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Synthesis and Evaluation of Pyrazolignans. A New Class of Cytotoxic Agents

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Abstract—A series of fused pyrazole derivatives of cyclolignans have been prepared through simple chemical routes and evaluated for their cytotoxic activities in culture cells of P-388 murine leukemia, A-549 lung carcinoma and HT-29 colon carcinoma. Despite the lack of the lactone moiety in their structures, they show IC_{50} values at μM levels.

Introduction

After the introduction of etoposide and teniposide into the clinical practice as useful antineoplastic agents and that of podophyllotoxin (**1**) as antiviral, the lignan family of natural products has been the object of numerous studies focused on the preparation of a large number of analogues with the aim of discovering better and safer anticancer and antiviral drugs.[1] Major efforts have been made to modify the lactone moiety, to change substituents at position C-7 and to prepare heteroanalogues by substitution of one carbon atom by nitrogen or sulfur at different positions of the cyclolignan skeleton.[1–4]

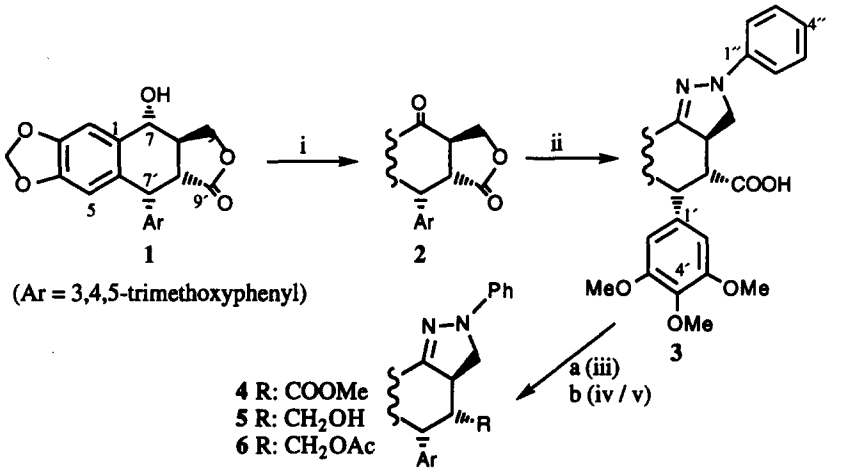
Methods and Results

Chemistry

Continuing our search for new analogues of podophyllotoxin[5,6] and with the aim of obtaining additional information on structure–activity relationships, we looked at the reaction between 7-ketocyclolignanolides and hydrazine derivatives as an easy method for the introduction of nitrogen substituents at that position. Rather than the expected hydrazones, but not surprisingly, the reactions led to a new family of compounds having a pyrazoline ring fused to the cyclolignan residue, while the lactone was opened to give a free carboxylic acid. Thus, podophyllotoxone (**2**), prepared from **1** by oxidation with pyridinium dichromate (PDC),[5] was converted in 65% yield (crystallized) into the pyrazoline-lignan **3** by reaction with phenylhydrazine in glacial acetic acid at room temperature. The resulting carboxylic acid **3** was successively transformed into the corresponding methyl ester **4** by treatment with diazomethane, into the alcohol **5** by LAH reduction of **4** and into the acetate **6** (Scheme 1).

Similarly, by reaction with the appropriate hydrazine derivative, pyrazolines **7–14**, were prepared which displayed electron attracting or withdrawing groups on the pyrazoline which influenced the antineoplastic activity. In these cases, the yields attained for the corresponding pyrazoline derivatives were lower than those obtained for phenylhydrazine itself, due in some extent to a lesser conversion degree, but mainly to the appearance of the by-product isopicropodophyllone (**15**), which resulted from the epimerization of the cyclolignanolide at the C-8 position, alpha to the 7-keto group, under the acidic reaction conditions and which did not react with the hydrazines used in such conditions.

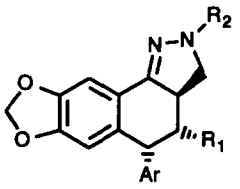
The structure assignment of compound **3** was based on the analysis of its IR, ^1H and ^{13}C NMR spectra in comparison with those of the ketolactone **2**. The absence of absorption bands for the aromatic ketone and the γ-lactone, as well as the presence of bands for the free carboxyl group in the IR spectrum of **3** prompted us to assume the formation of the pyrazoline rather than that of the simpler hydrazone. This was confirmed by the NMR spectra through the ^{13}C signal at δ 178.9 (COOH), the appearance of six additional signals corresponding to the phenyl group, and the shifting of the only methylene signal in the ^{13}C NMR spectrum of the lactone from δ 67.0 to 55.1 in that of the pyrazoline. The absorptions and splittings in the ^1H NMR spectrum were also in agreement with structure **3**. Finally, as several proton signals, important for ascertaining the configuration at C-8 and C-8', were hidden under the methoxyl absorptions the structure was confirmed by X-ray diffraction analysis. Figure 1 shows the crystal structure for **4**, solved by direct methods (MULTAN 80, see Experimental). The structure assignment of pyrazolines **7–14** was based on the spectral comparison with **3**.

