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CYTOTOXIC LIGNANS FROM FORMOSAN *HERNANDIA NYMPHAEIFOLIA*

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Key Word Index—*Hernandia nymphaeifolia*; Hernandiaceae; bark; lignans; dibenzylbutyrolactone lignan; (−)-6′-hydroxyyatein; furanoid lignan; (−)-hernone; (−)-nymphone; cytotoxic activity.

Abstract—A new dibenzylbutyrolactone lignan, (−)-6′-hydroxyyatein, along with two new furanoid lignans, (−)-hernone [(2*R*,3*S*,4*R*)-(−)-3-hydroxymethyl-4-(3″,4″,5″-trimethoxybenzoyl)-2-(3′,4′-dimethoxyphenyl) tetrahydrofuran] and (−)-nymphone [(2*R*,3*S*,4*R*)-(−)-3-hydroxymethyl-4-(3″,4″,5″-trimethoxybenzoyl)-2-(3′,4′-methylenedioxyphenyl)tetrahydrofuran], have been isolated and characterized from the trunk bark of Formosan *Hernandia nymphaeifolia*. The structures of these compounds were determined by means of spectral analyses. These three lignans exhibited cytotoxic activities against P-388, KB16, A549 and HT-29 cell lines.

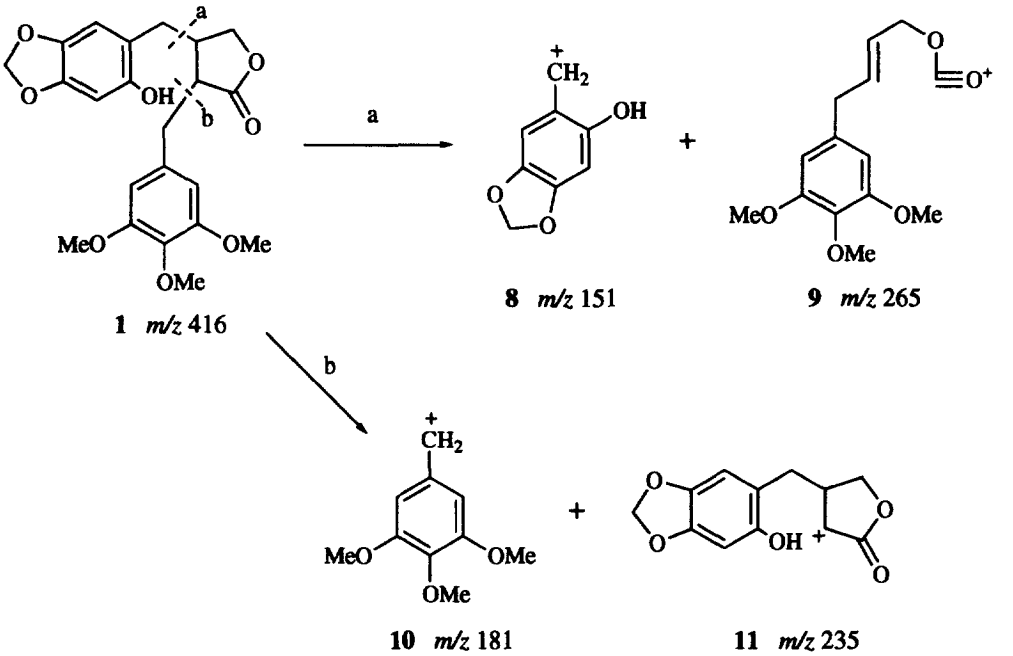
INTRODUCTION

Hernandia nymphaeifolia [1–3] is a maritime plant grown throughout the subtropical and tropical zones, and was often misnamed as *H. ovigera* or *H. sonora* in Taiwan and Japan for many years [4]. Past papers [5–26] have reported many aporphine alkaloids and lignans as major constituents from this species. In our previous studies, we have reported 24 new compounds, mainly aporphine alkaloids, and 20 known compounds, including several antiplatelet aggregation and cytotoxic agents from the trunk bark of Formosan *H. nymphaeifolia* [4, 27–32]. Further examination on the neutral chloroform-soluble fraction of the trunk bark of this species has led to the isolation of three new lignans, including one dibenzylbutyrolactone lignan, (−)-6′-hydroxyyatein (**1**), as well as two 2,3,4-trisubstituted furanoid lignans, (−)-hernone (**2**) and (−)-nymphone (**3**). These three new lignans were subjected to cytotoxic tests and **2** and **3** exhibited potent cytotoxic activities against P-388, KB16, A549 and HT-29 cell lines when assessed using standard protocols [33]. In this paper, we report on the isolation and structural elucidation and cytotoxic activities of these three new lignans.

