Synthesis, Antitumoral and Antiviral Evaluation of Halo- and Demethyl-Yatein Derivatives

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Summary

Several chloro- and iodo-lignanolides have been obtained by direct halogenation of aromatic rings from yatein and 4′-*O*-demethylyatein. They were assayed as antineoplastics, in order to check the influence in the activity of substitution in both aromatic rings. Although these compounds show a modest antineoplastic activity, it is far from that displayed by yatein and podophyllotoxin. These results confirm that demethylation and the introduction of halo substituents diminish the activity of lignans of the dibenzylbutyrolactone type.

Introduction

The cyclolignans are a family of compounds that has attracted much attention because of their multiple and diverse biological activities. Many papers have been published concerning their synthesis and transformations, as well as their pharmacological activities[1]. In fact, etoposide, a semisynthetic derivative of 4′-*O*-demethylpodophyllotoxin, is presently the most widely used antitumoral drug[2].

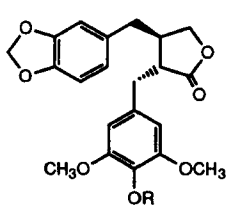
The lignanolides are another group of lignans that has been less thoroughly studied from the pharmacological point of view. Unlike podophyllotoxin derivatives, these substances do not inhibit the tubulin polymerization. Arctigenin and trachelogenin, two lignanolides with phenolic groups, show activity against AIDS virus[3]. Yatein, a compound isolated from *Juniperus thurifera* leaves[4], has shown a significant activity towards P-388 cell lines[5]. The fact that yatein shows a spatial disposition different to that of podophyllotoxin and is not one of the so called spindle poisons, suggests that its activity probably arises from some unknown mechanism of action.

Several modifications have been carried out on the basic structure of yatein in order to determine the structural requirements for this type of activity. One kind of modification recently described by us is the substitution of the trimethoxyphenyl moiety by a heterocyclic ring[6]. Another modification consists in the introduction of new substituents on the aromatic rings of yatein, in order to change the electronic and steric properties of this molecule. In the present work we describe the synthesis of yatein and demethylyatein derivatives with chlorine and iodine atoms in the aromatic rings. The bromo derivatives have been previously described[7,8].

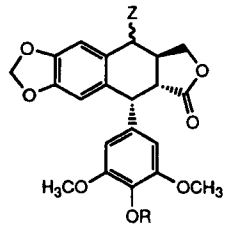
As starting materials, we chose yatein (**1**) and 4′-*O*-demethylyatein (**2**). The former because it shows a remarkable antitumoral activity *in vitro*[4], and the latter because compounds that inhibit the topoisomerase-II of DNA as well as dibenzylbutyrolactones which show anti-HIV activity, possess free phenolic groups[9].

Results and Discussion

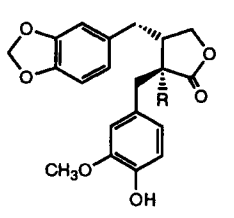
The synthesis of yatein was carried out by using the general methodology "conjugate addition-alkylation" to 5*H*-furan-2-one (γ-crotonolactone)[10], followed by desulfurization[11] with Raney Ni to remove the 1,3-dithiane moiety. 4′-*O*-Demethylyatein (**2**) was obtained by selective demethylation of yatein at 4′. For this purpose we used Me_3SiI generated *in situ* from Me_3SiCl and NaI. When the reaction was allowed to proceed up to the total disappearance of the starting material, formation of the bisdemehylated derivative **3** was also observed. This product, with two phenolic groups, was also used in the activity assays because, as is known for podophyllotoxin, an additional phenolic group does not affect the inhibi-



R=CH_3 Yatein (**1**)
R=H 4′-*O*-Demethylyatein (**2**)



