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# Bioactive Isoxazoline and Oxime Derivatives from 7-Ketolignans

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***Abstract***: A new type of cyclolignans with an isoxazoline ring fused to the cyclolignan core has been prepared from 7-ketolignanolides by reaction with hydroxylamine. The corresponding 7-oxime derivatives have also been obtained. The compounds prepared have been evaluated for their cytotoxic activities over four cell lines. © 1997 Elsevier Science Ltd.

Lignans are natural products that include compounds with important biological activities. Due to this great variety of biological properties, this family of natural products has been the objective of numerous studies which have been compiled in several reviews concerning the distribution, structural and synthetic aspects of lignans.[1]

Some of the cyclolignans that have attracted much attention are podophyllotoxin and two semisynthetic derivatives, etoposide and teniposide, which are used as potent chemotherapeutic agents against a variety of tumors.[2] Numerous modifications to the cyclolignan skeleton have been reported,[3] in order to overcome the cytotoxicity that these compounds show against normal cells, and hence to develop novel analogues with better therapeutic indices.

It is well accepted from structure-activity studies in this field that the presence of the lactone moiety is an important factor for displaying high cytotoxic activity, furthermore the *trans*-lactones are more potent as antineoplastics than the *cis*-lactones.[4] Many efforts have been addressed to modify the lactone moiety and to prepare analogues with heteroatoms at different positions of the cyclolignan skeleton.[5,6]

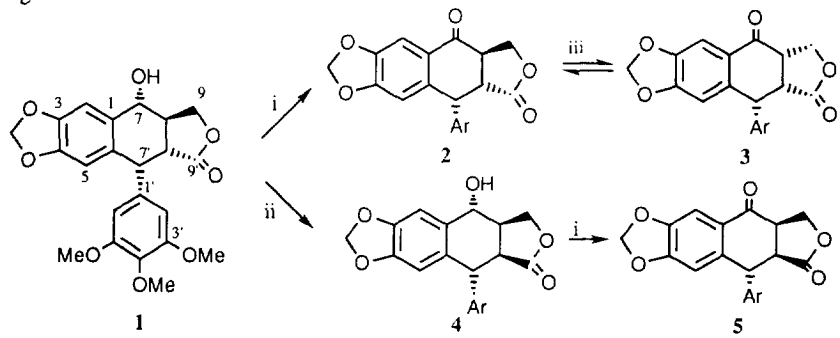
During the last few years we have been working on chemical transformations of podophyllotoxin and analogues and have prepared a large number of cyclolignan derivatives, some of which display potent antiviral and cytotoxic activities.[7] Recently, we have reported the reaction between 7-ketocyclolignanolides and various substituted hydrazines.[8] Rather than the expected hydrazones, the reaction led to a new family of compounds having a pyrazoline ring fused to the cyclolignan core, while the lactone was opened to give a free carboxylic acid, due to nucleophilic attack of the second nitrogen atom on the C-9 position of the lignan. Now, as part of our continuing efforts along this line, we present here the reactivity of several 7-ketolignans with other nucleophiles such as hydroxylamines.

## RESULTS AND DISCUSSION

We have chosen the reaction between carbonyl groups and hydroxylamines as an easy method for the introduction of nitrogen substituents at the C-7 position.[9] We also have the aim of studying the influence of lactone stereochemistry on the reaction with different nucleophiles.

The 7-ketolignanolides used in this work were one *trans*-lactone, podophyllotoxone (**2**) and two *cis*-lactones, isopicropodophyllone (**3**) and picropodophyllone (**5**), which differ in the stereochemistry at C-8 and C-8'. All of them were prepared by described procedures[10] from podophyllotoxin (**1**). The latter was isolated from *Podophyllum* resin by means of chromatographic procedures.[11]

Podophyllotoxone (**2**) was prepared from **1** by oxidation with PDC.[8] Equilibration of **2** in refluxing AcOH led to a mixture of starting ketone **2** and its C-8 epimer **3** *via* the enol tautomer.[10] To obtain the other *cis*-lactone, **1** was treated with bases to give picropodophyllin **4** which was oxidized with PDC to the corresponding ketone **5**.

