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ADDITIONAL CYTOTOXIC NEOLIGNANS FROM *PERSEA OBOVATIFOLIA*

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Key Word Index—*Persea obovatifolia*; Lauraceae; leaves; neolignans; obovatifol; obovaten; perseals C and D; cytotoxicity.

Abstract—Four additional neolignans, comprising obovatifol [(2*S*,3*S*)-2,3-dihydro-2-(3,4-dihydroxy-5-methoxyphenyl)-7-methoxy-3-methyl-5-*trans*-propenyl benzofuran], obovaten [2-(3,4-dihydroxy-5-methoxyphenyl)-7-methoxy-3-methyl-5-*trans*-propenyl benzofuran], perseal C [(2*S*,3*R*)-2,3-dihydro-2-(3,4-methylenedioxyphenyl)-5-formyl-3-hydroxymethyl-7-methoxy benzofuran] and perseal D [2-(3,4-dihydroxy-5-methoxyphenyl)-5-formyl-7-methoxy-3-methyl benzofuran] were isolated in a continuing study of the leaves of *Persea obovatifolia*. Obovatifol had been reported previously in a mass spectrometric analysis without any other spectroscopic data. Obovaten and perseals C and D are new compounds, bearing a C-1′ formyl side-chain, instead of a propenyl group. Their structures were elucidated from spectroscopic data; they showed significant cytotoxic activities against P-388, KB16, A549 and HT-29 cancer cell lines *in vitro*.

INTRODUCTION

Previously, we reported the isolation of three new cytotoxic formyl neolignans [1] from the chloroform-soluble fraction of the leaves of *Persea obovatifolia*, which is a small evergreen tree, endemic in the Hengchun Peninsula of Taiwan [2–4]. Continuing chemical investigation has led to the isolation of four additional neolignans, obovatifol (**1**), obovaten (**2**), perseal C (**3**) and perseal D (**4**); the latter three are new compounds, in which **3** and **4** had a C-1′ formyl substituted group, instead of a propenyl group. Compound **1** had previously been reported only from the presence of a $[M+H]^+$ peak [5] without other spectroscopic data. The isolation and structure elucidation of these neolignans with their cytotoxic activities are described in the present paper.

RESULTS AND DISCUSSION

Compound **1** had the molecular formula ($C_{20}H_{22}O_5$) as determined by EI $[M]^+$, *m/z* 342) and HR mass spectrometry. UV absorptions indicated the presence of a benzenoid moiety. The IR spectrum exhibited hydroxyl absorption at 3350 cm^{-1}. The structure of **1** resembled that of licarin A [2,3-dihydro-2(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methyl-5-*trans*-propenyl benzofuran] [6], except that **1** had an additional hydroxyl group on the C ring. The ^1H NMR spectrum showed a coupling constant ($J = 8.8$ Hz) between H-7 (δ 5.05) and H-8 (δ 3.41), indicating the relative *trans*-vicinal coupling of the dihydrofuran ring [7]. The absolute configuration of **1** was established as a 2*S*-aryl, 3*S*-methyl-substituted dihydrobenzofuran from the negative specific rotation ($[\alpha]_D^{25}$ -50°) [8, 9]. The acetylated derivative **1a**, showed resonances for the acetyl groups [δ 2.26 and 2.29 (each 3H, *s*) and corresponding IR absorptions at 1780 cm^{-1} (OCO)]. In the mass spectrum of **1a**, the M_r had increased by 84 mu, indicating that **1** contains two phenolic hydroxyl groups. On the basis of the above evidence, **1** was elucidated as (2*S*,3*S*)-2,3-dihydro-2-(3,4-dihydroxy-5-methoxyphenyl)-7-methoxy-3-methyl-5-*trans*-propenyl benzofuran. The structure was also confirmed by COSY, DEPT, HETCOR and NOESY (Fig. 1) experiments. Although **1** had previously been reported from the mass spectral analysis of Cooks *et al.* [5], its full spectroscopic data are reported for the first time, in the present study.

