Synthesis and Biological Activity of Bromolignans and Cyclolignans

Arturo San Feliciano[a], Manuel Medarde[a]*, Rafael Pelaez Lamamie de Clairac[a], Jose Luis López[a], Pilar Puebla[a], Mª Dolores García Gravalos[b], Pilar Ruiz Lázaro[b], Mª Teresa García de Quesada[b]

[a] Department of Organic Chemistry, University of Salamanca, Faculty of Pharmacy, Avda. Campo Charro s/n. 37007, Salamanca, Spain
[b] Pharma-Mar, c/ de la Calera nº 8, 28780, Tres Cantos (Madrid), Spain

Received July 8, 1992

Nine lignan derivatives (**4-12**) have been obtained from (-)-yatein by treatment with DDQ and NBS. They showed moderate antineoplastic activity (P-388, A-549, HT-29) compared with podophyllotoxin, but some of them have a better therapeutic index. None of the tested compounds shows antiviral (HSV-1, VSV) or enzyme inhibitor (ADA, DHFR, GST) activities.

Synthese und biologische Aktivität von Bromlignanen und cyclischen Lignanen

Neun Lignanverbindungen (**4-12**) wurden aus (-)-Yatein durch Umsetzung mit DDQ und NBS hergestellt. Diese Verbindungen zeigten im Vergleich mit Podophyllotoxin eine verminderte antineoplatische Wirksamkeit (P-388, A-549, HT-29), aber einige haben einen besseren therapeutischen Index. Keine der geprüften Verbindungen zeigte antivirale (HSV-1, VSV) oder Enzym-hemmende (ADA, DHFR, GST) Wirkung.

(-)-Yatein (**1**) is a lignan of the dibenzylbutyrolactone type, that has been isolated as main component of a hexane insoluble fraction obtained from an extract of *Juniperus thurifera*[1]. This compound has the aromatic rings with the same substitution pattern as podophyllotoxin (**2**), stegane (**3**) and other lignans[2], which are well known by their activities and clinical applications[3]. It has also been demonstrated that (-)-yatein is a biosynthetic precursor of deoxypodophyllotoxin and podophyllotoxin. Thus, (-)-yatein could find application as starting material in the preparation of more interesting lignans. Examples of these transformations have been described[4]: so **1** yields (+)-isostegane by non phenolic oxidation with VOF_3 or with TTFA, and 7'-benzylic derivatives of yatein produce cyclolignans.

As part of our work searching for new active compounds related to natural products, we have studied the reaction of (-)-yatein with DDQ and with NBS[5], reagents used for benzylic functionalization. The products obtained from these treatments have been tested for their activities as antineoplastics, antivirals and enzyme inhibitors, which are well known for many other lignans.

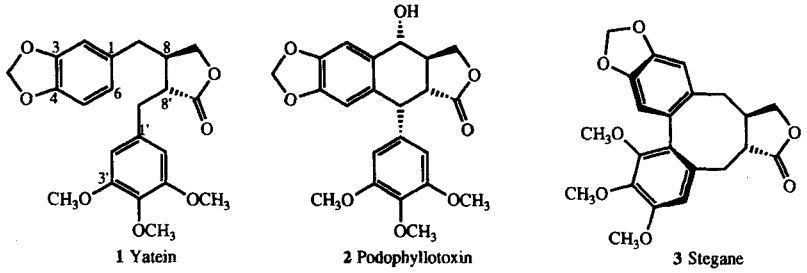
Chemistry

The reaction of **1** with DDQ was conducted in refluxing dioxane for 70 h using a 1/2 molar ratio (Scheme 2). The products obtained were identified as dehydroanhydropodophyllotoxin (**4**)[6], retrodeoxypodophyllotoxin (**5**), and 5-methoxyretrojusticidin B (**6**). The structure of **5** was deduced from its spectroscopic properties, which agree with a *trans-trans* disposition of benzyl and lactonic groups according to the coupling constants of H-7', H-8 and H-8'[7]. Its properties are identical to those described for a cyclization product of 7-hydroxy-yatein[8], although its NMR properties have not been fully described.

The previously unknown (-)-5-methoxyretrojusticidin B (**6**) displays a shielded methoxy group at C-5 and very deshielded H-7 and H-9' protons[2,7], enough to unequivocally identify this compound. **6** was also obtained after treatment of **5** with DDQ.