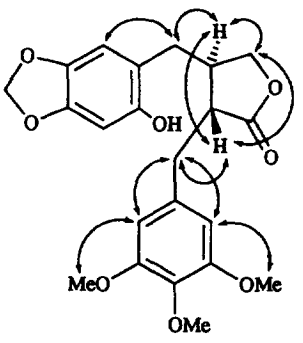
RESULTS AND DISCUSSION

(−)-6′-Hydroxyyatein (**1**) was isolated as colourless amorphous powder. The molecular formula was established as $C_{22}H_{24}O_8$ by EI ($[M]^+$, *m/z* 416) and HR-mass spectrometry. The presence of a dibenzylbutyrolactone lignan skeleton was suggested by the UV spectrum showing absorptions at 206, 230 sh and 303 nm, along with a lactone carbonyl absorption at 1758 cm^{-1} in the IR spectrum [23]. The presence of a phenolic hydroxyl group in the molecule was indicated by the IR absorption at 3340 cm^{-1} and a bathochromic shift of UV absorption in alkaline solution. The IR spectrum also revealed the presence of a methylenedioxy group at 1030 and 930 cm^{-1}. The ^1H NMR spectrum of **1** showed eight aliphatic protons at δ 2.51–4.18, typical of dibenzylbutyrolactone lignans [34, 35]. In addition, three methoxyl groups at δ 3.82 (9H, *s*) and two aromatic protons at δ 6.38 (2H, *s*) were similar to those of yatein [23], suggesting the presence of a 3,4,5-trimethoxybenzyl moiety; this was supported by a significant fragment at *m/z* 181 (**10**) in the EI-mass spectrum (Scheme 1). The presence of a 2-hydroxy-4,5-methylenedioxybenzyl moiety was easily revealed by a methylenedioxy signal, a hydroxyl group and two singlets of aromatic protons at δ 6.31 (H-5′) and δ 6.40 (H-2′), together with a significant fragment at *m/z* 151 (**8**) in the EI-mass spectrum (Scheme 1). According to the above data and the significant fragments **8** (*m/z* 151), **9** (*m/z* 265), **10** (*m/z* 181) and **11** (*m/z* 235) in the EI-mass spectrum (Scheme 1) [23], **1** was elucidated as 6′-hydroxyyatein. Its structure was further confirmed by ^1H-^1H COSY and NOESY experiments (Fig. 1). The *R*-configurations at C-2 and C-3 of **1** were supported by the following observations:

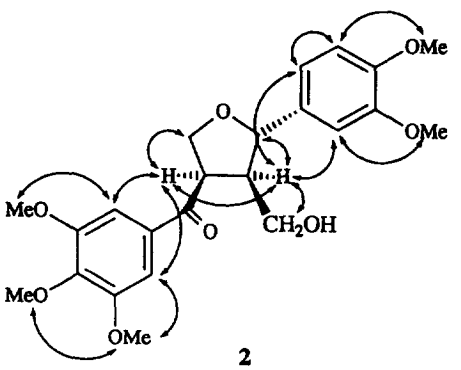
* Author to whom correspondence should be addressed.



Scheme 1. Mass spectral fragmentations of compound **1**.