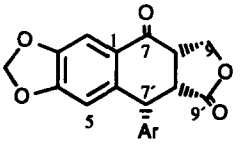


1 (Ar = 3,4,5-trimethoxyphenyl) 2 3

i) PDC/CH_2Cl_2, rt, 4 h; ii) Ph-NH-NH_2/AcOH, rt, 24 h; iii) CH_2N_2/ether, rt; iv) LAH/THF, rt, 3 h; v) Ac_2O/Py, rt, 12 h.

Scheme 1. The synthesis of pyrazoline derivatives of cyclolignans.





15

7 R_1= COOH, R_2 = *m* -NO_2Ph
8 R_1 = COOMe, R_2 = *m* -NO_2Ph (Ar = 3,4,5-trimethoxyphenyl)
9 R_1 = COOH, R_2 = *p*-BrPh
10 R_1 = COOMe, R_2 = *p*-MePh
11 R_1 = COOH, R_2 = Me
12 R_1 = COOMe, R_2 = Me
13 R_1 = COOH, R_2 = $CONH_2$
14 R_1 = COOMe, R_2 = $COCH_3$

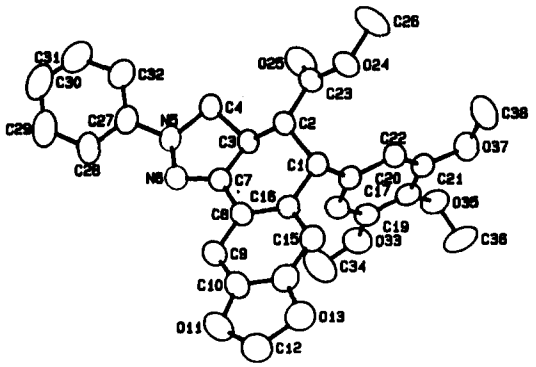


Figure 1. Crystal structure for compound **4**.

To increase the types and number of compounds for testing we tried to transform the pyrazoline moiety into the corresponding pyrazole. Thus, PDC oxidation of **5** produced the aromatization of the heterocyclic ring leading to the alcohol **16**, isolated in 33% yield and further acetylated to **17**, and the conjugated aldehyde **18** (31%), which was successively transformed into the unsaturated alcohol **19** and its acetate **20** (Scheme 2).

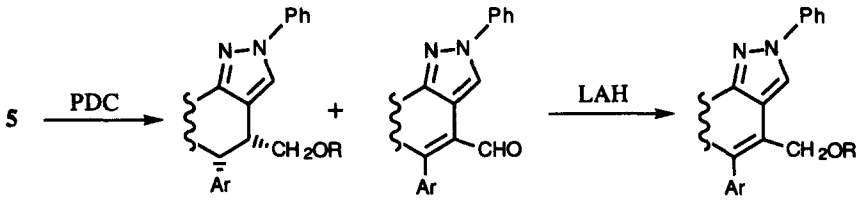
Bioactivity Results and Discussion

Cytotoxic activity for representative compounds was tested in cell cultures of P-388 murine leukemia, A-549 human lung carcinoma and HT-29 colon carcinoma. The results are shown in Table 1. As can be seen, the tested pyrazoline and pyrazole derivatives show cytotoxic activity levels two and three orders lower than those of podophyllotoxin (**1**), respectively, thus confirming that the presence of the lactone moiety is a prominent fact for displaying high cytotoxic activity.[5,6] Although some differences in potency can be observed within the family of *N*-phenylpyrazolines, the subsequent analysis on the influence of the electronic character of the substituents on the phenyl group would have no significance. Nevertheless, other aspects relating to a possible proposal for the cytotoxic mechanism of cyclolignanolide derivatives can be discussed.

The formation of a pyrazoline ring in the chemical condensation of phenylhydrazines with ketolactonic lignans, can be mechanistically interpreted in terms of the nucleophilic attack of the second nitrogen atom of the hydrazone on the C-9 of the lignan (Scheme 3a).

The attack should be facilitated by the great tendency of the γ-lactone moiety to opening, due mainly to the following reasons: (1) the ring strain provoked by the *trans*-junction of the tetralin and lactone fragments; (2) the good leaving group nature of the carboxylate anion; and (3) the formation of a new, almost not strained, pentagonal ring. It should be noted that the nitrogen atom participating in the nucleophilic substitution on C-9 is a poor nucleophile, due to its linking to the phenyl ring which would tend to delocalize and disperse the negative charge constituted by the electron pair of the nitrogen atom.

It is generally accepted that podophyllotoxin related lignans can interact with the neoplastic cell through two main mechanisms. One consists of the inhibition of tubulin polymerization, leading to the arrest of cellular division in metaphase and it is known to be based on the reversible interaction of the lignan with an active site (colchicine site) of tubulin.[7] The other accepted