R=CH_3, Z=αOH Podophyllotoxin
R=H, Z=βOH Demethylepipodophyllotoxin



R=H Arctigenin
R=OH Trachelogenin

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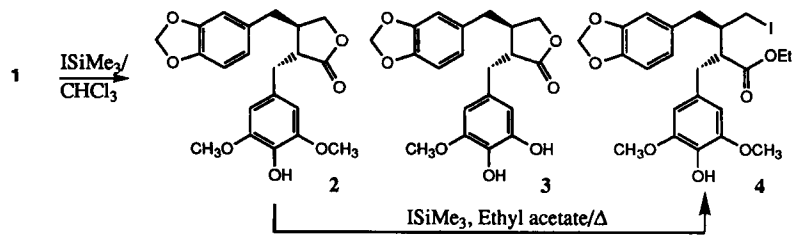
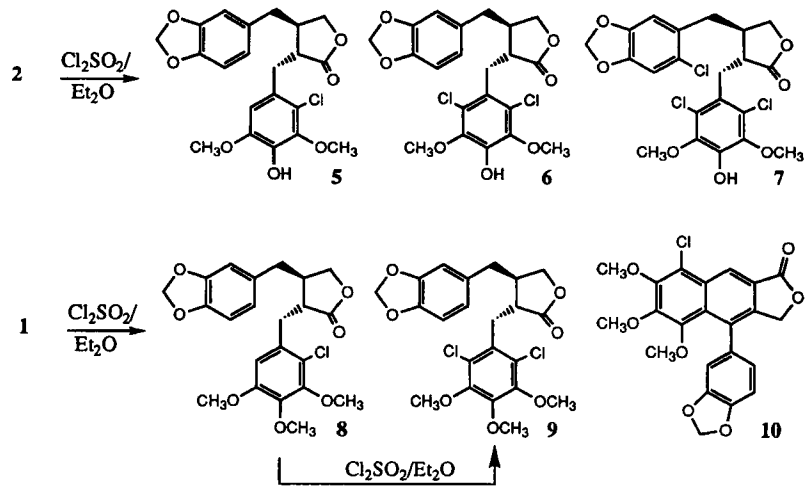
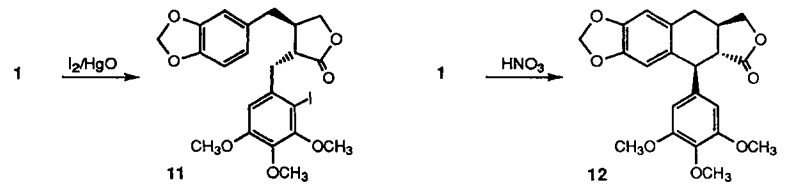


Scheme 1. Demethylation of yatein (**1**).



Scheme 2. Synthesis of demethylchloro- (**5–7**) and chloroyateins (**8–9**).



Scheme 3. Synthesis of iodoyatein (**11**) and isodeoxypodophyllotoxin (**12**) from yatein.

tory activity *versus* topoisomerase-II. When the reaction was conducted at 45 °C, in addition to the demethylated products already described, another more polar compound **4** was obtained. Instead of the 1780 cm^{-1} absorption, common in the IR spectra of other lignanolides, an ester absorption at 1750 cm^{-1} was observed. The rest of its spectroscopic properties also support structure **4** for the more polar product obtained under those conditions.

In order to obtain chloro derivatives from yatein and 4′-*O*-demethylyatein, we used sulfuryl chloride. In this reaction, both, the amount of reagent and the time of reaction, control the degree of chlorination produced in the lignan. On treatment of 4′-*O*-demethylyatein with 1 equivalent of sulfuryl chloride, the monochloro derivative **5** was the only isolated product; whereas by using 2.5 equivalents of sulfuryl chloride, the dichlorinated derivative **6** was obtained. In order to produce the trichloro derivative **7**, 4 equivalents of sulfuryl chloride were added.

In the same way, the monochloro derivative **8** (rafa **45**) was obtained by treating yatein with one equivalent of sulfuryl chloride. When two equivalents of this reagent were added, the monochloro derivative **8** (rafa **45**) was the main product and the dichloroderivative **9** was only formed in 4%. The retrocyclolignan **10** (rafa **47**) was also isolated. It displays a highly deshielded H-7 (8.9 ppm) and H-9 (5.05 and 5.10 ppm, *d*, *J*=16.1 Hz) signals in its NMR spectrum that unequivocally allowed us to identify this compound. The formation of this product can be explained by chlorination at the benzylic position 7, followed by cyclization and dehydrogenation. This process was also observed in the bromination of yatein[7]. With the increment of sulfuryl chloride ratio (up to 10 times), only a complex mixture was produced. The dichloro derivative **9** was easily obtained by further treatment of monochloride **8** (rafa **45**) with one equivalent of sulfuryl chloride.

In order to obtain iodo derivatives, both yatein and 4′-*O*-demethylyatein were treated with sublimated I_2 in presence of HgO[12]. When **2** was used as substrate, no derivatives containing iodine were obtained, and only the monoiodination product at 2′ (**11**) was isolated when yatein was treated with an excess of I_2. All these chloro and iodo derivatives display spectroscopic data very similar to that of the analogous bromo derivatives previously described[8].

Finally, the use of nitrating agents as nitric acid or isoamyl nitrite in order to produce nitroderivatives of yatein, did not lead to the desired product. Only isodesoxypodophyllotoxin **12** was obtained as reaction product. The benzylic oxidation[13] by nitric acid followed by the usual cyclization to products with *trans-trans* stereochemistry[14] observed for lignanolides functionalized at benzylic position, accounts for the isolation of **12**.

Biological assays

Compounds **1–10** and **12** were assayed as antineoplastics on: P-388 (lymphoid neoplasm from DBA/2 mouse), A-549 (human lung carcinoma) and HT-29 (human colon carcinoma) and MCF-7 (human breast carcinoma) cells; as antivirals on: HSV-1 (herpes simplex virus, type1) and VSV (vesicular stomatitis virus); and as enzyme inhibitors of: ADA (adenosine deaminase), DHFR (dihydrofolatereductase) and GST (glutation-S-transferase). None of these compounds showed antiviral activity and all of these compounds

Table 1. Antitumoral activities against P-388, A-549, HT-29, MCF-7 cell lines.