1



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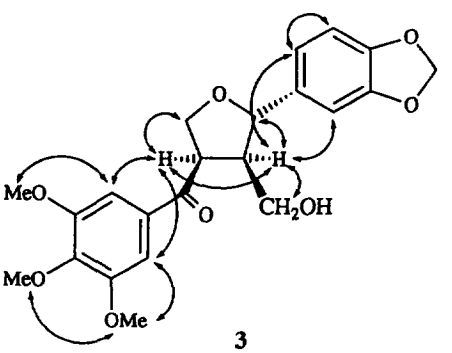
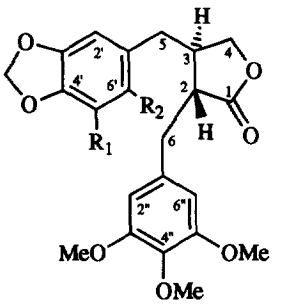


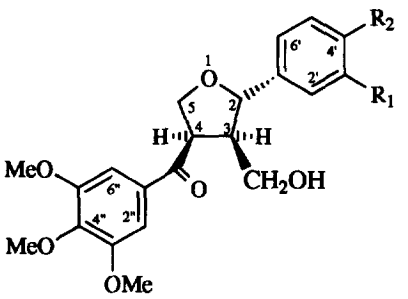
3

Fig. 1. NOESY correlations for compounds **1**–**3**.

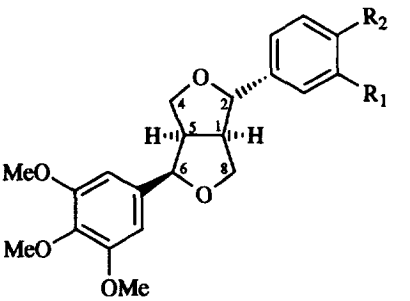
(i) the specific rotation of **1** is negative, $[\alpha]_D^{25}$ −29.3° (*c* 0.08, $CHCl_3$), similar to those of (−)-yatein (**4**) {$[\alpha]_D$ −29.5° ($CHCl_3$)} [23] and (−)-5′-methoxyyatein (**5**) {$[\alpha]_D$ −21° ($CHCl_3$)} [34]; (ii) the chemical shifts of eight aliphatic protons of **1** are similar to those of (−)-yatein (**4**) [35]; (iii) the configurations of C-2 and



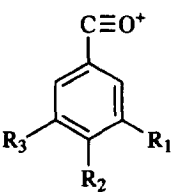
1 $R_1 = H, R_2 = OH$
4 $R_1 = R_2 = H$
5 $R_1 = OMe, R_2 = H$



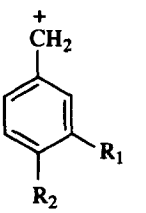
2 $R_1 = R_2 = OMe$
3 $R_1 + R_2 = OCH_2O$



6 $R_1 = R_2 = OMe$
7 $R_1 + R_2 = OCH_2O$



12 $R_1 = R_2 = R_3 = OMe$ *m/z* 195
13 $R_1 = R_2 = OMe, R_3 = H$ *m/z* 165
15 $R_1 + R_2 = OCH_2O, R_3 = H$ *m/z* 149



14 $R_1 = R_2 = OMe$ *m/z* 151
16 $R_1 + R_2 = OCH_2O$ *m/z* 135

C-3 of all previous dibenzylbutyrolactone lignans with negative $[\alpha]_D$ isolated from this species, are 2*R* and 3*R*.

(−)-Hernone (**2**) was obtained as colourless prisms with $[\alpha]_D^{25}$ −29.7° ($CHCl_3$). The EI-mass spectrum afforded the $[M]^+$ at *m/z* 432, implying a molecular formula of $C_{23}H_{28}O_8$, which was confirmed by the HR-mass spectrum. The UV absorptions were similar to those of sylvone and suggested the presence of a 2,3,4-trisubstituted furanoid lignan skeleton [36]. The presence of a conjugated carbonyl group was revealed by IR absorption at 1660 cm^{-1}, along with the ^{13}C NMR signal at δ 198.2. The IR spectrum also showed absorptions for a hydroxyl group at 3450 cm^{-1}. The ^1H NMR spectrum of **2** showed the presence of five methoxyl groups and five aromatic protons. Two lower field aromatic protons at δ 7.27 (2H, *s*, H-2″ and H-6″) suggested that one of the aryl groups was present as an aroyl unit; this was supported by the EI-mass spectrum which gave an ion *m/z* 195 (**12**) as the base peak. The EI-mass spectrum also showed the significant fragmentions **13** (*m/z* 165) and **14** (*m/z* 151),