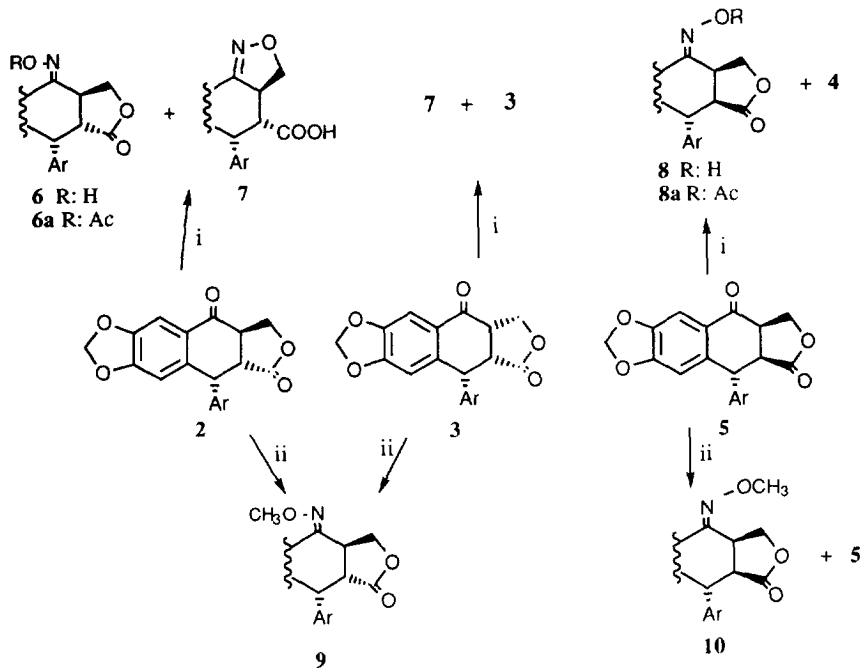



**1** **2** **3** **4** **5**

i) PDC. ii) KOH/MeOH. iii) AcOH

Scheme 1. Preparation of 7-ketolignanolides

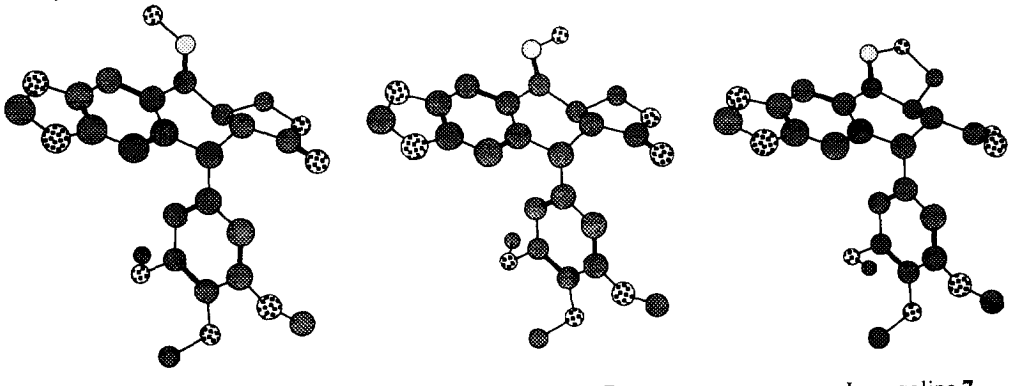
When **2** was treated with hydroxylamine hydrochloride in ethanol and pyridine, the corresponding oxime **6** was formed in low yield (8%), the main product (75%) being the isoxazoline derivative **7** (scheme 2). This is a new type of lignan that bears an isoxazoline ring fused to the cyclolignan core and a free carboxylic group resulting from the opening of the lactone moiety. The formation of the isoxazole is explained through initial formation of the oximes. In the case of the *E*-oxime, the oxygen atom is in the right position for a nucleophilic attack on the methylene at C-9. We know that this methylene is easily attacked by weak nucleophiles due to the great tendency of the *trans*-γ-lactone to opening with loss of the ring strain produced by the *trans* ring junction.[8] Using the same reasoning, we deduce that the isolated oxime **6** has *syn* stereochemistry since it does not lead to formation of the isoxazoline ring.




i) $NH_2OH.ClH$, EtOH/Py. ii) $NH_2OCH_3$, EtOH/Py (Ar = 3,4,5-trimethoxyphenyl)

Scheme 2. Reaction of ketocyclolignanolides with hydroxylamines

The formation of the isoxazoline **7** is understandable on the basis of calculated heats of formation of these compounds. They were calculated using MOPAC with the AM1 Hamiltonian Eigenvector following geometry optimisation and PRECISE convergence criteria.[12] The 6*Z*-oxime is slightly more stable (0.5 Kcal/mol) than the 6*E*-oxime, but the isoxazoline **7** is around 2.6 Kcal/mol lower still (Figure 1). Hence it is not surprising, given the expectation of a low activation energy for the cyclisation process, that the major final product was the heterocycle fused species instead of the oximes.