Compound	P-388 [a]	A-549 [a]	HT-29 [a]	MCF-7 [a]
1	0.025	0.05	0.05	0.05
2	10	10	20	10
3	5	5	5	–
4	2	2	2	10
5	10	10	10	>20
6	20	20	20	>20
7	5	5	5	5
8	10	10	10	20
9	>20	>20	>20	–
10	20	20	>20	–
12	>20	>20	>20	–

a) IC_{50} in μg/ml. – not tested

failed to inhibit the tested enzymes. The antineoplastic results, as well as their activities, are shown in table 1.

From the antineoplastic studies it can be deduced that the demethylation of yatein produces a strong diminution of activity in all of the assayed cell lines; 100 times in compound **2**, which only lost the 4′-*O*-methyl, and 10 times in compound **3** with a double demethylation. Apart from other electronic changes, this demethylation implies a weakness of the π-stacking interactions betweenthe two aromatic rings. On the other hand, the change of bromine by chlorine or iodine always implies a weak diminution of activity, which can be due to the racemic mixture of the synthetic yatein used as starting material. Although these compounds show a modest antineoplastic activity, it is far from that displayed by yatein and podophyllotoxin (10–20 ng/ml), which have been taken as references in these assays. These results confirm the negative influence on the antineoplastic activity of this type of lignanolides, when halogen atoms are introduced in its aromatic rings. As the mechanism of antitumoral and antiviral activity of these lignans is not fully understood further studies are needed in order to obtain a better knowledge and more active compounds.

Acknowledgments

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Experimental

Chemistry

General Experimental Procedures.– Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra were performed on a Beckmann (Acculab 8) spectrophotometer, in $CHCl_3$ solution (ν_{max} are given in cm^{-1}). NMR spectra were recorded on a Bruker WP 200 SY (200MHz for ^1H and 50.3 MHz for ^{13}C). Chemical shifts (δ) are given in ppm, referred to internal TMS, and coupling constants (*J*) in Hz. EIMS were recorded on a VG-TS-250 instrument; ionization energy was 70 eV. CC was performed over silica gel Merck 60 (0.063–0.2 mm). For flash CC, an Eyela EF-10 apparatus was used, with 3–85 ml/min flow rate, over silica gel Merck 60 (0.040–0.063 mm). TLC was performed on precoated silica gel polyester plates (0.25 mm thickness) with fluorescent indicator UV254 (Polychrom SI F_{254}). A solution of 10% phosphomolybdic acid in EtOH or 10% H_2SO_4 in EtOH were used for visualization, after heating at 110°C. PLC was developed on Merck 60 SiF_{254} plates.

To a solution of 1 ml of $ClSiMe_3$ in 15 ml of acetonitrile, 600 mg of NaI and 978 mg of **1** were added. The reaction mixture was maintained 24 h at room temp. After washing with 5% aqueous $NaHCO_3$, extraction with EtOAc an usual work up, the crude of the reaction was chromatographied using Hex/EtOAc mixtures. 504 mg (53%) of **2** and 406 mg (45%) of **3** were obtained.

*(±) 4′-O-Demethylyatein (**2**)*

IR ($CHCl_3$): $\tilde{\nu}$ = 3580 cm^{-1} (OH), 1780 (COO), 1640 (Ar), 1525 (Ar). – ^1H NMR ($CDCl_3$): δ = 2.4–2.7 (m, 3H), 2.8–3.0 (m, 3H), 3.7–3.9 (m, 1H), 3.83 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.13 (dd, J_1 = 5.6; J_2 = 9.2, 1H), 5.91 (s, 2H,O–CH_2–O), 6.36 (s, 1H, aromatic H), 6.36 (s, 1H, aromatic H), 6.45 (sa, 1H, aromatic H), 6.46 (br. d, *J* = 8.0, 1H, aromatic H), 6.68 (d, *J* = 8.0, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 34.8 (t, C-7′), 38.0 (t, C-7), 40.7 (d, C-8), 46.3 (d, C-8′), 56.1 (q, C-10′), 56.1 (q, C-12′), 70.9 (t, C-9), 100.8 (t, C-10), 105.9 (d, C-2′), 105.9 (d, C-6′), 108.0 (d, C-5), 108.5 (d, C-2), 121.2 (d, C-6), 128.4 (s, C-4′), 131.4 (s, C-1), 133.5 (s, C-1′), 146.1 (s, C-4), 146.9 (s, C-3′), 146.9 (s, C-5′), 147.6 (s, C-3), 178.3 (s, C-9′).– Anal. ($C_{21}H_{22}O_7$) C: calc, 65.28; found, 65.10; H: calc, 5.74; found 5.68.