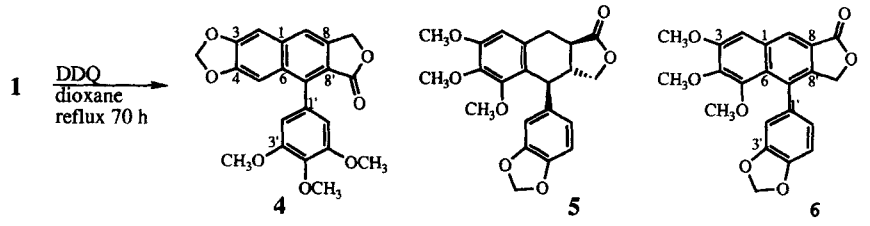
The results of DDQ treatment of (-)-yatein agree with the initial formation of benzylic cations or DDQ-lignan intermediates[9] which undergo cyclization to products with *trans-trans* stereochemistry[4,10]. The excess of DDQ dehydrogenates these compounds to the naphthalenelactones obtained as main reaction products. The reaction at both benzylic positions is very similar as the stabilization of the benzylic cations is produced by one *p*-oxygen in both cases.

Bromination reactions were conducted as described[11], with *N*-bromosuccinimide/benzoyl peroxide and cupric bromide/*tert*-butyl hydroperoxide (Scheme 3). Compounds 7-

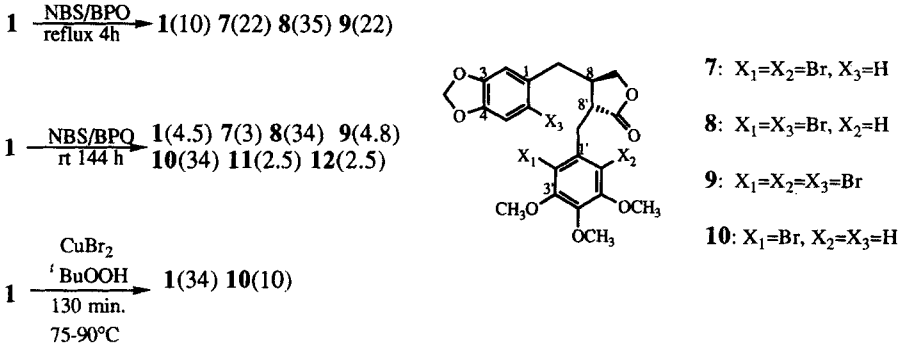


Scheme 1

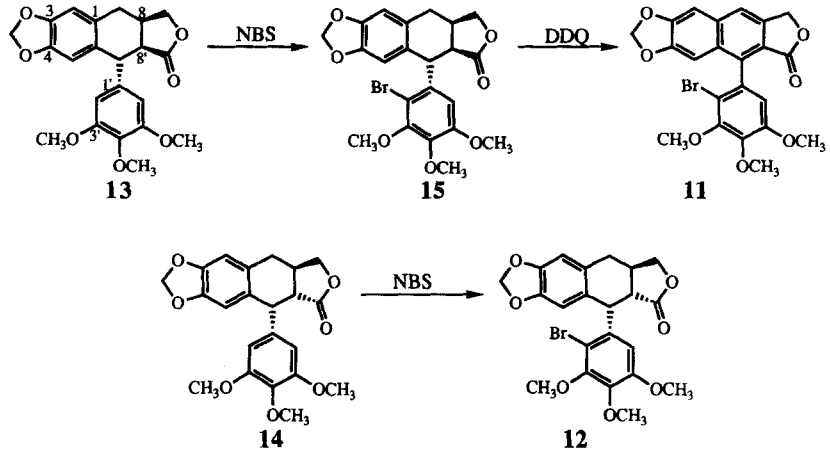
  0365-6233/93/0707-0421 $ 5.00 + .25/0



Scheme 2: DDQ treatment of (-)yatein



Scheme 3: NBS brominations of (-)yatein (**1**)



Scheme 4: Semisynthesis of **11** and **12**

10 are easily produced because both rings are activated by the substituents. **9** has been described as product of yatein bromination with $KBrO_3/KBr$[12].

Minor products **11** and **12**[13] were obtained when the reaction mixture was allowed to stand for several days. Their structures were confirmed through chemical correlation with deoxypicropodophyllin (**13**) and deoxypodophyllotoxin (**14**) (Scheme 4).

The benzylic bromination of yatein is not easily produced in the conditions that are well known to work on other compounds (as we assayed with xylene) making the aromatically substituted compounds the main products of the reaction. As in our case, aromatic brominations and/or cyclizations followed by dehydrogenations are observed in NBS treatment of other lignans[9,13,14].

As benzylic bromination is produced through an intermediate radical, an orthogonal benzylic hydrogen is needed to allow a constant overlap between the π-system and the developing radical, in order to produce stabilization of the transition state. The low yield of products derived from the reaction on the benzylic positions (only 11 and 12) can be explained in terms of a preferred conformation for yatein without any orthogonal hydrogen in benzylic positions, as it has been proposed for other lignans[15].