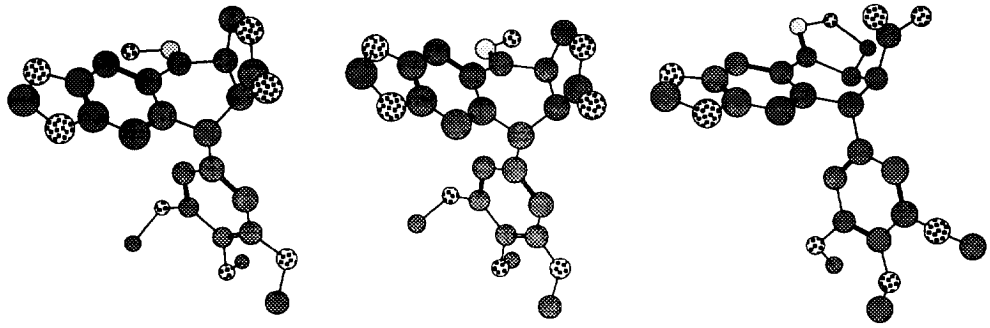
Oxime 6*Z*.
$\Delta H_{for}$= -183.66 Kcal/mol

Oxime 6*E*.
$\Delta H_{for}$= -183.18 Kcal/mol

Isoxazoline **7**.
$\Delta H_{for}$= -186.30 Kcal/mol

Figure 1. Lowest Energy Conformations of compounds **6** and **7**.

The *cis*-lactones were less reactive since the *cis*-junction of the lactone was less strained than the *trans*-junction. In the case of **3**, the resulting product was the same isoxazole carboxylic acid **7** together with unreacted ketone **3**. The change in the stereochemistry at C-8 can be justified due to the equilibrium between **2** and **3** already mentioned. When the condensation was made with **5**, the oxime **8***E* was isolated as major product, as predicted by calculations (Scheme 2). That none of the corresponding isoxazoles were formed is consistent with the theoretical stability of the species involved. The **8***E*-oxime is calculated to be slightly more stable (on the same basis as before) than the **8***Z*-oxime (by 0.8 Kcal/mol) and much more stable than the corresponding isoxazoline **8a** (by 3.5 Kcal/mol) (figure 2).



Oxime **8***Z*.
$\Delta H_{for}$= -189.53 Kcal/mol

Oxime **8***E*.
$\Delta H_{for}$= -190.28 Kcal/mol

Isoxazoline **8a**.
$\Delta H_{for}$= -186.83 Kcal/mol

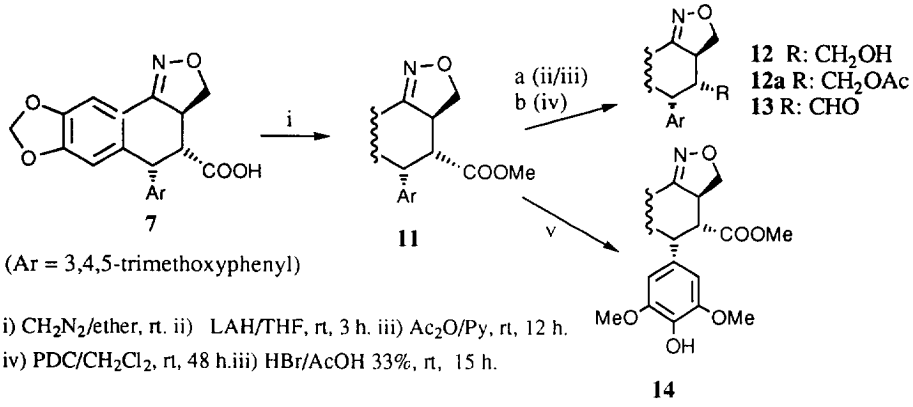
**Figure 2**. Lowest Energy Conformations of compound **8**

With the aim of confirming chemically the stereochemistry of the isolated oximes, the ketolactones were made to react with *O*-methylhydroxylamine which was unable to open the lactone ring. The reaction product of ketolactones **2** and **3** was the same methyloxime **9** while **5** gave the isomer **10** (scheme 2). The *syn* configuration of the oxime **9** was determined by NOE experiments; an increase in the intensity of the signal for H-2 (δ 7.59 ppm) was observed when the singlet corresponding to the methyl of the oxime (δ 3.92 ppm) was irradiated.

The preferential formation of the *Z*-oxime in the case of **9** over the geometrical **9***E*-isomer is also consistent with the theoretical stability of the species involved. Since in this case the energy difference calculated *in vacuo* was small (0.49 kcal/mol) the calculation was confirmed using the THERMO and FORCE options of MOPAC to generate calculated thermodynamic properties at 300 K. The difference in calculated heats of formation at this temperature remained at 0.49 kcal/mol, while the difference in free energy derived from the calculated enthalpies and entropies of the two isomers was still in the same direction, although smaller (0.23 kcal/mol). The dipoles calculated for the two isomers *in vacuo* were both within 0.04D of 5.53, and thus it seemed unlikely that there would be any reordering of the calculated stabilities of these molecules if the calculation were to take account of solvation. However, the AMSOL[13] semiempirical method was applied using SM2.1[14] as the solvation model, using the gas-phase optimised geometries from the earlier calculations. The energies derived from this method also supported the greater stability of *Z*-methyloxime, predicting a slightly larger preference of 0.76 kcal/mol (difference in heats of formation + ΔGsolv). These more extensive

calculations also therefore appear to support the use of the *in vacuo* calculated heats of formation referred to above for these comparisons.