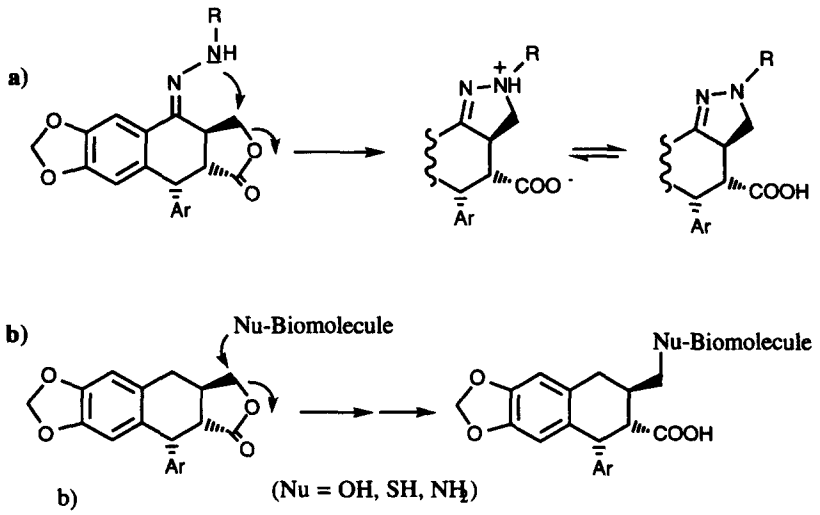


Scheme 2. The synthesis of pyrazole derivatives of cyclolignans.

Table 1. *In vitro* cytotoxic activities for some pyrazole derivatives of cyclolignans (IC_{50} μM)

Compound	P-388	A-549	HT-29
1	0.01	0.01	0.02
3	1.9	3.8	3.8
4	1.0	1.9	1.9
5	4.1	5.2	5.2
6	4.7	4.7	4.7
8	4.5	4.5	9.0
10	1.0	1.9	2.4
12	5.6	11	20
14	21	21	42
16	10	10	10
18	21	42	42

*Cell lines: P-388 (lymphoid neoplasma from DBA/2 mouse), A-549 (human lung carcinoma) and HT-29 (human colon carcinoma).



Scheme 3. Proposed mechanisms for (**a**) the formation of pyrazole derivatives from podophyllotoxone hydrazone and (**b**) the cytotoxic activity of cyclolignanolides

mechanism consists of the irreversible inhibition of DNA-topoisomerase II and it is based on the formation of a ternary nucleic acid–drug–enzyme complex, which subsequently induces DNA breaking as the starting event for a series of biochemical changes leading to cell death.[8] However, the structure of the complex and the detailed mechanism by which DNA breaking occurs are not known.

Eich *et al.* have proposed a covalent linkage of the enzyme to the carbonyl group of the lignan with simultaneous breaking of the lactone.[9] If we consider the easy opening of the lactone by the attack of poor nucleophiles at position C-9, as is shown in this paper, the cyclolignanolides of the podophyllotoxin group, will probably work as alkylating agents through the C-9 methylene, rather than as acylating agents through the C-9' carbonyl (Scheme 3**b**).

Experimental Section

Melting points were determined by heating in an external silicone bath and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in $CHCl_3$ solution and UV spectra on a Hitachi 100-60 spectrophotometer in EtOH solution. IR spectra were obtained on a Beckmann (Acculab VIII) spectrophotometer in $CHCl_3$ solution. EIMS were run in a VG-TS-250 spectrometer working at 70 eV. NMR spectra were recorded at 200 MHz for ^1H 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.

Chemistry

Pyrazoline derivatives preparation

Podophyllotoxone (2). Pyridinium dichromate (PDC) (1.3 g, 3.45 mmol) was added to a solution of podophyllotoxin (**1**) (1 g, 2.4 mmol) in dry CH_2Cl_2 (25 mL) and stirred at room temperature for 4 h. The excess of PDC was removed by filtration followed by CC of the residue on silica gel to give 750 mg (76%) of **2** and 200 mg (20%) of unreacted **1**.

Phenylpyrazopodophyllic acid (3). Phenylhydrazine (0.4 mL, 4.06 mmol) was added to a solution of **2** (1 g, 2.4 mmol) in 5 mL of glacial acetic acid and stirred at room temperature for 24 h. After addition of water, the unreacted ketone (90 mg) precipitated and was filtered off. The filtrate was treated with satd aq. $NaHCO_3$ and extracted with EtOAc. After removing the solvent, 900 mg (74%) of **3** was obtained. Mp 142–145 °C (CH_2Cl_2). Anal. calcd for $C_{28}H_{26}O_7N_2$: C, 66.93; H, 5.18; N, 5.58; found: C, 66.88; H, 5.05; N, 5.03. $[\alpha]^{22}$ (λ) –212.4° (589), –223.2° (578), –258.6° (546) (*c* 0.16, $CHCl_3$). UV λ_{max} (ε): 225 (21,100), 270 (11,100), 354 (14,800). IR 3400–2500, 1710, 1600, 1500, 1480, 1460, 1420, 1390, 1330, 1210, 1130, 1040, 1000, 940, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Treatment of acid **3** with an ethereal solution of diazomethane yielded methyl phenylpyrazopodophyllate **4** (87%). Mp 205–207 °C ($MeOH:Cl_2CH_2$). Anal. calcd for $C_{29}H_{28}O_7N_2$: C, 67.44; H, 5.43; N, 5.43; found: C, 67.19; H, 5.40; N, 5.33. MS m/z: 516 (M^+), 502, 486, 457, 335, 289, 181, 93. $[\alpha]^{22}$ (λ) –214.8° (589), –224.4° (578), –260.0° (546) (*c* 0.50, $CHCl_3$). UV λ_{max} (ε): 211 (28,100), 268 (10,200), 361 (14,000). IR 3000, 2840, 1740, 1610, 1505, 1485, 1470, 1430, 1390, 1340, 1210, 1140, 1050, 1010, 940, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