In order to check this explanation we have carried out the theoretical study of yatein conformations. Yatein molecule was built up, minimized and systematically analyzed. The global minimum, as well as every local minima (up to 10 Kcal/mol over the global minimum), showed an almost parallel disposition of both aromatic rings.

The global minimum was optimized by means of semiempirical method MNDO, resulting the geometry shown in figure 1. The aromatic rings are quasi-parallel and the distance between their centers is 4.3 Å (Figure 1). It is clear that this preferred conformation is controlled by π-stacking interactions between both aromatic rings and the alternated disposition of substituents in rotatable bonds C7-C8 and C7'-C8'. In this disposition, the benzylic hydrogens are forced to be far away from orthogonality with the aromatic rings (Table 1).

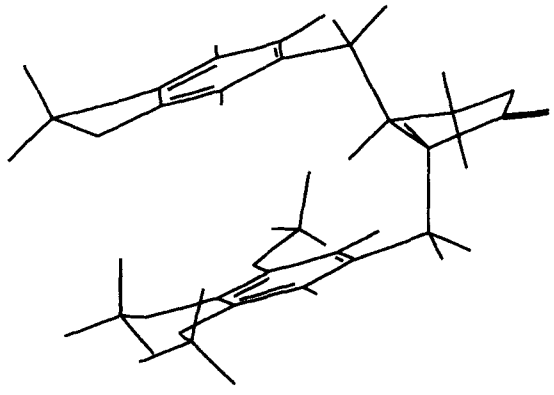


Fig. 1: Optimized geometry of yatein, showing the quasi-parallel disposition of both aromatic rings and the absence of orthogonality of benzylic hydrogens.

Table 1: Torsion angles between benzylic hydrogens and aromatic rings deduced from MNDO optimization of yatein geometry.

Torsion angle	degrees
C_2-C_1-C7-H7a	-18.56
C_6-C_1-C7-H7b	45.08
$C_{2'}$-$C_{1'}$-C7'-H7'a	-20.73
$C_{6'}$-$C_{1'}$-C7'-H7'b	42.23

Biological assays

Compounds **4-10** and **12** were assayed as antineoplastics on: P-388 (lymphoid neoplasma from DBA/2 mouse), A-549 (human lung carcinoma) and HT-29 (human colon carcinoma) cells; as antivirals on: HSV-1 (herpex simplex virus, type 1) and VSV (vesicular stomatitis virus); and as enzyme inhibitors of: ADA (adenosine deaminase), DHFR (dihydrofolate reductase) and GST (glutation-*S*-transferase). The antineoplastic results, as well as their activities, are shown in table 2. All the compounds failed to inhibit the tested enzymes.

None of these compounds showed antiviral activity. Those with high toxicity, **7** and **12**, were checked again at lower concentrations. Yatein (**1**) itself has a very low antiviral activity[3], thus it was expected that compounds **7-10** had also low activity. But other cyclolignans and arylnaphthalides related with compounds **4-6** and **11-12** are antivirals[16], and a possible activity could have been found for these products. As the mechanism of antiviral activity of lignans is not fully understood[3,16] further studies are needed in order to obtain a better knowledge and more active compounds.

Table 2: Antineoplastic activities (on P-388, A-549, HT-29) and cytotoxicities (on CV-1, BHK) of lignans **4-10**, **12**, podophyllotoxin (**2**) and deoxypodophyllotoxin (**14**)

	IC_{50} (μg / mL)				
Compound	**P-388**	**A-549**	**HT-29**	**CV-1**	**BHK**
2	0.02	0.02	0.02	0.025	-
4	2.1	3.6	3.5	4.5	6.1
5	>10	>10	>20	-	-
6	>20	>20	>20	-	-
7	0.6	1.3	2.3	1.9	2.6
8	5.1	5.0	3.5	4.5	4.3
9	>20	>20	>20	-	-
10	3.7	6.1	4.1	6.7	>10
12	0.4	0.7	0.9	0.5	1.3
14	$2.5\text{-}5\ 10^{-3}$	$<2.5\ 10^{-3}$	$2.5\ 10^{-3}$	$2.5\ 10^{-3}$	-

From the antineoplastic studies it can be deduced that the more cytotoxic compounds, **7** and **12**, are the more active ones. As a more interesting result, compound **10** has the higher ratio activity/toxicity, which could be important in a possible therapeutic application. Although these compounds are antineoplastics, their activity is far from that displayed by podophylltoxin (10-20 ng/ml) and deoxypodophyllotoxin (2.5-5 ng/ml), which have been taken as references in these assays.