The oximes **6** and **8** were transformed into their acetates **6a** and **8a** and the carboxylic acid **7** was successively transformed into the corresponding methyl ester **11** by treatment with diazomethane, into the alcohol **12** by LAH reduction of **11**, into the acetate **12a** and into the aldehyde **13** by PDC oxidation of **12**. Also the methyl ester **11** was treated with 33% HBr/AcOH to give the 4'-demethyl derivative **14** (scheme 3).




(Ar = 3,4,5-trimethoxyphenyl)

i) $CH_2N_2$/ether, rt. ii) LAH/THF, rt, 3 h. iii) $Ac_2O$/Py, rt, 12 h.
iv) PDC/$CH_2Cl_2$, rt, 48 h.iii) HBr/AcOH 33%, rt, 15 h.

Scheme 3. Obtention of the isoxazole derivatives

**Biological activity**. The prepared compounds have been evaluated for their bioactivity against cell cultures of P-388 murine leukaemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma. The results obtained are shown in table 1. As can be seen, the tested derivatives show cytotoxic activity levels two or three orders of magnitude lower than those of podophyllotoxin (**1**), thus confirming that the presence of the lactone moiety is a prominent factor for displaying high cytotoxic activity.

**Table 1**.Antineoplastic Activity of Compounds **6-14**.($IC_{50}$ μM)

| Compound | P-388 | A-549 | HT-29 | MEL-28 |
|---|---|---|---|---|
| **1** | 0.012 | 0.012 | 0.012 | |
| **6** | 2.3 | 2.3 | 2.3 | 2.3 |
| **6a** | 2.1 | 2.1 | 5.3 | 2.1 |
| **7** | 2.2 | 5.6 | 11 | 5.6 |
| **8** | 2.3 | 2.3 | 2.3 | 2.3 |
| **9** | 0.2 | 0.2 | 0.2 | 0.2 |
| **10** | 2.3 | 2.3 | 2.3 | 2.3 |
| **11** | 2.2 | 5.6 | 11 | 5.6 |
| **12** | 12 | 12 | 12 | 12 |
| **12a** | 2.6 | 2.6 | 2.6 | 2.6 |
| **13** | 2.4 | 2.4 | 2.4 | 2.4 |
| **14** | 23 | >23 | >23 | 23 |

Analyzing the data in table 1, some general effects were observed with respect to the chemical structures and their substituents.