With close R_f values on TLC, compounds **1** and **2** could not be successfully separated using usual methods. The mixture of **1** and **2** showed no evidence for acetyl group in the ^1H NMR spectrum. Compound

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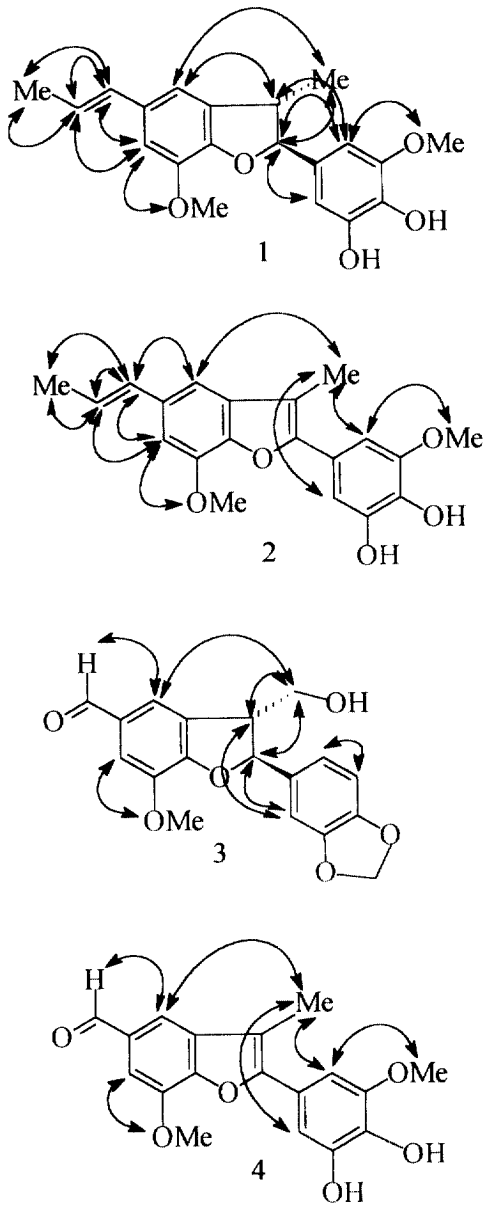

Fig. 1.

2a was isolated from the acetylation products of **1** and **2**. Then, **2a** was hydrolyzed by *p*-toluenesulfonic acid monohydrate in methanol at room temperature to obtain the original deacetylated compound **2**.

Compound **2** had the molecular formula ($C_{20}H_{20}O_5$) as determined by EI ([M]$^+$, *m/z* 340) and HR mass spectrometry. In its mass spectrum, the M_r of **2** was 84 mn less than **2a**, indicating the existence of two hydroxyl groups [^{1}H NMR: δ 5.46 and 5.58 (each 1H, D_2O-exchangeable; IR: 3300 cm^{-1}]. The UV spectrum also showed the presence of a phenolic benzenoid moiety. The structure of **2** was similar to that of eupomatenoid-7 [2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methyl-5-*trans*-propenyl benzofuran] [6], except that **2** had an additional hydroxyl group on the C ring. The ^{1}H NMR spectrum of **2** also showed the existence of a benzofuran moiety, with the presence of an olefinic C-8 methyl [δ 2.41, (3H, *s*)] and the lack of H-7 and H-8 signals. On the basis of the above data, **2** was thus determined to be 2-(3,4-dihydroxy-5-methoxyphenyl)-7-methoxy-3-methyl-5-*trans*-propenyl benzofuran. It was also further confirmed by DEPT, HETCOR, COSY and NOESY (Fig. 1) experiments.