Table 1. ^{13}C NMR spectral data of compounds **2** and **3** and sylvone

C	2	3	Sylvone*
2	83.7	83.7	80.5
3	52.4	52.5	49.4
CH_2OH	61.3	61.2	60.9
4	49.7	49.7	48.2
C=O	198.2	198.0	198.1
5	70.6	70.7	67.9
1′	132.9	134.4	130.0
2′	109.6	107.1	110.7
3′	149.3	148.0	148.3
4′	149.0	147.5	147.6
5′	110.9	108.1	108.6
6′	119.3	120.3	117.3
1″	131.7	131.7	130.6
1″	106.2	106.2	105.9
3″	153.2	153.2	152.4
4″	143.0	143.1	142.1
5″	153.2	153.2	152.4
6″	106.2	106.2	105.9
OMe-3′	55.9	—	55.2
OMe-4′	55.9	—	55.2
OMe-3″	56.4	56.4	55.5
OMe-4″	60.9	61.0	60.1
OMe-5″	56.4	56.4	55.5
OCH_2O	—	101.1	—

* Sylvone measured in $CDCl_3$ (20 MHz)

indicating the presence of a 3,4-dimethoxyphenyl substituent. In addition, seven aliphatic protons appeared in the region δ 2.87–δ 4.70 and the chemical shift of each proton was assigned according to ^1H-^1H COSY and NOESY experiments (Fig. 1). The $J = 8.8$ Hz of H-2 (δ 4.70) in **2** was larger than $J = 6.3$ Hz of H-2 (δ 4.89, *cis*-relationship with H-3) in sylvone [36], but corresponded with $J = 7$ Hz of H-2 (δ 4.47, *trans*-relationship with H-1), was larger than $J = 5$ Hz of H-6 (δ 4.87, *cis*-relationship with H-5) in epimagnolin (**6**) [10], suggesting that H-2 and H-3 of **2** were in *trans*-orientation, which was also supported by the smaller NOESY interactions. On the other hand, the larger NOESY interactions for the H-3, H-4 pair confirmed their *cis*-relationship. Furthermore, all previous tetrahydrofurofuran-type lignans isolated from this species are *epi*-form lignans and the formation of **2** may be the result of oxidative cleavage of our previous reported epimagnolin (**6**) [31] in the biogenetic process. Thus, **2** and epimagnolin (**6**) would possess analogous stereo-skeletons. The structure of (−)-hernone was reasonably established at (2*R*,3*S*,4*R*)-(−)-3-hydroxymethyl-4-(3″,4″,5″-trimethoxybenzoyl)-2-(3′,4′-dimethoxyphenyl)tetrahydrofuran (**2**) on the basis of the above observations and NOESY experiments (Fig. 1). The assignments of ^{13}C NMR (Table 1) of **2** were confirmed by the DEPT, HETCOR experiments and comparison with those of sylvone [36].