We wish to thank Spanish D.G.I.C.Y.T. (PB89-0394) for financial support and Dr. *M. A. Castro* for a sample of **14**. One of us (R.P.L.C.) thanks spanish M.E.C. for a grant.

Experimental Part

Melting points: Büchi 510 apparatus, uncorrected.- Optical rotations: Perkin-Elmer 241 polarimeter, 1-dm cell, $CHCl_3$ solution (λ are given in nm). UV-spectra: Hitachi 100-60 spectrophotometer, 1-cm cells, EtOH as solvent (λ max in nm, ε in $M^{-1} \cdot cm^{-1}$).- IR-spectra: Beckman Acculab 8 spectrophotometer, films, unless otherwise stated (ν_{max} in cm^{-1}).- NMR spectra: Bruker WP 200 SY (200 MHz for ^1H and 50.3 MHz for ^{13}C) in $CDCl_3$, unless otherwise stated. Chemical shifts (δ) in ppm, referred to internal TMS, coupling constants (J) in Hz.- Mass spectra (EI): VG-TS-250; ionization energy 70 eV.- Column chromatography: silica gel Merck 60 (0.063-0.2 mm). Flash chromatography: Eyela EF-10 apparatus, 3-85 mL/min flow rate, silica gel Merck 60 (0.040-0.063 mm).- TLC: precoated silica gel polyesther plates (0.25 mm thickness), fluorescent indicator UV254 (Polychrom SI F_{254}). 10% Phosphomolybdic acid in EtOH or 10% H_2SO_4 in EtOH were used for visualization, after heating at 110°C.

Oxidations

1.03 g (2.57 mmol) of **1** and 1.12 g (4.95 mmol) of DDQ were dissolved in dioxane (22.5 mL). The stirred solution was refluxed for 70 h. The crude product (1.35 g) was filtered, the solution evaporated and chromatographed (Flash, CH_2Cl_2/MeOH mixtures) to yield: **6** (23 mg), **5** (220 mg), **1** (51 mg) and **4** (200 mg).

*5-Methoxy-retrojusticidin B (**6**)*

M.p. 194-196°C (CH_2Cl_2/EtOAc).- IR: 3040; 1760; 1490; 1260; 1225; 1100; 950; 930.- UV: λ max (ε): 204 (6575), 252 (4309).- ^{1}H-NMR: 3.39 (3H, s), 3.94 (3H, s), 40.6 (3H, s), 5.03 (1H, d, J = 15.2), 5.13 (1H, d, J = 15.2), 6.04 (2H, s), 6.63 (1H, dd, J_1 = 1.7, J_2 = 7.8), 6.77 (1H, d, J = 1.7), 6.89 (1H, d, J = 7.8), 7.17 (1H, s), 8.30 (1H, s).- ^{13}C-NMR: 55.9, 60.8, 61.1, 69.8, 101.1, 104.7, 107.8, 108.9, 120.8, 122.5, 124.9, 131.8, 132.2, 132.9, 133.3, 139.2, 145.1, 146.7, 147.3, 149.7, 153.5, 187.2. MS (m/z): $M^{+\cdot}$ = 394 (48%).

*Retrodeoxypodophyllotoxin (**5**)*

M.p. 236-238°C (CH_2Cl_2/EtOAc).- IR: 2960; 1780; 1600; 1570; 1490; 1340; 1120; 1050; 990.- UV: λ max (ε): 215 (16488).- $[\alpha]^{20}$ (λ): -68.6° (578); -77.8° (546); 136° (436); -66° (589) [c = 0.5%].- ^{1}H-NMR: 2.37 (1H, dddd, J_1 = 6.4, J_2 = 10.2, J_3 = 10.3, J_4 = 13.7), 2.43 (1H, ddd, J_1 = 4.2, J_2 = 10.3, J_3 = 13.7), 2.92 (1H, dd, J_1 = 10.3, J_2 = 15.7), 3.05 (1H, dd, J_1 = 4.2, J_2 = 15.7), 3.20 (3H, s), 3.74 (3H, s), 3.87 (3H, s), 4.00 (1H, d, J = 10.2), 4.05 (1H, dd, J_1 = 8.5, J_2 = 10.3), 4.30 (1H, dd, J_1 = 6.4, J_2 = 8.5), 5.92 (2H, s), 6.52 (1H, s), 6.54 (1H, d, J_1 = 1.7), 6.55 (1H, dd, J_1 = 1.7, J_2 = 7.8), 6.73 (1H, d, J = 7.8).- ^{13}C-NMR: 30.4, 41.2, 46.5, 49.7, 55.9, 59.4, 60.4, 71.9, 100.9, 107.0, 107.5, 107.8, 108.3, 119.5, 125.0, 131.7, 140.0, 145.8, 147.8, 151.5, 152.8, 176.3. MS (m/z): $M^{+\cdot}$ = 398 (18%).