*(±) 3′,4′-O-Didemethylyatein (**3**)*

Mp 64–66 °C (acetone).– IR ($CHCl_3$): $\tilde{\nu}$ = 3560 cm^{-1} (OH), 1770 (COO), 1625 (Ar), 1520 (Ar), 1510 (Ar). – ^1H NMR ($CDCl_3$): δ = 2.4–2.6 (m, 4H), 2.77 (dd, J_1 = 6.4; J_2 = 14.0, 1H), 2.87 (dd, J_1 = 2.7; J_2 = 14.0, 1H), 3.7–3.9 (m, 1H), 3.79 (s, 3H, OCH_3), 4.09 (dd, J_1 = 6.2; J_2 = 9.0, 1H), 5.90 (s, 2H, O–CH_2–O), 6.25 (1H, d, *J* = 1.0, aromatic H), 6.39 (d, *J* = 1.0, 1H, aromatic H), 6.44 (dd, J_1= 1.7; J_2 = 8.2, 1H, aromatic H), 6.44 (d, *J* = 1.7, 1H, aromatic H), 6.66 (d, *J* = 8.2, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 34.9 (t, C-7′), 38.2 (t, C-7), 41.1 (d, C-8), 46.5 (d, C-8′), 56.2 (q, C-10′), 71.3 (t, C-9), 101.0 (t, C-10), 104.3 (d, C-6′), 108.3 (d, C-5), 108.9 (d, C-2′), 109.8 (d, C-2), 121.6 (d, C-6), 129.4 (s, C-4′), 131.5 (s, C-1), 131.8 (s, C-1′), 144.1 (s, C-3′), 146.3 (s, C-4), 147.3 (s, C-3), 147.8 (s, C-5′), 179.0 (s, C-9′).– Anal. ($C_{20}H_{20}O_7$) C: calc, 64.51; found, 64.43; H: calc, 5.41; found 5.40.

To a solution of 874 mg of **1** in $CHCl_3$ (10 ml), 2.5 ml of $ISiMe_3$ were added. The reaction mixture was maintained 24 h at room temp., and then washed with 5% aqueous $NaHCO_3$. By usual work up, and by flash chromatography (CH_2Cl_2/MeOH 98:2) 498 mg (59%) of **2** and 328 mg (20%) of **4** were obtained.

*(±) 3-(1,3-Benzodioxol-5-il)-2-(4-hydroxy-3.5-dimethoxybenzyl)-4-idomethylbutanoic acid ethyl ester (**4**)*

IR ($CHCl_3$): $\tilde{\nu}$ = 3650 cm^{-1} (OH), 1750 (COO), 1630 (Ar), 1510 (Ar), 1500 (Ar).– ^1H NMR ($CDCl_3$): δ = 1.16 (t, *J* = 7.2, 3H), 2.6–2.9 (m, 6H), 3.1 (dd, J_1 = 6.5; J_2 = 10.4, 1H), 3.26 (dd, J_1 = 4.6; J_2 = 10.4, 1H), 3.87 (s, 6H, OCH_3), 4.0 (dq, 2H), 5.87 (s, 2H, O–CH_2–O), 6.29 (s, 2H, aromatics H), 6.56 (dd, J_1 = 2.1; J_2 = 8.4, 1H, aromatic H), 6.58 (d, *J* = 2.1, 1H, aromatic H), 6.68 (d, *J* = 8.4, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 10.3 (t, C-9), 14.1 (q, C-a′) 35.2 (t, C-7′), 37.0 (t, C-7), 43.9 (d, C-8), 49.8 (d, C-8′), 56.2 (q, C-10′), 56.2 (q, C-12′), 60.4 (t, C-a), 100.8 (t, C-10), 105.6 (d, C-2′), 105.6 (d, C-6′), 108.1 (d, C-5), 109.2 (d, C-2), 121.9 (d, C-6), 129.6 (s, C-4′), 132.6 (s, C-1), 133.3 (s, C-1′), 146.0 (s, C-4), 146.9 (s, C-3′), 146.9 (s, C-5′), 147.6 (s, C-3), 173.7 (s, C-9′).– Anal. ($C_{23}H_{27}IO_7$) C: calc, 50.93; found, 50.75; H: calc, 5.02; found 4.99.

*(±) 2′-Chloro-4′-O-demethylyatein (**5**)*

To a 63 mg (0.16 mmol) of **2** in dry ether (5 ml) at 0 °C, 1 equivalent of sulfuryl chloride was added. The reaction mixture was maintained at room temp. for 30 min and then washed with 5% aqueous $NaHCO_3$. After neutralization, evaporation and flash chromatography on Si gel (*n*-hexane/EtOAc 7:3) 48 mg (70%) of **5** were obtained.– IR ($CHCl_3$): $\tilde{\nu}$ = 3540 cm^{-1} (OH), 1765 (COO), 1610 (Ar), 1500 (Ar).– ^1H NMR ($CDCl_3$): δ = 2.4–2.6 (m, 2H), 2.42 (dd, J_1 = 8.0; J_2 = 12.0, 1H), 2.70 (dd, J_1 = 4.3; J_2 = 12.0, 1H), 3.01 (dd,