**Table 2.** $^1$H RMN Data of Compounds **6-14.**

| H | 6 | 6a | 7 | 8 | 8a | 9 | 10 | 11 | 12 | 12a | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 7.32 *s* | 7.55 *s* | 7.43 *s* | 7.25 *s* | 7.47 *s* | 7.59 *s* | 7.31 *s* | 7.43 *s* | 7.41 *s* | 7.46 *s* | 7.43 *s* | 7.44 *s* |
| 5 | 6.65 *s* | 6.69 *s* | 6.56 *s* | 6.69 *s* | 6.73 *s* | 6.57 *s* | 6.67 *s* | 6.54 *s* | 6.53 *s* | 6.55 *s* | 6.59 *s* | 6.56 *s* |
| 8 | 3.54 *m* | 3.55 *m* | 3.80 *m* | 3.27 *dd* (8.5;2.5) | 3.31 *dd* (8.6;2.5) | 3.24 *m* | 3.30 *m* | 3.80 *m* | 3.80 *m* | 3.70 *m* | 3.78 *m* | 3.81 *m* |
| 9a | 4.84 *t* (9.2) | 4.75 *t* (9.4) | 4.82 *t* (12.3) | 4.58 *d* (2.5) | 4.62 *d* (2.5) | 4.80 *t* (9.4) | 4.44 sa | 4.82 *dd* (13.8;8.3) | 4.61 *t* (8.6) | 4.65 *t* (8.5) | 3.46 *dd* (14.1;7.1) | 4.83 *t* (7.3) |
| 9b | 3.90 *t* (9.2) | 3.95 *t* (9.4) | 3.80 *m* | 4.21 *t* (2.5) | 4.23 *t* (2.5) | 4.30 *t* (9.4) | | 3.91 *t* (8.3) | 3.83 *t* (8.6) | 3.70 *t* (8.5) | 4.13 *t* (7.1) | 3.83 *d* (7.3) |
| 10a | 6.01 *s* | 6.05 *s* | 5.99 *s* | 5.98 *s* | 6.03 *s* | 5.98 *s* | 5.97 *s* | 5.98 *s* | 5.96 *s* | 5.98 *s* | 5.97 *s* | 6.01 *s* |
| 10b | 6.04 *s* | 6.03 *s* | 5.99 *s* | 5.98 *s* | 6.03 *s* | 5.98 *s* | 5.97 *s* | 5.98 *s* | 5.96 *s* | 5.98 *s* | 5.97 *s* | 6.01 *s* |
| 2',6' | 6.31 *s* | 6.26 *s* | 6.20 *s* | 6.25 *s* | 6.21 *s* | 6.32 *s* | 6.25 *s* | 6.12 *s* | 6.22 *s* | 6.15 *s* | 6.11 *s* | 6.15 *s* |
| 7' | 4.36 *d* (5.9) | 4.50 *d* (5.8) | 4.67 *d* (5.0) | 4.52 *d* (3.9) | 4.25 *d* (4.0) | 4.64 *d* (4.0) | 4.50 *d* (2.3) | 4.62 *d* (5.2) | 4.85 *d* (5.1) | 4.29 *d* (5.0) | 4.86 *d* (5.3) | 4.86 *d* (5.2) |
| 8' | 3.26 *dd* (9.6;5.9) | 3.29 *dd* (9.6;5.8) | 3.23 *dd* (11.4;5.0) | 3.98 *dd* (8.5;3.9) | 3.90 *m* | 2.96 *dd* (15.1;4.0) | 3.22 *dd* (8.6;2.3) | 3.20 *dd* (11.9;5.2) | 2.32 *m* | 2.52 *m* | 3.14 *dd* (11.1;5.3) | 3.21 *dd* (11.9;5.2) |
| 9'a | | | 8.38 *sa* | | | | | | 3.46 *dd* (10.9;8.2) | 3.98 *dd* (11.0;7.5) | 9.78 *s* | |
| 9'b | | | | | | | | | 3.29 *dd* (10.9;6.0) | 3.82 *dd* (11.0;8.0) | | |
| MeO-3',5' | 3.70 *s* | 3.70 *s* | 3.66 *s* | 3.72 *s* | 3.74 *s* | 3.71 *s* | 3.75 *s* | 3.72 *s* | 3.72 *s* | 3.74 *s* | 3.72 *s* | 3.77 *s* |
| MeO-4' | 3.78 *s* | 3.78 *s* | 3.76 *s* | 3.79 *s* | 3.79 *s* | 3.77 *s* | 3.79 *s* | 3.78 *s* | 3.78 *s* | 3.80 *s* | 3.77 *s* | |
| COOMe | | | | | | | | 3.66 *s* | | | | 3.67 *s* |
| COMe | | 2.22 *s* | | | 2.25 *s* | | | | | 2.07 *s* | | |
| $CH_3$ | | | | | | 3.92 *s* | 3.98 *s* | | | | | |

**Table 3.** $^{13}$C RMN Data of Compounds **6-14.**

| C | 6 | 6a | 7* | 8 | 8a | 9 | 10 | 11 | 12 | 12a | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 133.2 | 133.4 | 120.2 | 132.7 | 133.4 | 133.7 | 132.5 | 119.1 | 119.0 | 118.9 | 119.2 | 119.1 |
| 2 | 105.0 | 106.2 | 104.2 | 104.7 | 105.0 | 104.2 | 105.0 | 104.1 | 103.9 | 104.1 | 104.0 | 104.0 |
| 3 | 148.0 | 148.3 | 148.8 | 148.1 | 148.3 | 147.8 | 148.1 | 147.7 | 147.4 | 147.6 | 147.8 | 147.7 |
| 4 | 150.1 | 151.6 | 151.9 | 150.2 | 151.6 | 149.8 | 150.1 | 150.5 | 150.5 | 150.6 | 150.6 | 150.6 |
| 5 | 108.7 | 108.7 | 110.4 | 109.5 | 109.6 | 109.5 | 109.4 | 109.2 | 109.5 | 109.4 | 109.2 | 109.2 |
| 6 | 124.7 | 123.1 | 138.5 | 123.9 | 123.1 | 124.8 | 124.1 | 138.1 | 137.7 | 138.0 | 138.1 | 135.1 |
| 7 | 152.4 | 158.2 | 159.5 | 154.6 | 158.2 | 153.4 | 153.2 | 156.4 | 157.6 | 156.9 | 156.2 | 156.3 |
| 8 | 36.8 | 37.8 | 46.0 | 33.7 | 37.8 | 36.8 | 34.1 | 43.6 | 44.6 | 41.5 | 42.2 | 43.6 |
| 9 | 71.4 | 70.3 | 76.2 | 71.0 | 70.3 | 70.1 | 71.1 | 74.5 | 73.9 | 73.7 | 74.1 | 74.5 |
| 10 | 101.8 | 102.0 | 103.1 | 101.7 | 102.0 | 101.7 | 101.5 | 101.9 | 101.6 | 101.6 | 101.7 | 101.7 |
| 1' | 133.9 | 135.5 | 138.2 | 137.7 | 135.0 | 134.8 | 137.8 | 135.1 | 136.6 | 136.0 | 135.0 | 134.8 |
| 2',6' | 106.5 | 107.0 | 109.2 | 105.1 | 106.0 | 108.1 | 105.0 | 107.2 | 107.6 | 107.5 | 106.9 | 106.9 |
| 3',5' | 153.1 | 153.4 | 154.0 | 153.7 | 153.4 | 153.1 | 153.7 | 153.2 | 153.2 | 153.4 | 153.5 | 147.2 |
| 4' | 137.4 | 138.2 | 138.0 | 137.7 | 138.2 | 137.9 | 137.5 | 134.9 | 135.6 | 134.9 | 134.4 | 130.7 |
| 7' | 45.2 | 44.9 | 49.0 | 44.8 | 44.9 | 43.9 | 44.8 | 48.1 | 44.8 | 44.6 | 46.5 | 48.0 |
| 8' | 45.2 | 44.9 | 53.8 | 45.7 | 44.9 | 46.8 | 45.8 | 50.1 | 47.8 | 48.2 | 56.1 | 52.2 |
| 9' | 176.6 | 175.1 | 178.8 | 176.3 | 175.1 | 173.1 | 176.3 | 171.4 | 63.7 | 65.4 | 199.5 | 171.4 |
| MeO-3',5' | 56.1 | 56.3 | 56.6 | 56.3 | 56.3 | 56.4 | 56.2 | 56.3 | 56.4 | 56.4 | 56.4 | 56.5 |
| MeO-4' | 60.9 | 60.8 | 61.0 | 60.8 | 60.8 | 60.7 | 60.9 | 60.7 | 60.8 | 60.8 | 60.7 | |
| COOMe | | | | | | | | 51.9 | | | | 51.8 |
| $\underline{C}OCH_3$ | | 167.5 | | | 167.5 | | | | | 170.3 | | |
| $CO\underline{C}H_3$ | | 19.6 | | | 19.6 | | | | | 20.7 | | |
| $CH_3$ | | | | | | 62.4 | 62.5 | | | | | |