(−)-Nymphone (**3**) was isolated as colourless prisms with $[\alpha]_D^{25}$ −47.4° ($CHCl_3$). Its HR-mass spectrum revealed a $[M]^+$ at *m/z* 416.1464 leading to the molecular formula $C_{22}H_{24}O_8$ (calcd 416.1471). The presence of a 2,3,4-trisubstituted furanoid lignan skeleton [36] was indicated by UV absorptions, as in the case of (−)-hernone (**2**). The presence of a conjugated carbonyl group was indicated by IR absorption at 1660 cm^{-1}, as well as ^{13}C NMR signal at δ 198.0. The IR spectrum also showed the absorptions of a hydroxyl group and a methylenedioxy group. The ^1H NMR spectrum of **3** was similar to that of **2**, except that a methylenedioxy group (C-3′, C-4′) in **3** at δ 5.95 (2H, *s*) replaced OMe-3′ (δ 3.92) and OMe-4′ (δ 3.88) in **2**. The presence of a 3,4-methylenedioxybenzyl moiety was easily revealed by three mutually coupling aromatic protons and a methylenedioxy group, together with the significant fragments at *m/z* 149 (**15**) and *m/z* 135 (**16**) in the EI-mass spectrum. Three methoxyl groups at δ 3.93 (9H, *s*) and two low field aromatic protons at δ 7.27 (2H, *s*, H-2″ and H-6″) suggested the presence of a 3,4,5-trimethoxybenzoyl moiety; this was supported by the EI-mass spectrum, which exhibited a base peak at *m/z* 195 (**12**). In addition, the NOESY interactions and chemical shifts of seven aliphatic protons were similar to those of **2**, suggesting that they possess the same stereochemistry. Furthermore, the formation of **3**, like **2**, may be the result of oxidative cleavage of our previous reported epiaschantin (**7**) [31] in the biogenetic process. Thus, the stereochemistry of **3** would correspond with epiaschantin (**7**). On the basis of the above data, the structure of (−)-nymphone was elucidated as (2*R*,3*S*,4*R*)-(−)-3-hydroxymethyl-4-(3″,4″,5″-trimethoxybenzoyl)-2-(3′,4′-methylenedioxyphenyl) tetrahydrofuran (**3**), which was further confirmed by the ^1H-^1H COSY and NOESY experiments (Fig. 1). The ^{13}C NMR spectrum (Table 1) of **3** was similar to that of **2**, except for minor shifts of C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′ due to replacing OMe-3′ (δ 55.9) and OMe-4′ (δ 55.9) in **2** by OCH_2O (δ 101.1) in **3**. The assignments of ^{13}C NMR of **3** were supported by DEPT and HETCOR experiments.

Table 2. Cytotoxicities of compounds **1**–**3** against P-388, KB16, A549 and HT-29 cell lines

Compound	ED_{50} ($\mu g\ ml^{-1}$) A549	HT-29	KB15	P-388
Mithramycin*	0.076	0.082	0.084	0.061
(−)-6′-Hydroxyyatein (**1**)	3.153	4.073	>50	2.164
(−)-Hernone (**2**)	1.120	0.909	1.218	0.806
(−)-Nymphone (**3**)	3.024	0.740	0.639	0.321

* Mithramycin used as reference.

The cytotoxic effects of the three new lignans were tested *in vitro* against P-388, KB16, A549, and HT-29 cell lines. The cytotoxicity data are shown in Table **2**; the clinically used anticancer agent, mithramycin, was used as reference. The 2,3,4-trisubstituted furanoid

lignans, (−)-hernone (**2**) and (−)-nymphone (**3**) exhibited more potent cytotoxic activities than the dibenzylbutyrolactone lignan, (−)-6′-hydroxyyatein (**1**) against all the cell lines. Although the cytotoxicities of the new lignans were less than that of mithramycin, they still display effective activities (ED_{50} values < 4 $\mu g\ ml^{-1}$), except for **1** (ED_{50} values > 4 $\mu g\ ml^{-1}$ against KB16 and HT-29 cell lines).

EXPERIMENTAL

Mps: uncorr. ^1H (400 MHz) and ^{13}C NMR (100 MHz): $CDCl_3$; Chemical shifts are given in δ with TMS as int. standard. MS were measured using a direct inlet system. Optical rotations were measured in $CHCl_3$. UV spectra were determined in EtOH, and IR in KBr discs. Silica gel (60–230, 230–400 mesh) (Merck) was used for CC and silica gel 60 F−254 for TLC.