Compound **3** had the molecular formula ($C_{18}H_{16}O_6$) by EI ([M]$^+$, *m/z* 328) and HR mass spectrometry. Its UV absorptions showed maxima around 207, 236, 290 and 300 nm. The existence of C-1′ formyl [^{1}H NMR: δ 9.84 (1H); IR: 1680 cm^{-1}], a methylenedioxy [^{1}H NMR: δ 5.92 (2H); IR: 1040 and 940 cm^{-1}] and a hydroxyl [^{1}H NMR: δ 3.90 (1H, D_2O-exchangeable); IR: 3450 cm^{-1}] groups were observed. The ^{1}H NMR resonances of **3** showed the presence of two protons at δ 3.88 and 3.97 (each 1H, *dd*, J = 14.8, 6.0 Hz), which were assigned to H-9a and H-9b on the hydroxymethyl group. From the deshielding effect of the loan pair on the C-8 hydroxymethyl group, the signals of H-7 and H-8 were downshifted by ca 0.25–0.64 ppm, when compared with the 2-aryl, 3-methyl-disubstituted dihydrobenzofuran-type neolignans [9]. Three aromatic protons in an ABX-system at δ 6.78 (1H, *dd*, J = 8.0, 1.2 Hz), 6.87 (1H, *d*, J = 8.0 Hz) and 6.88 (1H, *d*, J = 1.2 Hz) were assigned to H-6, H-5 and H-2, respectively. Two *meta*-coupled doublets at δ 7.40 (1H, J = 1.0 Hz) and 7.41 (1H, J = 1.0 Hz) were assigned as H-6′ and H-2′, respectively. The coupling constant (J = 6.4 Hz) between H-7 (δ 5.69) and H-8 (δ 3.66) indicated the relative *trans*-vicinal coupling of 2-aryl, 3-hydroxymethyl to the dihydrobenzofuran ring [7–9]. The absolute configuration of **3** was proposed to be 2*S*-aryl, 3*R*-hydroxymethyl-disubstituted dihydrobenzofuran, by comparing its specific rotation ($[\alpha]_D^{25}$ −20°) with 2*S*,3*SR*-dihydrodehydrodiconiferyl alcohol ($[\alpha]_D$ −4.1°) [9]. From the above evidence, **3** was identified as (2*S*,3*R*)-2,3-dihydro-2(3,4-methylene dioxyphenyl)-5-formyl-3-hydroxymethyl-7-methoxy benzofuran, which was further confirmed by COSY and NOESY (Fig. 1) experiments.

Compound **4** had the molecular formula $C_{18}H_{16}O_6$, as deduced by EI ([M]$^+$, *m/z* 328) and HR mass spectrometry. UV absorption maxima around 237 and 295 nm, and a bathochromic shift in alkaline solution, indicated the presence of a phenolic benzenoid moiety. The IR spectrum indicated a formyl group at 1680 cm^{-1} and hydroxyl absorption at 3350 cm^{-1}. The ^{1}H NMR spectrum of **4** was similar to that of **2**. However, **4** showed the presence of a C-1′ formyl group [δ 10.03 (1H, *s*)] on the benzofuran, instead of propenyl signals. The presence of an olefinic methyl signal at δ 2.49 (3H, *s*) in **4** was observed. A broad singlet at δ 5.45 (2H), which disappeared on addition of D_2O, indicated two hydroxyl groups. Two pairs of *meta*-coupled protons at δ 6.98 (1H, *d*, J = 1.8 Hz) and 7.05 (1H, *d*, J = 1.8 Hz), with δ 7.37 (1H, *d*, J = 1.2 Hz) and 7.67 (1H, *d*, J = 1.2 Hz), were assigned to H-6,

Table 1. Cytotoxicity of compounds **1**–**4**

Compound	ED_{50} ($\mu g\ ml^{-1}$)			
	P-388	KB16	A549	HT-29
Mithramycin*	0.061	0.084	0.076	0.082
Obovatifol (**1**)	0.121	0.090	0.329	0.269
Obovatifol diacetate (**1a**)	0.391	0.075	0.970	0.483
Obovaten (**2**)	0.246	0.766	0.386	0.683
Obovaten diacetate (**2a**)	0.207	0.049	0.421	0.667
Perseal C (**3**)	0.346	0.808	0.753	0.725
Perseal D (**4**)	0.386	0.976	0.590	1.002

* Positive control.

For significant activity of pure compound an ED_{50} value $\leq 4.0\ \mu g\ ml^{-1}$ is required.

H-2, and H-2′, H-6′, respectively. From the above evidences, **4** was determined to be 2-(3,4-dihydroxy-5-methoxyphenyl)-5-formyl-7-methoxy-3-methyl benzofuran, which was further confirmed by COSY and NOESY (Fig. 1) experiments.