*Anhydrodehydropodophyllotoxin (**4**)*

M.p. 266-268°C (CH_2Cl_2/EtOAc).- IR: 2930; 1760; 1580; 1500; 1460; 1360; 1350; 1150; 1125; 1040; 940; 900.- UV: λ max (ε): 279 (5220), 232 (2660).- ^{1}H-NMR: 3.84 (3H, s), 3.84 (3H, s), 3.96 (3H, s), 5.37 (2H, s), 6.08 (2H, s), 6.55 (2H, s), 7.11 (1H, s), 7.20 (1H, s), 7.71 (1H, s).- ^{13}C-NMR: 56.3, 56.3, 61.0, 68.0, 101.7, 103.7, 103.8, 107.7, 107.7, 119.1, 130.4, 132.7, 134.7, 138.3, 139.1, 139.9, 140.4, 148.8, 150.1, 153.1, 153.1, 169.6. MS (m/z): $M^{+\cdot}$ = 394 (37%).

Brominations with NBS and benzoyl peroxide BPO (reflux)

516 mg (2.90 mmol) of NBS and 20 mg of benzoyl peroxide were added to a solution of 558 mg (1.40 mmol) of **1** in 6 mL of CCl_4. The reaction mixture was refluxed under N_2 for 2 h 15 min. The precipitate was filtered off and the solution evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2, washed with a saturated solution of $Na_2S_2O_8$, dried (Na_2SO_4), and evaporated, to yield 638 mg of crude product which was chromatographed several times (hexane/EtOAc and/or CH_2Cl_2/MeOH as eluents) to give pure **7** (20 mg), **8** (14.3 mg), **9** (19 mg). Mixtures of **7** and **9** were separated by crystallization of **9** in hexane/CH_2Cl_2.

*2',6'-Dibromoyatein (**7**)*

IR: 3000; 2780; 1765; 1500; 1460; 1240; 1030; 920; 750.- UV: λ max (ε): 230 (27976), 188 (11453).- $[\alpha]^{20}$ (λ): -26° (578); -31° (546); -24° (589) [c = 1.4%].- ^{1}H-NMR: 2.34 (2H, m), 2.80 (1H, dd, J_1 = 8.0, J_2 = 15), 2.90 (1H, ddd, J_1 = 5.0, J_2 = 7.1, J_3 = 10.5), 3.35 (1H, dd, J_1 = 10.5, J_2 = 14.0), 3.53 (1H, dd, J_1 = 5.0, J_2 = 14.0), 3.89 (6H, s), 3.93 (3H, s), 3.90-4.00 (1H, m), 4.30 (1H, dd, J_1 = 7.1, J_2 = 9.2), 5.89 (2H, s), 6.39 (1H, d, J = 1.7), 6.39 (1H, d, J = 8.4), 6.62 (1H, dd, J_1 = 1.7, J_2 = 8.4).- ^{13}C-NMR: 36.7, 38.5, 42.2, 43.3, 60.9, 60.9, 61.2, 71.2, 101.0, 108.3, 108.7, 115.6, 115.6, 121.5, 131.8, 132.9, 146.3, 146.8, 147.9, 150.9, 150.9, 177.8.- MS (m/z): $M^{+\cdot}$ = 556 (4%), 558 (7.5%), 560 (3.8%).

*2',6-Dibromoyatein (**8**)*

IR: 2900; 1760; 1500; 1470; 1220; 1100; 1030; 920; 720.- UV λ max (ε): 221 (27976).- $[\alpha]^{20}$ (λ): -24° (578); -28° (546); -56° (436); -23° (589) [c = 0.36%].- ^{1}H-NMR: 2.50-2.90 (4H, m), 3.01 (1H, dd, J_1 = 8.5, J_2 = 13.9), 3.20 (1H, dd, J_1 = 5.1, J_2 = 13.9), 3.80-4.40 (2H, m), 3.81 (3H, s), 3.87 (3H, s), 3.89 (3H, s), 5.96 (2H, s), 6.46 (1H, s), 6.60 (1H, s), 6.89 (1H, s).- ^{13}C-NMR: 35.8, 38.7, 40.6, 45.6, 56.2, 60.1, 60.1, 71.1, 102.0, 110.2, 110.3, 111.2, 112.9, 114.5, 130.4, 133.1, 142.4, 147.6, 147.6, 151.1, 152.9, 178.2.- MS (m/z): $M^{+\cdot}$ = 556 (4.6%), 558 (8.3%), 560 (4.2%).