Plant material. Trunk bark of *H. nymphaeifolia* (Presl) Kubitzki (*H. peltata* Meissn.) was collected from Green Island, Taitung Hsien, Taiwan, in August 1992. A voucher sample (Chen 5521) is deposited in the herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation. Dried trunk bark (7 kg) was powdered, extracted with MeOH and the extract concd under red. pres. The MeOH extract when partitioned between H_2O–$CHCl_3$ (1:1) afforded a $CHCl_3$-sol. fr. Bases in the $CHCl_3$-sol. fr. were extracted with 2% H_2SO_4. The acid-insol. part was dried ($MgSO_4$) and concd to give a neutral $CHCl_3$-sol. fr. (C, 220 g). Part (80.4 g) of fr. C was chromotographed on silica gel (2450 g) eluting with $CHCl_3$–MeOH (100:0–0:100) to obtain 17 frs (each 2000 ml, C1–C17). Fr. C9 (442 mg) was rechromatographed on silica gel (13 g) and eluted with *n*-hexane–EtOAc (1:1) to give 6 frs (C9-1–C9-6). Fr. C-9-4 (50.3 mg) was further purified by prep. TLC ($CHCl_3$–Me_2CO, 10:1) to obtain **3** (6.8 mg) (R_f 0.40) after recrystallization from CH_2Cl_2–Me_2CO. Fr. C10 (3.43 g) was rechromatographed on silica gel (102 g) and eluted with $CHCl_3$–Me_2CO (10:1) to give 9 frs (C10-1–C10-9). Fr C10-3 (1.17 g) was further rechromatographed on silica gel (35 g) and eluted with benzene–Me_2CO (5:1) to give 6 frs (C10-3-1–C10-3-6). Fr. C-10-3-4 (163 mg) was purified by prep. TLC (*n*-hexane–EtOAc, 1:1) to obtain **1** (4.1 mg) (R_f 0.45). Fr. C-10-3-5 (395 mg) was purified by prep. TLC ($CHCl_3$–Me_2CO, 10:1) to obtain **2** (18.7 mg) (R_f 0.39) after recrystallization from CH_2Cl_2–Me_2CO.

(−)-6′-*Hydroxyyatein* (**1**). Colourless amorphous powder. UV λ_{max}^{EtOH} nm (log ε): 206 (4.42), 230 sh (3.78), 303 (3.48); UV $\lambda_{max}^{EtOH+KOH}$ nm (log ε): 206 (4.62), 318 (3.43). IR ν_{max} cm^{-1}: 3340 (OH), 1758 (C = O), 1585, 1500, 1440 (aromatic ring C = C stretch), 1030, 930 (OCH_2O). EIMS *m/z* (rel. int.): 416 $[M]^+$ (53), 266 (8), 265 (48), 264 (7), 247 (14), 233 (7), 219 (17), 189 (10), 182 (17), 181 (76), 176 (17), 175 (13), 168 (26), 167 (9), 153 (10), 152 (49), 151 (100), 148 (17), 147 (9), 137 (10), 136 (11), 121 (16); HRMS: $C_{22}H_{24}O_8$, found: 416.1469, calcd: 416.1471. ^1H NMR: δ 2.51 (1H, *dd*, *J* = 12.4, 6.8 Hz, H-5β), 2.58 (1H, *m*, H-3), 2.61 (1H, *m*, H-2), 2.66 (1H, *dd*, *J* = 12.4, 5.6 Hz, H-5α), 2.89 (1H, *dd*, *J* = 14.1, 6.4 Hz, H-5α), 2.95 (1H, *dd*, *J* = 14.1, 5.0 Hz, H-6α), 3.82 (9H, s, OMe-3″, 4″ and 5″), 3.91 (1H, *dd*, *J* = 9.1, 7.2 Hz, H-4β), 4.18 (1H, *dd*, *J* = 9.1, 6.6 Hz, H-4α), 4.86 (1H, *br s*, OH-6′, disappeared after addition of D_2O), 5.87, 5.88 (each 1H, *d*, *J* = 1.2 Hz, OCH_2O), 6.31 (1H, *s*, H-5′), 6.38 (2H, *s*, H-2″ and 6″), 6.40 (1H, *s*, H-2′). $[\alpha]_D^{25}$ −29.3° (*c* 0.08, $CHCl_3$).

