max} (ε): 214 (15 200), 256 (13 100), 326 (7 400). IR: 1 730, 1 680, 1 600, 1 510, 1 495, 1 470, 1 250, 1 140, 1 050 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

Following the same procedure described before for the Swern oxidations, 130 mg of **19** afforded 40 mg (31%) of **14**.

4.1.5.4. 9′-O-Methyl-9-deoxy-9-oxo-α-apopicropodophyllol **20**

Pyridinium *p*-toluensulphonate (PPTS, 2 mg) was added to 65 mg (0.16 mmol) of **18** in 15 mL of benzene/MeOH, 10:5. The reaction mixture was stirred under Dean-Stark reflux and argon for 8 h. Then the solvent was evaporated, EtOAc added, washed with brine, dried and evaporated to afford 17 mg (26%) of **20** and 35 mg (54%) of unreacted **19** after flash chromatography (CH_2Cl_2/EtOAc, 8:2 as eluent). $[\alpha]^{22}$ (λ): –91.3° (589), –96.3° (578), –119.1° (546) (c = 0.16%). UV λ_{max} (ε): 222 (14 800), 271 (13 900), 313 (6 900). IR: 1 670, 1 600, 1 505, 1 490, 1 470, 1 240, 1 135, 1 050 cm^{-1}. ^{1}H-NMR (*table II*). ^{13}C-NMR (*table III*).

4.2. Bioactivity

Antineoplastic assays: 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 MEM10FCS 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 3 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.

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