J_1 = 7.7, J_2 = 14.0, 1H), 3.23 (dd, J_1 = 5.0, J_2 = 14.0, 1H), 3.83 (s, 3H, OCH_3), 3.85 (dd J_1 = 4.5; J_2 = 9.2, 1H), 3.89 (s, 3H, OCH_3), 4.18 (dd, J_1 = 6.8; J_2 = 9.2, 1H), 5.92 (s, 2H, O–CH_2–O), 6.39 (d, J = 8.4, 1H, aromatic H), 6.39 (d, J = 1.7, 1H, aromatic H), 6.60 (s, 1H, aromatic H), 6.62 (dd, J_1 = 1.7; J_2 = 8.4, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 32.4 (t, C-7′), 38.5 (t, C-7), 41.7 (d, C-8), 45.9 (d, C-8′), 58.4 (q, C-12′), 60.8 (q, C-10′), 71.4 (t, C-9), 101.1 (t, C-10), 108.3 (d, C-5), 108.8 (d, C-2), 108.9 (d, C-2′), 121.8 (d, C-6), 127.0 (d, C-6′), 131.7 (s, C-1), 136.8 (s, C-4′), 138.9 (s, C-1′), 145.0 (s, C-5′), 146.5 (s, C-4), 146.5 (s, C-3′), 148.1 (s, C-3), 178.4 (s, C-9′).– Anal. ($C_{21}H_{21}ClO_7$) C: calc, 59.93; found, 59.90; H: calc, 5.03; found 5.02.

*(±) 2′,6′-Dichlo-4′-O-demethylyatein (**6**)*

By using the same method described previously, from 59 mg (0,15 mmol) of **2** and 2 equivalent of sulfuryl chloride, 30 mg (47%) of **5** and 28 mg (41%) of **6** were obtained.– IR ($CHCl_3$): $\tilde{\nu}$ = 3520 cm^{-1} (OH), 1780 (COO), 1610 (Ar), 1500 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 2.3–2.5 (m, 4H), 3.17 (dd, J_1 = 10.6; J_2 = 14.0, 1H), 3.30 (dd, J_1 = 4.6; J_2 = 14.0, 1H), 3.9 (m, 1H) , 3.92 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.29 (dd, J_1 = 7.0; J_2 = 9.1, 1H), 5.90 (s, 2H, O–CH_2–O), 6.37 (sa, 1H, aromatic H), 6.39 (da, J = 8.4, 1H), 6.63 (d, J = 8.4, 1H).– Anal. ($C_{21}H_{20}Cl_2O_7$) C: calc, 55.40; found, 55.40; H: calc, 4.43; found 4.42.

*(±) 2′,6,6′-Trichlo-4′-O-demethylyatein (**7**)*

By the same method described previously, from 124 mg (0,32 mmol) of **2** and 4 equivalent of sulfuryl chloride, 100 mg (64%) of **7** were obtained. Mp 142–144 °C (Hex/CH_2Cl_2).– IR ($CHCl_3$): $\tilde{\nu}$ = 3520 cm^{-1} (OH), 1775 (COO), 1610 (Ar), 1510 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 2.47 (dd, J_1 = 8.5; J_2 = 13.6, 1H), 2.59 (dd, J_1 = 7.1; J_2 = 13.6, 1H), 2.7–2.9 (m, 1H), 2.71 (ddd, J_1 = 5.1; J_2 = 5.7; J_3 = 10.8, 1H), 3.16 (dd, J_1 = 10.8; J_2 = 13.9, 1H), 3.34 (dd, J_1 = 5.1; J_2 = 13.9, 1H), 3.91 (s, 6H, OCH_3), 4.02 (dd, J_1=5.5; J_2=9.5, 1H), 4.38 (dd, J_1 = 7.0; J_2 = 9.5, 1H), 5.95 (s, 2H, O–CH_2–O), 6.40 (s, 1H, aromatic H), 6.68 (s, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 31.1 (d, C-8), 36.6 (t, C-7′), 40.3 (t, C-7), 43.4 (d, C-8′), 61.0 (q, C-10′), 61.0 (q, C-12′), 71.3 (t, C-9), 101.9 (t, C-10), 109.8 (d, C-2), 109.9 (d, C-5), 124.2 (s, C-1), 125.2 (s, C-1′), 125.6 (s, C-6), 128.4 (s, C-4′), 143.0 (s, C-2′), 143.0 (s, C-6′), 143.3 (s, C-3′), 143.3 (s, C-5′), 146.8 (s, C-3), 146.9 (s, C-4), 178.2 (s, C-9′).– Anal. ($C_{21}H_{19}Cl_3O_7$) C: calc, 51.50; found, 51.51; H: calc, 3.91; found 3.90.