In the same way described for acid **3** and after flash chromatography of the reaction product, the following compounds were obtained using the corresponding starting materials:

m-*Nitrophenylpyrazopodophyllic acid (7).* From 500 mg of **2** and 210 mg of *m*-nitrophenylhydrazine at room temperature for 120 h were obtained 235 mg (47%) of unreacted **2** and 50 mg (7.5%) of **7**. Mp 201–203 °C (CH_2Cl_2). Anal. calcd for $C_{28}H_{25}O_9N_3$: C, 61.43; H, 4.57; N, 7.68; found: C, 61.08; H, 4.89; N, 7.39. MS m/z: 547 (M^+), 532, 500, 334, 288, 154, 84. $[\alpha]^{22}$ (λ) –183.0° (589), –202.0° (578), –247° (546) (c 0.10, $CHCl_3$). UV λ_{max} (ε): 228 (29,200), 354 (24,700). IR 3500–2400, 1710, 1620, 1600, 1530, 1500, 1480, 1430, 1390, 1350, 1230, 1130, 1040, 940, 880 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Treatment of **7** with CH_2N_2 afforded ester **8** (97%). Mp 205–207 °C (CH_2Cl_2). Anal. calcd for $C_{29}H_{27}O_9N_3$: C, 62.03; H, 4.82; N, 7.49; found: C, 61.85; H, 4.73; N, 6.87. $[\alpha]^{22}$ (λ) –184.9° (589), –203.1° (578), 248.4° (546) (*c* 0.42, $CHCl_3$). UV λ_{max} (ε): 226 (28,500), 354 (20,500). IR 3400, 3010, 2940, 1740, 1620, 1595, 1540, 1505, 1480, 1430, 1390, 1350, 1270, 1230, 1130, 940, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

p-*Bromophenylpyrazopodophyllic acid (9).* From 500 mg of **2** and 210 mg of *p*-bromophenylhydrazine at 50°C for 8 h were obtained 96 mg (19%) of **2**, 40 mg (8%) of **15** and 15 mg (2%) of acid **9**. IR 3500–2500, 1720, 1600, 1505, 1480, 1430, 1340, 1220, 1140, 1050, 950, 880 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Methyl p-*methylphenylpyrazopodophyllate (10).* From 300 mg of **2** and 110 mg of *p*-methylphenylhydrazine at room temperature for 96 h were obtained 182 mg (61%) of **2**, 57 mg (19%) of **15** and 57 mg (15%) of ester **10**. $[\alpha]^{22}$ (λ) –203.6° (589), –216.5° (578), –252.9° (546) (*c* 0.05, $CHCl_3$). UV λ_{max} (ε): 205 (35,000), 275 (7700), 324 (7800), 365 (8900). IR 2940, 1740, 1600, 1510, 1480, 1460, 1420, 1380, 1320, 1200, 1130, 1040, 1010, 940, 910, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Methylpyrazopodophyllic acid (11). From 300 mg of **2** and 0.12 mL of methylhydrazine at room temperature for 114 h were obtained 140 mg (47%) of **2** and 50 mg (16%) of **11**. Mp 161–163 °C (CH_2Cl_2). $[\alpha]^{22}$ (λ) −128.0° (589), −136.3° (578), −159.7° (546) (*c* 0.41, $CHCl_3$). UV λ_{max} (ε): 223 (18,400), 325 (10,500). IR 3500–2200, 1730, 1600, 1510, 1490, 1430, 1340, 1240, 1140, 1050, 1010, 940, 880 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Treatment of **11** with CH_2N_2 afforded ester **12** (97%). Mp 162–165 °C (CH_2Cl_2). Anal. calcd for $C_{24}H_{26}O_7N_2$: C, 63.44; H, 5.73; N, 6.17; found: C, 63.29; H, 6.00; N, 5.81. MS m/z: 454 (M^+), 440, 393, 362, 284, 253, 227, 169. $[\alpha]^{22}$ (λ) −129.9° (589), −137.6° (578), 161.6° (546) (*c* 0.50, $CHCl_3$). UV λ_{max} (ε): 212 (25,000), 326 (6900). IR 2920, 1740, 1600, 1510, 1480, 1460, 1420, 1380, 1330, 1240, 1130, 1040, 1010, 980, 940, 870, 820 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Table 2. ^1H NMR data of compounds **3–14** and **16–20**