*2',6,6'-Tribromoyatein (**9**)*

M.p. 180-181°C (CH_2Cl_2/Hexane).- IR: 2980; 1765; 1500; 1480; 1235; 1040; 920; 870; 730.- UV: λ max (ε): 208 (32993).- $[\alpha]^{20}$ (λ): -8° (578); -9° (546); -25° (436); -62.5° (365); -7° (589) [c = 1.03%].- ^{1}H-NMR: 2.45 (1H, dd, J_1 = 8.2, J_2 = 13.6), 2.65 (1H, dd, J_1 = 7.4, J_2 = 13.6), 2.65-2.9 (1H, m), 2.95-3.1 (1H, m), 3.20-3.60 (2H, m), 3.88 (3H, s), 3.88 (3H, s), 3.94 (3H, s), 4.06 (1H, dd, J_1 = 4.9, J_2 = 9.4), 4.43 (1H, dd, J_1 = 7.0, J_2 = 9.4), 5.96 (2H, s), 6.47 (1H, s), 6.82 (1H, s).- ^{13}C-NMR: 36.6, 39.0, 39.9, 43.3, 60.9, 60.9, 61.2, 71.2, 101.9, 110.4, 113.0, 114.4, 116.0, 116.0, 130.3, 132.6, 146.6, 147.4, 147.4, 150.9, 150.9, 177.8.- MS (m/z): $M^{+\cdot}$ = 634 (1.0%), 636 (2.7%), 638 (2.5%), 640 (0.8%).

Brominations with NBS and BPO (room temperature)

A solution of 1.03 g (2.57 mmol) of **1**, 1.6 g (8.90 mmol) of NBS and 201 mg of BPO in 30 mL of CCl_4 was stirred at room temp. for six days. The mixture was filtered and washed with a saturated solution of $Na_2S_2O_3$. Standard workup gave 2 g of crude product which was chromatographed (some different hexane/EtOAc solvent systems), giving [by crystallization (CH_2Cl_2/Hexane) of **9** and **11**]: **7** (45 mg), **8** (485 mg), **9** (76 mg), **10** (416 mg), **11** (38 mg), and **12** (50 mg).

*2'-Bromoyatein (**10**)*

IR: 3040; 2780; 1770; 1565; 1500; 1480; 1390; 1240; 940; 730.- UV: λ max (ε): 202 (20913).- $[\alpha]^{20}$ (λ): -21.9° (578); -25.3° (546); -49.9° (436); -20.7° (589) [c = 1.04%].- ^{1}H-NMR: 2.40-2.90 (4H, m), 3.05 (1H, dd, J_1 = 6.9, J_2 = 13.5), 3.30 (1H, dd, J_1 = 5.3, J_2 = 13.5), 3.81 (3H, s), 3.88 (3H, s), 3.90 (3H, s), 3.90 (1H, t, J = 6.7), 4.20 (1H, dd, J_1 = 6.7, J_2 = 9.2), 5.90 (2H, m), 6.42 (1H, d, J = 1.7), 6.45 (1H, dd, J_1 = 1.7, J_2 = 7.4), 6.62 (1H, d, J = 7.4), 6.64 (1H, s).- ^{13}C-NMR: 35.4, 38.5, 41.9, 45.7, 56.2, 61.0, 61.1, 71.4, 101.1, 108.3, 108.7, 110.2, 111.2, 121.6, 131.7, 133.2, 142.4, 146.5, 148.0, 151.1, 152.9, 178.2.- MS (m/z): $M^{+\cdot}$ = 478 (4.2%), 480 (4.1%).

*2'-Bromoanhydrodehydropodophyllotoxin (**11**)*

M.p. 252-254°C (Hexane/EtOAc).- IR (CH_2Cl_2): 3020; 1770; 1620; 1570; 1500; 1470; 1390; 1260; 1150; 1110; 1040; 940.- UV: λ max (ε): 205 (19887), 258 (21681).- ^{1}H-NMR: 3.78 (3H, s), 3.98 (3H, s), 4.02 (3H, s), 5.40 (2H, s), 6.09 (2H, s), 6.59 (1H, s), 6.84 (1H, s), 7.22 (1H, s), 7.74 (1H, s).- ^{13}C-NMR: 56.3, 61.2, 61.2, 68.2, 102.0, 103.2, 103.9, 110.1, 110.5, 119.6, 119.6, 129.9, 131.7, 134.8, 138.7, 139.9, 143.2, 149.2, 150.2, 151.4, 152.9, 169.2.- MS (m/z): $M^{+\cdot}$ = 472 (4.6%), 474 (4.3%).- Semisynthesis of **11**: A solution of 162 mg (0.041 mmol) of desoxypicropodophyl-