(−)-*Hernone* (**2**). Colourless prisms (CH_2Cl_2–Me_2CO), mp 126–128°. UV λ_{max}^{EtOH} nm (log ε): 204 (4.73), 218 sh (4.44), 282 (4.09). IR ν_{max} cm^{-1}: 3450 (OH), 1660 (conjugated C=O) 1585, 1505, 1455, 1420 (aromatic ring C=C stretch). EIMS *m/z* (rel. int.): 432 $[M]^+$ (71), 418 (2), 414 (2), 402 (4), 223 (29), 219 (29), 211 (11), 210 (80), 196 (15), 195 (100), 194 (46), 193 (18), 192 (82), 181 (17), 168 (11), 167 (59), 166 (37), 165 (80), 152 (26), 151 (42), 139 (38), 138 (18), 137 (19), 124 (12), 122 (12), 109 (11); HRMS: $C_{23}H_{28}O_8$, found: 432.1782, calcd: 432.1785. ^1H NMR: δ 2.87 (1H, *m*, H-3), 3.69 (1H, *dd*, *J* = 10.9, 5.4 Hz, $C\underline{H}_A H_B OH$), 3.79 (1H, *dd*, *J* = 10.9, 4.0 Hz, $CH_A\underline{H}_B OH$), 3.87 (3H, *s*, OMe-4′), 3.91 (3H, *s*, OMe-3′), 3.92 (9H, *s*, OMe-3″, 4″ and 5″), 4.20 (1H, *m*, H-4), 4.23 (1H, *m*, H-5β), 4.28 (1H, *m*, H-5α), 4.70 (1H, *d*, *J* = 8.8 Hz, H-2), 6.84 (1H, *d*, *J* = 8.1 Hz, H-5′), 6.92 (1H, *dd*, *J* = 8.1, 2.1 Hz, H-6′), 7.01 (1H, *d*, *J* = 2.1 Hz, H-2′), 7.27 (2H, *s*, H-2″ and 6″). ^{13}C NMR: Table 1. $[\alpha]_D^{25}$ −29.7° (*c* 0.19, $CHCl_3$).

(−)-*Nymphone* (**3**). Colourless prisms (CH_2Cl_2–Me_2CO), mp 123–125°. UV λ_{max}^{EtOH} nm (log ε): 204 (4.63), 216 sh (4.38), 287 (4.11). IR ν_{max} cm^{-1}: 3510 (OH), 1660 (conjugated C=O), 1580, 1490, 1450, 1415 (aromatic ring C=C stretch), 1030, 920 (OCH_2O). EIMS *m/z* (rel. int.): 416 $[M]^+$ (55), 398 (2), 238 (26), 223 (43), 222 (19), 210 (37), 203 (39), 196 (26), 195 (100), 194 (74), 181 (15), 179 (16), 178 (74), 177 (13), 176 (57), 169 (12), 167 (14), 153 (12), 152 (31), 151 (75), 150 (44), 149 (74), 137 (25), 135 (37); HRMS: $C_{22}H_{24}O_8$, found: 416.1464, calcd: 416.1471. ^1H NMR: δ 2.84 (1H, *m*, H-3), 3.69 (1H, *dd*, *J* = 10.9, 5.4 Hz, $C\underline{H}_A H_B OH$), 3.79 (1H, *dd*, *J* = 10.9, 4.4 Hz, $CH_A\underline{H}_B OH$), 3.93 (9H, *s*, OMe-3″, 4″ and 5″), 4.19 (1H, *m*, H-4), 4.21 (1H, *m*, H-5β), 4.29 (1H, *m*, H-5α), 4.68 (1H, *d*, *J* = 9.2 Hz, H-2), 5.95 (2H, *s*, OCH_2O), 6.78 (1H, *d*, *J* = 7.8 Hz, H-5′), 6.86 (1H, *dd*, *J* = 7.8, 1.6 Hz, H-6′), 6.96 (1H, *d*, *J* = 1.6 Hz, H-2′), 7.27 (2H, *s*, H-2″ and 6″). ^{13}C NMR: Table 1. $[\alpha]_D^{25}$ −47.4° (*c* 0.08, $CHCl_3$).

Cytotoxicity assay. Activities of compounds **1–3** against P-388 (mouse lymphocytic leukemia), KB16 (human nasopharyngeal carcinoma), A549 (human lung adenocarcinoma) and HT-29 (human colon carcinoma) cells were assayed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [37, 38].

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