H	3	4	5	6	7	8	9	10	11
2	7.56 *s*	7.54 *s*	7.54 *s*	7.54 *s*	7.54 *s*	7.51 *s*	7.52 *s*	7.54 *s*	7.41 *s*
5	6.54 *s*	6.52 *s*	6.48 *s*	6.51 *s*	6.54 *s*	6.51 *s*	6.54 *s*	6.53 *s*	6.52 *s*
8	3.71 *m*	3.84 *m*	3.25 *m*	3.33 *m*	3.71–3.79 *m*	3.65–3.72 *m*	3.70 *m*	3.70–3.80 *m*	3.50–3.70 *m*
9a	4.37 *t*(9.8)	4.38 *t*(9.6)	4.08 *t*(8.6)	4.12 *t*(8.5)	4.39 *t*(9.9)	4.38 *t*(9.9)	4.37 *t*(9.8)	4.38 *t*(9.7)	3.86 *t*(8.8)
9b	3.15 *dd*	3.11 *dd*	3.08 *dd*	3.15 *dd*	3.22 *dd*	3.13 *dd*	3.14 *dd*	3.08 *dd*	2.53 *dd*
	(13.8;9.8)	(13.6;9.6)	(13.9;8.6)	(13.9;8.5)	(12.7;9.9)	(13.0;9.9)	(13.6;9.8)	(13.7;9.7)	(13.9;8.8)
2',6'	6.29 *s*	6.19 *s*	6.29 *s*	6.21 *s*	6.28 *s*	6.16 *s*	6.27 *s*	6.19 *s*	6.22 *s*
7'	4.63 *d*(5.1)	4.60 *d*(5.2)	4.29 *d*(4.8)	4.26 *d*(5.0)	4.65 *d*(4.8)	4.60 *d*(5.1)	4.36 *s*(5.2)	4.61 *d*(5.2)	4.58 *d*(5.1)
8'	3.26 *dd*	3.23 *dd*	2.26 *m*	2.50 *m*	3.29 *dd*	3.22 *dd*	3.26 *dd*	3.23 *dd*	3.15 *dd*
	(12.9;5.1)	(13.1;5.2)			(13.0;4.8)	(12.0;5.1)	(12.5;5.2)	(12.5;5.3)	(13.3;5.1)
9'a			3.42 *d*(8.4)	3.90 *d*(8.7)					8.76 *sa*
9'b			3.42 *d*(8.4)	3.90 *d*(8.7)					
CH_3O-3',5'	3.71 *s*	3.75 *s*	3.74 *s*	3.74 *s*	3.71 *s*	3.73 *s*	3.71 *s*	3.75 *s*	3.67 *s*
CH_3O-4'	3.82 *s*	3.81 *s*	3.81 *s*	3.91 *s*	3.80 *s*	3.78 *s*	3.79 *s*	3.81 *s*	3.77 *s*
O-CH_2-O	5.98 *s*	5.98 *s*	5.93 *s*	5.93 *s*	6.00 *s*	5.98 *s*	5.99 *s*	5.98 *s*	5.95 *s*
2"	7.14 *d*(7.7)	7.12 *d*(7.8)	7.12 *d*(7.7)	7.14 *d*(7.9)	7.88 *s*	7.82 *s*	7.02 *d*(8.8)	7.07 *d*(8.8)	
3"	7.29 *t*(7.7)	7.28 *t*(7.8)	7.28 *t*(7.7)	7.28 *t*(7.9)			7.37 *d*(8.8)	7.10 *d*(8.8)	
4"	6.86 *t*(7.7)	6.86 *t*(7.8)	6.86 *t*(7.7)	6.85 *t*(7.9)	7.36 *d*(4.3)	7.36 *d*(4.7)			
5"	7.29 *t*(7.7)	7.28 *t*(7.8)	7.28 *t*(7.7)	7.28 *t*(7.9)	7.62 *sa*	7.62 *sa*	7.37 *d*(8.8)	7.10 *d*(8.8)	
6"	7.14 *d*(7.7)	7.12 *d*(7.8)	7.12 *d*(7.7)	7.14 *d*(7.9)	7.36 *d*(4.3)	7.36 *d*(4.7)	7.02 *d*(8.8)	7.07 *d*(8.8)	
$COOCH_3$		3.71 *s*				3.59 *s*		3.71 *s*	
CH_3COO				2.11 *s*					
CH_3								2.30 *s*	2.87 *s*