* Spectrum done in acetone-$d_6$

The stereochemistry of the ketolactones seems important for their activity. It is well known that the *trans*-lactones are more cytotoxic than the *cis*-lactones,[7] but when we compare their oxime derivatives, there is no difference in potency (**6** *versus* **8**). On the other hand, when the -OH of the oxime is methylated as in **9**, the resulting potency is about 3-fold better than the precursor **2**.

Among the compounds with the fused isoxazole ring, the acid **7** is less potent than **1**, but it is worth noting that it has some selectivity against P-388. Esterification of **7** to give the methyl ester **11** did not modify the activity. Its reduction to the alcohol **12** decreased the potency significantly, but it could be partially recovered when the hydroxyl group was acetylated, (**12a** *versus* **12**). The same effect is observed when the alcohol is transformed into the aldehyde **13**. The derivative **14** which did not have the methyl group at C-4' position, like etoposide and teniposide, showed the worst antitumor activity of all the series.

From these results it can be concluded that the activity of these compounds not only depends on the presence and stereochemistry of the lactone but of the substituent at C-7 which also plays an important role.

Compound **7** has been subjected to an *in vitro* and *in vivo* evaluation of its immunomodulatory (IM) activity which is very promising.[15]

## EXPERIMENTAL

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.

### Chemistry

*Reaction between 7-ketolignans and hydroxylamine*

*With podophyllotoxone* **2**. Hydroxylamine chlorhidrate (64 mg, 0.93 mmol) and 0.2 mL of pyridine were added to a solution of **2** (300 mg, 0.73 mmol) in 10 mL of ethanol and stirred at 95°-100°C for 72 h. The filtrate was treated with sat. aq. $NaHCO_3$ and extracted with ethyl acetate. After removing the solvent, the crude product was purified by flash chromatography ($CH_2Cl_2$/EA 98:2 as eluant) to give the following compounds:

- 25 mg (8 %) of oxime **6**. Mp. 138-140°C (MeOH). Anal. calcd. for $C_{22}H_{21}O_8N$ : C, 61.82; H, 4.95; N, 3.28; found: C, 61.58; H, 4.93; N, 3.26. MS m/z: 427 ($M^+$), 367, 326, 203, 167, 149, 83. $[\alpha]^{22}$ ($\lambda$): -128.2° (589), -132.7° (578), -154.4° (546), -298.5° (436) (c=0.20, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 223 (15800), 266 (9800), 309 (5900). IR: 3020, 2920, 2860, 1780, 1600, 1510, 1490, 1470, 1425, 1380, 1340, 1220, 1130, 1030, 1010, 980, 940, 880 $cm^{-1}$. $^{1}H$ NMR table 2. $^{13}C$ NMR table 3.