In the ^1H NMR spectral data, all the A- and C-ring chemical shifts of the benzofuran moiety, such as **2**, were downshifted by ca 0.05–0.41 ppm, in comparison with the dihydrobenzofuran, such as **1**. The A-ring aromatic protons of C-1′ formyl neolignans, such as **3** and **4**, were downshifted by ca 0.54–0.64 ppm, in comparison with those of a C-1′ propenyl neolignan, such as **2**. But chemical shifts on the C-ring between C-1′ formyl and propenyl neolignans were little different. For the existence an additional hydroxyl group on the C-ring, the H-2 and H-6 signals of ^1H NMR of **1** and **2** were upshifted by ca 0.26–0.35 ppm, compared with lican A and eupomatenoid-7 [6]. However, all of the ^{13}C NMR data showed no obvious differences.

The four additional neolignans isolated showed significant cytotoxic activities against P-388, KB16, A549 and Ht-29 cancer cell lines (Table 1). Compound **1** showed nearly the same ED_{50} value as mithramycin, against the KB16 cell line. Compounds **1a** and **2a** showed smaller ED_{50} values than mithramycin against the KB16 cancer cell line. The C-1′ propenyl neolignan, **2**, showed better cytotoxic activity than the C-1′ formyl neolignan 4 against P-388, KB16, A549 and HT-29 cancer cell lines.

EXPERIMENTAL

General

Mps: uncorr. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were taken in $CDCl_3$. Chemical shifts are given in δ with TMS as internal standard. MS were measured using a direct inlet system. UV spectra were determined in EtOH. CC was carried out on silica gel (Merck, 70–230 and 230–400 mesh) and TLC used silica gel plates (Merck, 60 GF-254).

Plant material

Leaves of *P. obovatifolia* Kost. (*Machilus bovatifolia* Kanehira et Sadsaki), were collected from Pingtung Hsien, Taiwan, in August 1994. A voucher specimen (No 5687) is deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiumg, Taiwan, R.O.C.

Extraction and isolation

Dried leaves (4.9 kg) were exhaustively extracted with MeOH (5 × 20 l) and concd *in vacuo* to give a dark residue (0.83 kg). The MeOH extract was partitioned between $CHCl_3$ (3 l) and H_2O (1 l), and extracted with $CHCl_3$ (10 l) to afford a $CHCl_3$-sol. fr. (0.41 kg). Part of the $CHCl_3$-sol. fr. (0.11 kg) was subjected to CC on silica gel, eluting with $CHCl_3$, gradually enriched with MeOH, to give 26 frs (1 ~ 26). Fr. 6 ($CHCl_3$, 3.1 g) was resubjected to CC on silica gel, eluting with CH_2Cl_2 gradually enriched with EtOAc, to provide 9 frs (6-1 ~ 6-9). Fr. 6-5 (CH_2Cl_2–EtOAc, 100:1; 0.74 g) was resubjected to CC on silica gel, eluting with CH_2Cl_2 gradually enriched with Me_2CO, to furnish 9 frs (6-5-1 ~ 6-5-9). Fr. 6-5-5 (CH_2Cl_2–Me_2CO, 100:1; 0.4 g) was washed with Et_2O, then purified by prep. TLC (*n*-hexane–EtOAc 5:4) and recrystallized from benzene to obtain **1** (24.3 mg). Part of the Et_2O washings (37.2 mg) containing the two close TLC R_f values spots, were acetylated and separated by prep. TLC (CH_2Cl_2–Me_2CO, 100:1) to obtain **1a** (15.1 mg) and **2a** (16.2 mg). Then, **2a** was hydrolyzed with TsOH in MeOH to give **2** (9.2 mg). Fr. 9 (8.64 g) was resubjected to CC on silica gel, eluting the *n*-hexane gradually enriched with EtOAc, to obtain 13 frs (9-1 ~ 9-13). Fr. 9-11 (*n*-hexane–EtOAc (10:1); 0.58 g) was resubjected to CC on silica gel, eluting with *n*-hexane gradually enriched with CH_2Cl_2, to obtain 7 frs (9-11-1 ~ 9-11-7). Fr. 9-11-5 (*n*-hexane–CH_2Cl_2, 1:10; 40.4 mg) afforded **3** (1.5mg). Fr. 15 (3.07 g) was resubjected to CC on silica gel, eluting with *n*-hexane gradually enriched with CH_2Cl_2, to obtain 9 frs (15-1 ~ 15-9). Fr. 15-5 (*n*-hexane–CH_2Cl_2, 1:2; 1.13 g) was resubjected to CC on silica gel, eluting with *n*-hexane gradually enriched with EtOAc, to obtain 18 frs (15-5-1 ~ 15-5-18). Fr. 15-5-17 (EtOAc, 15 mg), furnished **4** (1.5 mg).