lin (**13**) and 70 mg of NBS (0.040 mmol) in CCl_4 was stirred and refluxed for 135 min. Standard procedure gave 191 mg of a crude mixture from which 159 mg of 2'-bromodeoxypicropodophyllin (**15**) were isolated by chromatography (CH_2Cl_2/MeOH 1:1). 34 mg (0.015 mmol) of DDQ were added to a stirred solution of 30 mg (0.063 mmol) of **15** in dioxan (8 mL) and refluxed for 70 h. Standard work up gave **11**.

*2'-Bromodeoxypodophyllotoxin (**12**)*

M.p. 170-172°C (hexane/EtOAc).- IR (CH_2Cl_2): 3040; 2780; 1780; 1620; 1580; 1560; 1500; 1480; 1390; 1240; 1110; 1040; 940; 730; 700.- UV: λ max (ε): 225 (15041), 290 (5188).- $[\alpha]^{20}$ (λ): -83.7° (578); -95.8° (546); -175.7° (436); -79.5° (589) [c = 0.9%].- ^{1}H-NMR: 2.80 (1H, m), 2.95 (1H, m), 3.14 (2H, m), 3.63 (3H, s), 3.86 (3H, s), 3.90 (3H, s), 3.92 (1H, dd, J_1 = 8.5, J_2 = 8.7), 4.50 (1H, dd, J_1 = 6.2, J_2 = 8.5), 5.27 (1H, d, J = 6.3), 5.89 (2H, s), 6.14 (1H, s), 6.38 (1H, s), 6.63 (1H, s).- ^{13}C-NMR: 32.8, 34.4, 41.5, 46.7, 56.4, 60.9, 60.9, 71.5, 101.2, 108.4, 110.2, 110.9, 114.5, 127.5, 131.2, 136.5, 142.7, 147.0, 147.0, 151.0, 152.2, 173.2.- MS (m/z): $M^{+\cdot}$ = 476 (5.0%), 478 (4.6%).- Semisynthesis of **12**: 96 mg (0.054 mmol) of NBS were added to a stirred solution of 233 mg (0.059 mmol) of deoxypodophyllotoxin (**14**). The reaction mixture was refluxed for 33 h. Standard workup afforded 341 mg of crude product which was chromatographed (hexane/EtOAc) to give 83.5 mg of **12**.

Brominations with cupric bromide and tert butylhydroperoxide (TBHP)

0.25 mg of TBHP and 133 mg (0.60 mmol) of $CuBr_2$ were added to a stirred solution of 211 mg of **1** (0.33 mmol) in Ac_2O. After 2 h reflux the mixture was poured into water, acidified with 2N HCl and extracted (EtOAc). After standard work up, the crude product (192 mg) was separated by flash chromatography (hexane/EtOAc 7:3) to give **8** (26 mg) and unreacted **1** (65 mg).

Theoretical Conformation Study

The molecular modelling and geometry optimizations were carried out on a Macintosh IIcx. The molecule of yateine (**1**) was built up from standard fragments of Nemesis package, then minimised and finally subjected to a systematic conformational search performed with the Cosmic molecular mechanics force field, implemented in this package[17]. Each rotatable bond (C1-C7, C7-C8, C1'-C7' and C7'-C8') was rotated at 30° increments. The global minimum from the search results was fully optimized with the semiempirical method MNDO[18] of the package MOPAC. Dummy planes were created using the carbon atoms of both aromatic rings.

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) and HT-29 (human colon carcinoma). At the same time the cytotoxicity against normal cells has been determined using CV-1 (monkey fibroblast) cells.

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 $NaHCO_3$; supplemented with 10% fetal calf serum, 10^{-2} M $NaHCO_3$ and 0.1 g/l Penicillin-G and Streptomycin sulfate was employed.

Antitumor assays[19]

P-388 cells (suspension culture) were seeded into 16 mm wells at 1 x 10^4 cells/well in 1 ml aliquot of medium MEM 10FCS containing the indicated (table 2) concentration of sample. The remaining cell lines: A-549, HT-29 and CV-1 (monolayer cultures) were seeded into 16 mm wells at 2 x 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 (table 2) 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[20]

HSV-1: CV-1 cells were seeded in 16 mm diameter wells at 2 x 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 x 10^5 in 1 ml aliquots of MEM 10FCS. The day after, cells were infected with VSV at 100 PFU/well in 200 µl 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 crystal violet. Plates were washed, dried and number of plaques production was determined.