- 250 mg (81 %) of isoxazolopodophyllic acid **7**. Mp. 246-248°C ($CH_2Cl_2$). Anal. calcd. for $C_{22}H_{21}O_8N$ : C, 61.82; H, 4.95; N, 3.28; found: C, 61.67; H, 4.94; N, 3.30. MS m/z: 428 ($M+H^+$), 329, 286, 260, 176, 133, 89. $[\alpha]^{22}$ ($\lambda$): -152.6° (589), -161.4° (578), -185.0° (546), -330.3° (436) (c=0.61, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 215 (26500), 274 (12000), 313 (8900). IR: 3400-2800, 1710, 1600, 1510, 1490,

1470, 1425, 1390, 1330, 1230, 1130, 1040, 1010, 940, 910, 880, 860 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

Acetylation of **6** with acetic anhydride in pyridine afforded, after usual work up, the appropiated acetate **6a** (95%). Anal. calcd. for $C_{24}H_{23}O_8N$ : C, 61.41; H, 4.90; N, 2.99; found: C, 61.26; H, 4.88; N, 3.01. $[\alpha]^{22}$ ($\lambda$): -147.4° (589), -155.2° (578), -180.0° (546), -356.1° (436) (c=0.15, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 216 (12500), 274 (7000), 315 (5700). IR: 2940, 1780, 1600, 1510, 1490, 1470, 1430, 1370, 1340, 1240, 1200, 1140, 1050, 1010, 950, 930, 880 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

*With isopicropodophyllone* (**3**). The same compound **7** (64 mg, 48 %) were obtained with 130 mg (0,32 mmol) of **3**, 33 mg of hydroxylamine chlorhidrate (0,48 mmol) and 0,1 mL de pyridine in 10 mL of ethanol; 65 mg (50 %) of unreacted **3** were also obtained.

*With picropodophyllone* **5**. To a solution of **5** (100 mg, 0.24 mmol) in ethanol (10 mL) was added 0.1 mL of pyridine and 25 mg of hydroxylamine chlorhidrate (0,36 mmol). After the same work up described before for **6** and **7**, 64 mg (64%) of unreacted **5** and 23 mg (22 %) of **8** were isolated after flash chromatography of the reaction product. $[\alpha]^{22}$ ($\lambda$): -16.3° (589), -20.0° (578), 25.0° (546), -60.0° (436) (c=0.08, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 205 (19500), 267 (4000), 309 (2900). IR: 3500-3100, 3020, 2940, 1780, 1600, 1515, 1490, 1470, 1430, 1385, 1350, 1340, 1250, 1140, 1050, 1010, 975, 950, 880, 830 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

Acetylation of **8** in the usual way, afforded the appropiate acetate **8a** (96 %). $[\alpha]^{22}$ ($\lambda$): -18.3° (589), -23.0° (578), -27.7° (546), -65.2° (436) (c=0.09, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 207 (17800), 274 (3900), 312 (2800). IR: 3005, 1780, 1600, 1510, 1490, 1465, 1435, 1390, 1340, 1230, 1130, 1040, 1010, 940, 880 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

*Reaction between 7-ketolignans and O-Methylhydroxylamine.*

*With podophyllotoxone* **2**. To a solution of podophyllotoxone **2** (150 mg, 0.36 mmol) in ethanol (15 mL) was added 0.1 mL of pyridine and 46 mg of *O*-methylhydroxylamine (0.98 mmol), after the usual work up, afforded 150 mg (94 %) of **9**. Mp. 139-141°C ($H/CH_2Cl_2$). Anal. calcd. for $C_{23}H_{23}O_8N$ : C, 62.58; H, 5.25; N, 3.17; found: C, 62.81; H, 5.26; N, 3.15. MS m/z: 442 ($M+H^+$), 430, 410, 399, 383, 368, 354, 274, 168. $[\alpha]^{22}$ ($\lambda$): -142.2° (589), -150.6° (578), -179.4° (546), -406.1° (436) (c=0.18, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 230 (15100), 271 (12200), 311 (8100). IR: 3020, 1780, 1600, 1510, 1490, 1470, 1420, 1380, 1330, 1220, 1130, 1050, 1000, 980, 940, 910, 880 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

*With isopicropodophyllone* (**3**). The same compound **9** (35 mg, 36 %) was obtained from 90 mg (0.22 mmol) of isopicropodophyllone (**3**), 30 mg of *O*-methylhydroxylamine chlorhidrate (0.64 mmol) and 0,1 mL de pyridine in 10 mL of ethanol.

*With picropodophyllone* (**5**). To a solution of picropodophyllone **5** (150 mg, 0.36 mmol) in ethanol (10 mL) was added 0.1 mL of pyridine and 46 mg of *O*-methylhydroxylamine (0.98 mmol), after the usual work up, 81 mg (50 %) of **10** and 40 mg (26 %) of unreacted **5** were obtained.