*Obovatifol (**1**).* Amorphous. $[\alpha]_D^{25}$ −50° ($CHCl_3$, *c* 0.336). IR ν_{max} (film) cm^{-1}: 3350 (OH), 2950, 1605, 1495 (aromatic ring). UV λ_{max} nm (log ε): 210 (4.68), 271 (4.24). UV λ_{max} (KOH) nm (log ε): 217 (4.69), 271 (4.29). EIMS *m/z* (rel. int.): 342 $[M]^+$ (100), 327 (10), 309 (12). HRMS: $C_{20}H_{22}O_5$. Found: 342.1466, calcd. 342.1467. ^1H NMR: δ 1.37 (3H, *d*, *J* = 6.8 Hz, Me-8), 1.87 (3H, *d*, *J* = 6.8, 1.6 Hz, Me-8′), 3.41 (1H, *dq*, *J* = 8.8, 6.8 Hz, H-8), 3.84 (3H, *s*, OMe-5), 3.89 (3H, *s*, OMe-3′), 5.05 (1H, *d*, *J* = 8.8 Hz, H-7), 5.59 (2H, *br s*, OH-3 and 4, D_2O exchangeable), 6.11 (1H, *dd*, *J* = 16.0, 6.8 Hz, H-8′), 6.36 (1H, *dd*, *J* = 16.0, 1.6 Hz, H-7′), 6.58 (1H, *d*, *J* = 2.0 Hz, H-6), 6.65 (1H, *d*,

J = 2.0 Hz, H-2), 6.76 (1H, *s*, H-6′), 6.78 (1H, *s*, H-2′). ^{13}C NMR: δ 17.7 (C-9), 18.3 (C-9′), 45.6 (C-8), 55.9 (OMe-5), 56.2 (OMe-3′), 93.6 (C-7), 101.4 (C-6), 107.2 (C-2), 109.3 (C-2′), 113.3 (C-6′), 123.4 (C-8′), 130.9 (C-7′), 132.1 (C-1), 132.2 (C-1′), 132.4 (C-5′), 133.2 (C-3), 143.7 (C-4), 144.0 (C-3′), 146.5 (C-4′), 147.0 (C-5).

Acetylation of ***1*** *and* ***2***. Ac_2O (3 ml) was added to a soln of the mixt. (37.2 mg) of **1** and **2** in pyridine (3 ml), and the resultant soln stirred overnight at room temp., to afford an acetylated mixt. (41.4 mg). This mixt. was then separated by prep. TLC (CH_2Cl_2–Me_2CO, 100:1) to obtain the acetylated derivatives, **1a** (15.1 mg) and **2a** (16.2 mg).