*(±) 2′-Chloroyatein (**8**)*

To a 98 mg (0.25 mmol) of **1** in dry ether (5 ml) at 0°C, 1 equivalent of sulfuryl chloride was added. The reaction mixture was maintained at room temp. for 30 min and then washed with 5% aqueous $NaHCO_3$. After neutralization, evaporation and flash chromatography on Si gel (*n*-hexane/EtOAc 6:4) 57 mg of **8** (48%) were obtained.– IR ($CHCl_3$): $\tilde{\nu}$ = 1785 cm^{-1} (COO), 1600 (Ar), 1520 (Ar), 1500 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 2.3–2.8 (m, 4H), 3.05 (dd, J_1 = 7.9; J_2 = 13.8, 1H), 3.30 (dd, J_1 = 5.2; J_2 = 13.5, 1H), 3.84 (s, 3H, OCH_3), 3.90 (m, 1H), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.20 (dd, J_1 = 6.6, J_2 =9.1, 1H), 5.90 (s, 2H, O–CH_2–O), 6.42 (s, 1H, aromatic H), 6.43 (d, J = 6.6, 1H, aromatic H), 6.63 (s, 1H, aromatic H), 6.64 (d, J = 6.6, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 32.7 (t, C-7′), 38.3 (t, C-7), 41.9 (d, C-8), 45.5 (d, C-8′), 56.1 (q, C-12′) 61.1 (q, C-10′), 61.1 (q, C-11′), 71.4 (t, C-9), 101.1 (t, C-10), 108.2 (d, C-5), 108.7 (d, C-2), 109.7 (d, C-6′), 120.1 (s, C-2′), 121.5 (s, C-6), 131.4 (s, C-1), 131.7 (s, C-1′), 142.4 (s, C-4′), 146.4 (s, C-4), 147.9 (s, C-3), 150.0 (s, C-3′), 152.2 (s, C-5′), 178.4 (s, C-9′).– Anal. ($C_{22}H_{23}ClO_7$) C: calc, 60.76; found, 60.70; H: calc, 5.35; found 5.34.

To a 635 mg (1.59 mmol) of **1** in dry ether (15 ml) at 0 °C, 2.5 equivalents of sulfuryl chloride were added. The reaction mixture was maintained at room temp. for 30 min and then washed with 5% aqueous $NaHCO_3$. After neutralization, evaporation and flash chromatography on Si gel (*n*-hexane/EtOAc 7:3) 420 mg of **8** (61%), 30 mg of **9** (4%) and 44 mg of **10** (4%) were obtained.

*(±) 2′,6′-Dichloroyatein (**9**)*

By using the method previously described, from 144 mg (0.33 mmol) of **8**, and 1 equivalent of $SOCl_2$, 101 mg of **9** (65%) were obtained.– Mp 110–112 °C (hex/CH_2Cl_2).– IR ($CHCl_3$): J = 1775 cm^{-1} (COO), 1510 (Ar), 1500 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 2.3–2.5 (m, 2H), 2.82 (dd, J_1 = 7.0; J_2 = 12.0, 1H), 3.21 (dd, J_1 = 11.2; J_2 = 13.7, 1H), 3.21 (dd, J_1 = 6.9; J_2 = 12.0, 1H), 3.42 (dd, J_1 = 4.7; J_2 = 13.7, 1H), 3.89 (s, 6H, OCH_3), 3.90 (m, 1H), 3.94 (s, 3H, OCH_3), 4.30 (dd, J_1 = 7.1; J_2 = 8.9, 1H), 5.89 (s, 2H, O–CH_2–O), 6.42 (d, J = 1.9, 1H, aromatic H), 6.45 (dd, J_1 = 1.9; J_2 = 8.3, 1H, aromatic H), 6.63 (d, J = 8.3, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 31.6 (t, C-7′), 38.5 (t, C-7), 42.6 (d, C-8), 43.2 (d, C-8′), 61.1 (q, C-10′), 61.1 (q, C-12′), 61.3 (q, C-11′), 71.2 (t, C-9), 101.0 (t, C-10), 108.3 (d, C-5), 108.6 (d, C-2), 121.5 (d, C-6), 124.6 (s, C-2′), 124.6 (s, C-6′), 130.2 (s, C-1′), 131.5 (s, C-1), 146.3 (s, C-4), 147.0 (s, C-4′), 147.9 (s, C-3), 149.3 (s, C-3′), 149.3 (s, C-5′), 177.8 (s, C-9′).– Anal. ($C_{22}H_{22}Cl_2O_7$) C: calc, 56.30; found, 56.20; H: calc, 4.72; found 4.68.

*(±) 2-Chloro-5-Metoxy-retrojusticidin B (**10**)*

Mp 199–202 °C (CH_2Cl_2/EtOAc).– IR ($CHCl_3$): $\tilde{\nu}$ = 3040 cm^{-1} (OH), 1760 (COO), 1490 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 3.38 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 5.05 (d, J = 16.1, 1H), 5.10 (d, J = 16.1, 1H), 6.08 (s, 2H, O–CH_2–O), 6.72 (d, J = 1.7, 1H, aromatic H), 6.72 (d, J = 7.8, 1H, aromatic H), 6.72(dd, J_1 = 1.7; J_2 = 7.8, 1H, aromatic H), 8.9 (s, 1H, aromatic H).– Anal. ($C_{22}H_{17}ClO_7$) C: calc, 61.62; found, 61.44; H: calc, 4.00; found 3.78.