*Isoxazoline Derivatives Preparation.*

*Methyl isoxazolopodophyllate* (**11**) Treatment of acid **6** with an ethereal solution of diazomethane yielded **11** (87 %). Mp 82-84°C (*n*-hexanol). Anal. calcd. for $C_{23}H_{23}O_8N$: C, 62.58; H, 5.25; N, 3.17; found: C, 62.33; H, 5.50; N, 3.16. MS m/z: 441 ($M^+$), 412, 382, 352, 326, 273, 258, 214, 149, 119, 91. $[\alpha]^{22}$ ($\lambda$): -137.5° (589), -144.4° (578), -165.0° (546) (c=0.32, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 219 (42800), 274 (22000), 313 (15200). IR: 3040, 2980, 1740, 1600, 1510, 1490, 1460, 1390, 1340, 1240, 1140, 1050, 1010, 950, 930, 900, 870 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

*Isoxazolopodophyllol* (**12**): 100 mg (0.23 mmol) of methyl ester **11** in THF (5 mL) was slowly added to a suspension of LAH (120 mg, 3.16 mmol) in dry THF. The reaction mixture was stirred at room temperature under Argon for 3 h. Then, wet ethyl acetate was added, filtered, dried and evaporated to afford 96 mg (96 %) of **12**. Mp 146-148°C ($MeOH/CH_2Cl_2$). Anal. calcd. for $C_{22}H_{23}O_7N$ : C, 63.92; H, 5.61; N, 3.39; found: C, 63.70; H, 5.60; N, 3.33. $[\alpha]^{22}$ ($\lambda$): -88.8° (589), -93.8° (578), -108.8° (546), -190.0° (436) (c=0.08, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 213 (23600), 275 (7900), 313 (5700). IR: 3600-3300, 3020, 2940, 1600, 1510, 1490, 1465, 1430, 1330, 1230, 1135, 1050, 1010, 945 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

Acetylation of alcohol **12** (30 mg, 0.07 mmol) with acetic anhydride in pyridine afforded, after usual work up, 30 mg (91 %) of acetate **12a**. Anal. calcd. for $C_{24}H_{25}O_8N$ : C, 63.29; H, 5.53; N, 3.08; found: C, 63.13; H, 5.55; N, 3.07. $[\alpha]^{22}$ ($\lambda$): -98.5° (589), -103.3° (578), -118.8° (546), -212.2° (436) (c=0.36, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 213 (22000), 275 (7200), 313 (5000). IR: 3010, 2930, 1740, 1600, 1510, 1490, 1465, 1435, 1390, 1330, 1230, 1130, 1040, 1010, 940, 860 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

*Isoxazolopodophyllal* (**13**): A solution of alcohol **12** (200 mg, 0.47 mmol) in $Cl_2CH_2$ (15 mL) containing pyridinium dichromate (PDC, 300 mg, 3.1 mmol) was stirred for 48 h. The excess of PDC was removed by filtration followed by columm chromatography on silica gel. The crude product was purified by flash chromatography ($CH_2Cl_2$/EA 8:2 as eluant) to give 135 mg (68 %) of aldehyde **13**. Mp 145-147°C ($Hex/CH_2Cl_2$). Anal. calcd. for $C_{22}H_{21}O_7N$ : C, 64.23; H, 5.11; N, 3.41; found: C, 64.44; H, 5.11; N, 3.38. $[\alpha]^{22}$ ($\lambda$): -76.7° (589), -81.4° (578), 93.4° (546) (c=0.37, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 207 (20400), 273 (10300), 312 (7300). IR: 3010, 2940, 2830, 1730, 1600, 1510, 1490, 1465, 1420, 1390, 1330, 1240, 1135, 1045, 1010, 945, 880 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

To a solution of methyl ester **11** (150 mg, 0,34 mmol) in 1,2-dichloroethane (15 mL), 3 mL de 33% HBr/AcOH were added, the reaction mixture was stirred at room temperature for 15 h. Then ice and ethyl acetate was added and the filtrate was treated with sat. aq. $NaHCO_3$ and brine. After removing the solvent, the crude product was purified by flash chromatography ($CH_2Cl_2$ /AE 9:1 as eluant) to give 30 mg (21 %) of **14**. $[\alpha]^{22}$ ($\lambda$): -145.3° (589), -153.0° (578), -177.0° (546) (c=0.30, $CHCl_3$). UV $\lambda_{max}$ ($\varepsilon$): 215 (26200), 274 (11600), 313 (6900). IR: 3540, 3020, 2950, 1740, 1630, 1530, 1510, 1490, 1470, 1440, 1390, 1340, 1220, 1130, 1050, 950, 930, 880 $cm^{-1}$. $^1H$ NMR table 2. $^{13}C$ NMR table 3.

**Bioactivity**

*Antineoplastic assays*: Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of $1x10^4$ (P-388), $2x10^4$ (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 three 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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