References

1 A. San Feliciano, J.L. López, M. Medarde, J.M. Miguel del Corral, A.F. Barrero, and P. Puebla, Phytochemistry *28*, 2863 (1989).

2 C.B.S. Rao, "Chemistry of Lignans", Andhra University Press, Waltair, India 1978.

3 W.D. Mac Rae and G.H.N. Towers, Phytochemistry *23*, 1207 (1984); I. Jardine, "Podophyllotoxins" in "Anticancer Agents Based on Natural Products Models", Academic Press, N.Y. 1980.

4 R.E. Damon, R.H. Schlessinger, and J.F. Blount, J. Org. Chem. *41*, 3772 (1976); K. Tomioka, T. Ishiguro, and K. Koga, J. Chem. Soc. Chem. Commun. *1979*, 652; K. Tomioka, T. Ishiguro, Y. Iitaka, and K. Koga, Tetrahedron *40*, 1303 (1984); K. Tomioka and K. Koga, Tetrahedron Lett. *20*, 3315 (1979); E. Brown and A. Daugan, Tetrahedron Lett. *26*, 3997 (1985); A. Pelter, R.S. Ward, M.C. Pritchard, and I.T. Kay, Tetrahedron Lett. *26*, 6377 (1985); E. Brown and A. Daugan, Tetrahedron *46*, 141 (1989).

5 M.V. Naidu and G.S.K. Rao, Synthesis *1979*, 144; J.S. Pizey, "Synthetic Reagents" *2*, 15, J. Wiley & Sons. Chichester 1981.

6 H. Yamaguchi, M. Arimoto, S. Nakajima, M. Tanoguchi, and Y. Fukada, Chem. Pharm. Bull. *34*, 2056 (1986).

7 D.C. Ayres and J.D. Loike, "Lignans. Chemical, biological and clinical properties", p. 221, Cambridge University Press, Cambridge 1990.

8 F.E. Ziegler and J.A. Schwartz, J. Org. Chem. *43*, 985 (1978).

9 S. Ghosal and S. Banerjee, J. Chem. Soc. Chem. Commun. *1979*, 165; R.S. Ward, P. Satyanarayana, and B.V.G. Rao, Tetrahedron Lett. *22*, 3021 (1981); R.S. Ward, A. Pelter, I.R. Jack, P. Satyanarayana, B.V.G. Rao, and P. Subrahmanyam, Tetrahedron Lett. *22*, 4111 (1981).

10 E. Brown and A. Daugan, Tetrahedron Lett. *27*, 3719 (1986); b) P. Boissin, R. Dhal, and E. Brown, Tetrahedron Lett. *30*, 4371 (1989).

11 A. San Feliciano, M. Medarde, R. Pelaez Lamamie de Clairac, and P. Puebla, Studia Chemica *16*, 45 (1992).

12 J. Harmatha, M. Budesinsky, and A. Trka, Coll. Czech. Chem. Commun. *47*, 644 (1982).
13 H. Yamaguchi, M. Arimoto, S. Nakajima, M. Tanoguchi, and Y. Fukada, Chem. Pharm. Bull. *34*, 2056 (1986); H. Yamaguchi, S. Nakajima, M. Arimoto, M. Tanoguchi, Y. Ishida, and M. Inoue, Chem. Pharm. Bull. *32*, 1754 (1984).
14 K.V. Rao and F.M. Alvarez, J. Nat. Products *48*, 592 (1985).
15 T. Ishiguro, H. Mizuguchi, K. Tomioka, and K. Koga, Chem. Pharm. Bull. *33*, 609 (1985).
16 Ref. 7, p. 93; W.D. McRae, J.B. Hudson, and.G.H.N. Towers, Planta Medica 55, 531 (1989).
17 Nemesis, Oxford Molecular Ltd (1991); COSMIC (COmputation and Stucture Manipulation in Chemistry) J.G. Vinter, A. Davis, and M.R. Saunder, J. Comput. Aided Mol. Desing. *1*, 31 (1987).
18 M.J.S. Dewar and W. Thiel, J. Am. Chem. Soc. *99*, 4899 (1977).
19 R.J. Bergeron, P.F. Cavanaugh Jr., S.J. Kline, R.G. Hughes Jr., G.T. Elliott, and C.W. Porter, Bioch. Bioph. Res. Commun. *3*, 121 (1984).
20 A.C. Schroeder, R.C. Hughes Jr., and A. Bloch, J. Med. Chem. *24*, 1078 (1981).

[Ph74]