*(±) 2′-Iodoyatein (**11**)*

To 151 mg (0.38 mmol) of **1** in 5 ml of dry benzene at 0 °C, 116 mg of I_2 and 148 mg of HgO were successively added. The mixture was maintained 72 h at room temp. and then, solids were eliminated by filtration. An usual work up, the crude of the reaction was chromatographied using Hex/AcOEt mixtures. 105 mg (66%) of **11** were obtained.– IR ($CHCl_3$): $\tilde{\nu}$ = 1775 cm^{-1} (COO), 1600 (Ar), 1570 (Ar), 1510 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 2.4–2.8 (m, 4H), 3.06 (dd, J_1 = 8.4; J_2 = 13.9, 4H), 3.32 (dd, J_1 = 5.1; J_2 = 13.9, 1H), 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.0 (m, 1H), 4.25 (dd, J_1 = 6.4; J_2 = 9.5, 1H), 5.90 (s, 2H, O–CH_2–O), 6.40 (d, J = 1.7, 1H, aromatic H), 6.43 (dd, J_1 = 1.7; J_2 = 7.9, 1H, aromatic H), 6.64 (d, J = 7.9, 1H, aromatic H), 6.66 (s, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 38.5 (t, C-7), 39.9 (t, C-7′), 41.9 (d, C-8), 45.7 (d, C-8′), 56.1 (q, C-12′), 60.7 (q, C-10′), 60.9 (q, C-11′), 71.4 (t, C-9), 101.0 (t, C-10), 108.2 (d, C-2), 108.6 (d, C-5), 109.8 (d, C-6′), 121.5 (d, C-6), 131.6 (s, C-1), 136.5 (s, C-1′), 141.2 (s, C-4′), 146.3 (s, C-2′), 147.8 (s, C-3), 151.8 (s, C-4), 153.8 (s, C-3′), 153.8 (s, C-5′), 178.2 (s, C-9′).– Anal. ($C_{22}H_{23}IO_7$) C: calc, 50.21; found, 50.08; H: calc, 4.40; found 4.38.

*(±) Isodesoxipodophyllotoxin (**12**)*

To a 100 mg (0.25 mmol) of **1** in $CHCl_3$ (10 ml) at 0°C, 1 ml of nitric acid was added. The reaction mixture was maintained at room temp. for 4 days. After neutralization, 81 mg of **12** were obtained.– Mp 194–196 °C (Hex/CH_2Cl_2). – IR ($CHCl_3$): $\tilde{\nu}$ = 1785 cm^{-1} (COO), 1600 (Ar), 1510 (Ar).– ^{1}H NMR ($CDCl_3$): δ = 2.4–2.8 (m, 2H), 2.91 (dd, J_1 = 11.0; J_2 = 15.2, 1H), 2.97 (dd, J_1 = 4.9; J_2 = 15.2, 1H), 3.82 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.00 (dd, J_1 = 8.6; J_2 = 10.4, 1H), 4.05 (d, J = 10.7, 1H), 4.52 (dd, J_1 = 6.4; J_2 = 8.6, 1H), 5.89 (s, 2H, O–CH_2–O), 6.34 (s, 1H, aromatic H), 6.41 (s, 2H, aromatic H), 6.60 (s, 1H, aromatic H).– ^{13}C-NMR ($CDCl_3$): δ = 33.0 (t, C-7), 40.2 (d, C-8), 46.8 (d, C-8′), 48.8 (t, C-7′), 56.3 (q, C-10′), 56.3 (q, C-12′), 60.9 (q, C-11′), 70.9 (t, C-9), 101.1 (t, C-10), 107.0 (d, C-2′), 107.0 (d, C-6′), 108.5 (d, C-2), 110.0 (d, C-5), 127.9 (s, C-1′), 132.4 (s, C-1), 136.2 (s, C-6), 138.8 (s, C-4′), 146.6 (s, C-4), 146.8 (s, C-3), 153.3 (s, C-3′), 153.3 (s, C-5′), 175.4 (s, C-9′).– Anal. ($C_{22}H_{22}O_7$) C: calc, 66.32; found, 66.25; H: calc, 5.57; found 5.56.

Biological assays

A simple screening procedure has been used to determine the possible antineoplastic and/or antiviral activity of these compounds. The antitumoral cell lines employed have been P-388 (lymphoid neoplasma from DBA/2 mouse), A-549 (human lung carcinoma), HT-29 (human colon carcinoma) and MCF-7 (breast human carcinoma).