H	12	13	14	16	17	18	19	20
2	7.41 *s*	7.43 *s*	7.41 *s*	7.55 *s*	7.55 *s*	8.12 *s*	8.09 *s*	8.10 *s*
5	6.50 *s*	6.62 *s*	6.52 *s*	6.71 *s*	6.72 *s*	6.98 *s*	6.80 *s*	6.84 *s*
8	3.50–3.85 *m*	3.92 *d*(10.1)	3.61 *m*					
9a	3.50–3.85 *m*	4.20 *d*(10.1)	4.47 *t*(11.1)	7.74 *s*	7.70 *s*	8.69 *s*	8.65 *s*	8.48 *s*
9b	2.31 *dd*	2.21 *s*	3.34 *t*(11.7)					
	(13.9;8.6)							
2',6'	6.14 *s*	6.25 *s*	6.12 s	6.26 *s*	6.19 *s*	6.66 *s*	6.66 *s*	6.66 *s*
7'	4.55 *d*(5.2)	4.65 *d*(6.8)	4.59 *d*(5.3)	4.17 *d*(5.9)	4.16 *d*(6.1)			
8'	3.14 *dd*	3.42 *d*(6.8)	3.19 *dd*	3.59 *m*	3.73 *m*			
	(12.4;5.2)		(12.7;5.3)					
9'a	3.64 *s*			3.69–3.88 *m*	4.20 *dd* (10.7;7.3)	9.17 *s*	4.74 *s*	5.21 *s*
9'b				3.69–3.88 *m*	4.11 *dd* (10.7;7.3)			
CH_3O-3',5'	3.71 *s*	3.69 *s*	3.71 *s*	3.62 *s*	3.74 *s*	3.87 *s*	3.65 *s*	3.86 *s*
CH_3O-4'	3.78 *s*	3.77 *s*	3.77 *s*	3.74 *s*	3.81 *s*	3.98 *s*	3.96 *s*	3.97 *s*
O-CH_2-O	5.94 *s*	6.05 *s*	5.97 *s*	5.96 *d*(1.1)	5.99 *d*(1.4)	6.10 *s*	6.04 *s*	6.08 *s*
				5.93 *s*	5.95 *d*(1.4)			
2"				7.73 *d*(7.8)	7.71 *d*(8.4)	8.01 *d*(8.2)	7.97 *d*(8.0)	7.98 *d*(8.0)
3"				7.44 *t*(7.8)	7.46 *t*(7.8)	7.55 *t*(8.2)	7.54 *t*(7.7)	7.56 *t*(7.7)
4"				7.25 *t*(7.3)	7.26 *t*(8.0)	7.41 *t*(8.2)	7.38 *t*(7.6)	7.40 *t*(7.7)
5"				7.44 *t*(7.8)	7.46 *t*(7.8)	7.55 *t*(8.2)	7.54 *t*(7.7)	7.56 *t*(7.7)
6"				7.73 *d*(7.8)	7.71 *d*(8.4)	8.01 *d*(8.2)	7.97 *d*(8.0)	7.98 *d*(8.0)
$COOCH_3$	3.64 *s*		3.61 *s*					
CH_3COO					2.15 *s*			2.06 *s*
CH_3	2.89 *s*							
$COCH_3$			2.34 *s*					

Table 3. ^{13}C NMR data of compounds 3–8, 10–14 and 16–20

C	3	4	5	6	7	8	10	11
1	122.4	122.5	122.4	122.2	121.7	121.7	122.6	122.1
2	103.2	103.1	103.2	103.2	103.3	103.3	103.1	103.4
3	147.7	146.6	147.3	147.4	146.9	146.9	149.0	147.6
4	149.0	149.3	149.3	149.3	147.8	147.8	149.2	149.6
5	109.2	109.2	109.6	109.5	109.3	109.3	109.2	109.2
6	133.3	133.5	135.2	134.7	134.0	134.2	133.3	133.9
7	149.3	149.3	151.1	150.2	150.8	151.1	147.6	152.8
8	40.5	40.6	41.5	41.8	40.8	40.9	40.7	41.9
9	55.1	55.1	54.4	54.3	54.6	54.5	55.6	62.7
1'	135.5	135.7	136.2	135.5	135.1	135.3	135.8	135.5
2',6'	107.2	107.1	107.6	107.6	107.5	107.2	107.1	107.1
3',5'	153.1	153.1	153.2	153.2	153.2	153.2	153.1	153.0
4'	137.7	137.9	137.5	137.9	139.0	137.8	144.6	137.6
7'	47.8	48.0	45.2	41.9	47.8	47.9	48.0	47.9
8'	50.3	50.5	47.6	47.8	50.2	50.4	50.5	50.3
9'	178.8	171.9	63.5	65.1	176.2	171.8	172.0	178.0
CH_3O-3',5'	56.3	56.3	56.4	56.3	56.4	56.3	56.3	56.2
CH_3O-4'	60.7	60.8	60.6	60.7	60.7	60.7	60.8	60.7
O-CH_2-O	101.5	101.5	101.3	101.3	101.6	101.6	101.4	101.5
1"	146.5	—	149.9	146.8	149.9	149.9	—	
2"	113.5	113.5	113.6	113.6	107.8	107.5	113.6	
3"	129.1	129.1	129.1	129.1	149.4	149.4	129.6	
4"	119.7	119.6	119.6	119.7	113.6	113.6	118.9	
5"	129.1	129.1	129.1	129.1	129.7	129.7	129.6	
6"	113.5	113.5	113.6	113.6	118.8	118.7	113.6	
COO$\underline{C}H_3$		51.6				51.6	51.8	
$\underline{C}OCH_3$				170.4				
CO$\underline{C}H_3$				20.8				
CH_3							20.5	43.7