Cell culture multidishes 24 wells, 16 mm diameter, NUNC 42001 and cell culture flasks 80 cm^2 NUNC 44004, were used. Eagle's minimum essential medium (SEROMED T 437-10) with Eagle's Balanced Salts, without sodium bicarbonate; suplemented with 10% fetal calf serum, 10^{-2} M sodium bicarbonate and 0.1 g/l Penicillin-G and Streptomycin sulfate was employed.

Antitumoral assays[15]

P-388 cells (suspension culture) were seeded into 16 mm wells at 1×10^4 cells/well in 1 ml aliquot of medium MEM 10FCS containing the concentration of sample indicate in table 1. The remaining cell lines: A-549, HT-29 and MCF-7 (monolayer cultures) were seeded into 16 mm wells at 2×10^4 cells/well in 1 ml aliquots of MEM 10FCS. The day after the inoculum, media were replaced by 1 ml aliquots of MEM 10FCS containing the different concentrations of sample. In both cases a separate set of cultures without sample was counted daily to ensure that the cells remained in exponential growth. Cells were incubated at 37 °C in a 10% CO_2 humid atmosphere. All determinations were carried out in duplicate. After three days of incubation, cells were counted and the IC_{50} for each sample was determined.

Antiviral assays.[16]

HSV-1: CV-1 cells were seeded in 16 mm diameter wells at 2×10^5 in 1 ml aliquots of MEM 10FCS. The day after, cells were infected with HSV-1 at 100 PFU/well in 200 µl aliquots MEM 5FCS. After adsorption for 1.5 h, the inoculum was replaced with 0.5 ml aliquots of MEM 5FCS with 4% methylcellulose 15 cps. Samples were dropped in 6 mm paper disk and distributed in the wells. After 2 days, cells were stained with neutral red and 24 h later plates were washed, dried and number of plaques production was observed.

VSV: BHK cells were seeded in 16 mm diameter wells at 1.5×10^5 in 1ml aliquots of MEM 10FCS. The day after, cells were infected with VSV at 100 PFU/well in 200 µ l aliquots MEM. After adsorption for 1.5 h, the inoculum was replaced with 0.5 aliquots of MEM with 4% methylcellulose 15 cps. Samples were dropped in 6 mm paper disk and distributed in the wells. After 24 h cells were stained with violet crystal. Plates were washed, dried and number of plaques production was observed.

References

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

[2] M.G. Saulnier, K.L. LeBoulluec, D.P.P. McGee, B.H. Long, A.R. Crosswell, D.M. Vyas, *Bioorg. Med. Chem. Lett.* **1993,** *3*, 1619–1624.

[3] H.C. Schröder, H. Herz, R. Steffen, W.E.G. Müller, P.S. Sarim, S. Trumme, J. Schulz, E. Eich, *Naturforsch.* **90,** *45*, 1215–1221.

[4] A. San Feliciano, J.L. López, M. Medarde, J.M. del Corral, B. Pascual-Teresa, P. Puebla, *Phytochemistry* **1989,** *28*, 2863–2866.

[5] M. Medarde, R. Peláez-Lamamié de Clairac, D.G. Grávalos, F. Tome, J.L. López, A. San Felicianos, *Arch. Pharm. (Weinheim)* **1995**, *328*, 403–407.

[6] M. Medarde, R. Peláez-Lamamié de Clairac, F. Tome, J.L. López, A. San Feliciano, *Arch. Pharm.(Weinheim)* **1994,** *327*, 91–94.

[7] A. San Feliciano, M. Medarde, R. Peláez-Lamamié de Clairac, J.L. López, P. Puebla, *Studia Chemica* **1991,** *16*, 53–61.

[8] M. Medarde, R. Peláez-Lamamié de Clairac, F. Tome, J.L. López, A. San Feliciano, *Arch. Pharm.(Weinheim)* **1993,** *326*, 421–426.

[9] K.H. Lee, S.A. Beers, M. Mori, Z.Q. Wang, L. Li, S.Y. Liu, J.Y. Chang, F.S. Han and Y.C. Cheng, *J. Med. Chem.* **1990,** *33*, 1364–1375.

[10] M.J. Chapdelaine, M. Hulce, *Org. React.* **1992,** *38*, 225–654.

[11] G.R. Pettit, E.E. van Tamelen, *Org. React.* **1962,** *12*, 356–361.

[12] W. Minnis, *Org. Synt. Coll.* **1943,** *2*, 357.

[13] L.F.Y. Fieser and M. Fieser, *Reagents for Organic Synthesis* Vol. 1, J. Wiley & Sons, Inc., New York, **1967**, p. 733.

[14] E. Brown, A. Daugan, *Tetrahedron* **1989** *45*, 141–154.

[15] R.J. Bergeron, P.F. Cavanaugh Jr., S.J. Kline, R.G. Hughes Jr., G.T. Elliott, C.W. Porter, *Biochem. Bioph. Res. Commun.***1984,** *3*, 121.

[16] A.C. Schroeder, R.C. Hughes Jr., A. Bloch, *J. Med. Chem.* **1981,** *24*, 1078–1083.

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