C	12	13	14	16	17	18	19	20
1	122.5	126.4	121.5	123.0	122.8	125.5	—	—
2	103.2	106.4	103.4	103.6	103.6	101.4	101.2	101.2
3	147.5	148.6	147.8	147.4	147.5	145.9	—	—
4	149.3	150.3	150.6	148.0	148.0	148.4	—	—
5	109.2	108.9	109.4	109.5	109.5	106.8	106.3	106.5
6	133.6	133.3	135.0	134.4	134.0	131.4	—	—
7	151.6	154.4	155.0	150.5	150.3	150.2	—	—
8	42.0	43.5	40.2	118.0	117.2	114.8	—	—
9	62.8	75.8	50.2	125.9	126.1	127.6	127.4	127.5
1'	135.8	138.1	135.5	136.0	135.1	138.5	—	—
2',6'	107.2	107.2	107.2	106.2	106.0	108.8	—	—
3',5'	153.1	153.3	153.2	153.1	153.0	153.3	—	—
4'	137.6	140.3	—	137.5	—	140.6	—	—
7'	48.1	43.5	47.8	39.6	36.4	128.0	—	—
8'	50.5	53.2	50.4	48.7	48.6	129.6	—	—
9'	171.9	174.6	171.2	63.3	64.8	192.6	62.3	63.4
CH_3O-3',5'	56.3	56.3	56.3	56.1	56.0	56.4	56.3	56.3
CH_3O-4'	60.7	60.8	60.8	60.8	60.7	61.0	61.0	61.0
O-CH_2-O	101.3	102.5	101.7	101.2	101.2	101.8	101.2	101.4
1"				140.7	140.5	147.1	—	—
2"				118.9	118.0	120.6	120.5	120.6
3"				129.5	129.5	129.6	129.6	129.6
4"				124.4	124.0	123.5	121.5	121.0
5"				129.5	129.5	129.6	129.6	129.6
6"				118.9	118.0	120.6	120.5	120.6
COO$\underline{C}H_3$	51.6		51.9					
$\underline{C}OCH_3$			169.3		170.0			—
CO$\underline{C}H_3$			21.3		20.9			21.0
CH_3	43.8							
$CONH_2$		194.3						

— Signals not observed in the spectra.

Carbamoylpyrazopodophyllic acid (13). From 300 mg of **2** and 125 mg of semicarbazide at 60 °C for 100 h were obtained 35 mg (12%) of **2** and 60 mg (18%) of acid **13**. Mp 108–110 °C (CH_2Cl_2). $[\alpha]^{22}$ (λ) –69.0° (589), –72.0° (578), –84.0° (546) (*c* 0.07, $CHCl_3$). UV λ_{max} (ε): 211 (10,000), 271 (3600), 324 (2800). IR 3500–2400, 1780, 1670, 1600, 1505, 1480, 1420, 1330, 1240, 1130, 1040, 940, 870 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Methyl acetylpyrazopodophyllate (14). From 500 mg of **2** and 0.2 mL of hydrazine hydrate at room temperature for 72 h were obtained 95 mg (19%) of **2** and 90 mg (15%) of **14** after esterification and chromatography of the residue. Mp 250–252 °C (MeOH/CH_2Cl_2). Anal. calcd for $C_{25}H_{26}O_8N_2$: C, 62.24; H, 5.39; N, 5.81; found: C, 61.82; H, 5.23; N, 5.78. MS m/z: 482 (M^+), 440, 423, 381, 352, 314, 272, 239, 213, 181, 84. $[\alpha]^{22}$ (λ) –111.8° (589), 116.8° (578), 113.4° (546) (*c* 0.99, $CHCl_3$). UV λ_{max} (ε): 212 (26,300), 302 (12,900), 323 (14,200). IR 3400, 3000, 2940, 1730, 1650, 1600, 1505, 1470, 1420, 1360, 1330, 1240, 1130, 1040, 1010, 970, 930, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Phenylpyrazopodophyllol (5). Three hundred milligrams (0.6 mmol) of methyl ester **4** in THF (3 mL) was slowly added to a suspension of LAH (390 mg, 10.3 mmol) in dry THF. The reaction mixture was stirred at room temperature under Ar for 3 h. Then EtOAc was added, filtered, dried and evaporated to afford 280 mg (98%) of **5**. Mp 109–112 °C (CH_2Cl_2). Anal. calcd for $C_{28}H_{28}O_6N_2$: C, 68.85; H, 5.74; N, 5.74; found: C, 68.61; H, 5.78; N, 5.72. $[\alpha]^{22}$ (λ) –139.1° (589), –148.0° (578), –171.0° (546) (*c* 0.35, $CHCl_3$). UV λ_{max} (ε): 222 (20,600), 270 (9800), 350 (14,400). IR 3450, 3000, 2800, 1600, 1505, 1485, 1465, 1430, 1380, 1330, 1230, 1130, 1040, 1005, 940, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Acetylation of alcohol **5** (100 mg, 0.21 mmol) with acetic anhydride in pyridine afforded, after usual work-up, 95 mg (87%) of acetate **6**. Mp 147–151 °C (CH_2Cl_2). MS m/z: 530 (M^+), 468, 437, 304, 289, 264, 242. $[\alpha]^{22}$ (λ) –125.6° (589), –133.3° (578), –153.4° (546) (*c* 0.23, $CHCl_3$). UV λ_{max} (ε): 230 (27,100), 271 (14,800), 361 (21,600). IR 3005, 2940, 1740, 1600, 1505, 1485, 1465, 1430, 1380, 1360, 1330, 1230, 1130, 1040, 940, 875, 850 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Pyrazole derivatives preparation

Oxidation of phenylpyrazopodophyllol (5). A solution of alcohol **5** (115 mg, 0.24 mmol) in Cl_2CH_2 (5 mL) containing pyridinium dichromate (190 mg, 0.51 mmol) was stirred for 17 h using the procedure described for **2**. The crude product was purified by flash chromatography (CH_2Cl_2:EA 8:2 as eluant) to give the following compounds:

Phenyldehydropyrazopodophyllol (**16**), 38 mg (33%). $[\alpha]^{22}$ (λ) –39.0° (589), –42.0° (578), –50.0° (546) (*c* 0.54, $CHCl_3$). UV λ_{max} (ε): 234 (21,500), 292 (21,000), 323 (21,500). IR 3400, 3020, 2940, 1610, 1510, 1480, 1430, 1390, 1360, 1340, 1220, 1140, 1050, 970, 880 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Acetylation of **16** in the usual way, afforded the appropriate acetate **17** (85%). Mp 165–167 °C (CH_2Cl_2). MS m/z: 528 (M^+), 468, 438, 406, 368, 301, 266, 219, 181, 139. $[\alpha]^{22}$ (λ): –87.0° (589), –103.0° (578), –123.0° (546) (*c* 0.03, $CHCl_3$). UV λ_{max} (ε): 237 (11,600), 295 (11,000), 325 (13,200). IR 2920, 1740, 1600, 1505, 1480, 1430, 1380, 1240, 1130, 1045, 960, 880 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Phenyldidehydropyrazopodophyllal (**18**), 35 mg (31%). Anal. calcd for $C_{28}H_{22}O_6N_2$: C, 69.71; H, 4.56; N, 5.81; found: C, 69.52; H, 4.59; N, 5.69. UV λ_{max} (ε): 252 (20,500), 365 (19,600). IR 2940, 1670, 1580, 1505, 1480, 1420, 1370, 1330, 1240, 1140, 1050, 980, 940, 880, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Reduction of phenyldidehydropyrazopodophyllal (18). A solution of aldehyde **18** (35 mg, 0.07 mmol) in dry THF (3 mL) was slowly added to the suspension of LAH (40 mg, 1 mmol) in dry THF. Following the procedure described before, 30 mg (85%) of phenyldidehydropyrazopodophyllol (**19**) was obtained. Mp 139–142 °C (CH_2Cl_2). UV λ_{max} (ε): 230 (24,500), 286 (27,100), 345 (21,000). IR 3300, 3000, 1940, 1590, 1505, 1470, 1410, 1330, 1220, 1130, 1050, 940 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Acetylation of **19** yielded acetate **20** (85%). Mp 137–141 °C (CH_2Cl_2). MS m/z: 526 (M^+), 482, 468, 452, 436, 405, 288, 204. UV λ_{max} (ε): 234 (27,200), 290 (28,500), 335 (9600). IR 3640, 3540, 3010, 2940, 1740, 1600, 1590, 1505, 1470, 1410, 1370, 1330, 1240, 1220, 1130, 1050, 940, 860 cm^{-1}. ^1H NMR: Table 2. ^{13}C NMR: Table 3.

Bioactivity

A screening procedure[10] was used to assess the cytotoxic activity against the following cell lines: P-388 (lymphoid neoplasma from DBA/2 mouse), A-549 (human lung carcinoma) and HT-29 (human colon carcinoma).

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) 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 and HT-29 were stained with Crystal Violet before examination.

X-Ray crystallographic data

Crystal data for compound **4**. $C_{29}H_{28}N_2O_5$, orthorhombic, space group $P2_12_12_1$, a = 10.964(1), b = 13.732(1), c = 17.357 (8) Å, V = 2613.3 Å^3, Z = 4; Dc: 1.31 g cm^{-3}, Cu$K\alpha$ radiation, μ (Cu$K\alpha$): 7.4 cm^{-1}, F(000): 108 C. Colourless crystal, 0.37 × 0.2 × 0.2 mm size. Other data are included in the suplementary material.

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References and Notes.

1. Ayres, D. C.; Loike J. D. *Lignans. Chemical, Biological and Clinical Properties*; Cambridge University Press: Cambridge, 1990.

2. Biogenetic numbering according to Moss G. P. (1987, Paper to the *Biochemistry Commitee of the International Union of Pure & Applied Chemistry*) is used in this paper.

3. Ward, R. S. *Nat. Prod. Rep.* **1993**, *10*, 1.

4. Pearce, H.; Bach, N.; Cramer, T.; Danks, M.; Grindey, G.; Kattersohn, D.; Rinzel, S.; Beck, T. *Proc. Am. Cancer. Res.* **1990**, *47*, 758.

5. San Feliciano, A.; Gordaliza, M.; Miguel del Corral, J. M.; Castro, M. A.; García-Grávalos, M. D.; Ruiz-Lázaro, P. *Planta Med.* **1993**, *59*, 246.

6. Gordaliza, M.; Castro, M. A.; García-Grávalos, M. D.; Ruiz-Lázaro, P. M.; Miguel del Corral, J. M.; San Feliciano, A. *Arch. Pharm. (Weinheim)* **1994**, *327*, 175.

7. Kelleher, J. K. *Mol. Pharmacol.* **1977**, *13*, 232.

8. Chen, G. L.; Yang, L.; Rowe, T. C.; Halligan, B. D.; Tewey, K. M.; Liu, L. F. *J. Biol. Chem.* **1984**, *33*, 1364.

9. Eich, E.; Schulz, J.; Kaloga, M.; Merz, H.; Schröder, H. C.; Muller, W. E. G. *Planta Med.* **1991**, *57*, A7.

10. Bergeron, R. J.; Cavaragh, Jr P. F.; Kline, S. J.; Hughes, R. G.; Elliot, G. T.; Porter, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *3*, 121.

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