*Obovatifol diacetate (**1a**)*. Oil. $[\alpha]_D^{25}$ −9.8° ($CHCl_3$, *c* 0.51). IR ν_{max} (neat) cm^{-1}: 2950, 1780 (OCO), 1610, 1500 (aromatic ring). UV λ_{max} nm (log ε): 207 (4.72), 272 (4.18). EIMS *m/z* (rel. int.): 426 [M]$^+$ (98), 384 (76), 342 (100). HRMS: $C_{24}H_{26}O_7$. Found: 426.1682, calc. 426.1685. ^{1}H NMR: δ 1.42 (3H, *d*, J = 6.8 Hz, Me-8), 1.86 (3H, *dd*, J = 6.8, 1.6 Hz, Me-8′), 2.26 (3H, *s*, OAc), 2.29 (3H, *s*, OAc), 3.45 (1H, *dq*, J = 8.8, 6.8 Hz, H-8), 3.83 (3H, *s*, OMe-5), 3.90 (3H, *s*, OMe-3′), 5.14 (1H, *d*, J = 8.8 Hz, H-7), 6.10 (1H, *dq*, J = 15.6, 6.8 Hz, H-8′), 6.37 (1H, *dd*, J = 15.6, 1.6 Hz, H-7′), 6.75 (1H, *s*, H-6′), 6.78 (1H, *s*, H-2′), 6.84 (1H, *d*, J = 1.6 Hz, H-2), 6.94 (1H, *d*, J = 1.6 Hz, H-6). ^{13}C NMR: δ 18.2 (C-9), 18.3 (C-9′), 20.3 (OCOMe), 20.5 (OCOMe), 45.8 (C-8), 56.0 (OMe-5), 56.3 (OMe-3′), 92.5 (C-7), 107.5 (C-6), 109.5 (C-2′), 112.8 (C-2), 113.4 (C-6′), 123.6 (C-8′), 130.8 (C-7′), 131.6 (C-1), 132.5 (C-1′), 132.8 (C-5′), 139.1 (C-3), 143.3 (C-3′), 144.1 (C-5), 146.3 (C-4′), 152.5 (C-4), 167.7 (OCO), 168.0 (OCO).

*Obovaten diacetate (**2a**)*. Colourless needles (benzene), mp 144–145° $[\alpha]_D^{25}$ ±0° ($CHCl_3$, *c* 0.10). IR ν_{max} (KBr) cm^{-1}: 2950, 1780 (OCO), 1615, 1500 (aromatic ring). UV λ_{max} nm (log ε): 230 (4.68), 268 (4.74), 306 (4.69). EIMS *m/z* (rel. int.): 424 [M]$^+$ (27), 382 (63), 340 (100). HRMS: $C_{24}H_{24}O_7$. Found: 424.1511, calc. 424.1522. ^{1}H NMR: δ 1.92 (3H, *dd*, J = 6.4, 1.6 Hz, Me-8′), 2.32 (3H, *s*, OAc), 2.33 (3H, *s*, OAc), 2.44 (3H, *s*, Me-8), 3.93 (3H, *s*, OMe-5), 4.04 (3H, *s*, OMe-3′), 6.22 (1H, *dq*, J = 16.0, 6.4 Hz, H-8′), 6.49 (1H, *dd*, J = 16.0, 1.6 Hz, H-7′), 6.85 (1H, *d*, J = 1.2 Hz, H-2′), 7.06 (1H, *d*, J = 1.2 Hz, H-6′), 7.21 (1H, *d*, J = 1.6 Hz, H-2), 7.30 (1H, *d*, J = 1.6 Hz, H-6). ^{13}C NMR: δ 9.7 (C-9), 18.4 (C-9′), 20.3 (OCOMe), 20.6 (OCOMe), 56.2 (OMe-5), 56.5 (OMe-3′), 105.2 (C-2′), 108.2 (C-6), 109.4 (C-6′), 112.5 (C-8), 113.7 (C-2), 124.7 (C-8′), 129.6 (C-1), 131.3 (C-7′), 131.5 (C-1′), 132.8 (C-5′), 133.9 (C-4′), 142.4 (C-3′), 143.3 (C-5), 145.0 (C-3), 149.8 (C-4), 152.5 (C-7), 167.8 (OCO), 168.1 (OCO).

Acid hydrolysis of ***2a***. Compound **2a** (16.2 mg) was treated at room temp. with 5% TsOH in MeOH (8 ml) and stirred overnight. The reaction mixt. was quenched with ice-H_2O and concd *in vacuo* to remove MeOH. The residue was extracted with CH_2Cl_2, dried (K_2CO_3) and then concd to obtain **2** (9.2 mg).

*Obovaten (**2**)*. Colourless needles (benzene), mp 159–161° $[\alpha]_D^{25}$ ±0° ($CHCl_3$, *c* 0.16). IR ν_{max} (KBr) cm^{-1}: 3300 (OH), 2950, 2850, 1600, 1510 (aromatic ring). UV λ_{max} nm (log ε): 238 (4.67), 270 (4.61), 306 (4.60). UV λ_{max} (KOH) nm (log ε): 243 (4.40) 319 sh (4.04). EIMS *m/z* (rel. int.): 340 [M]$^+$ (100), 325 (7), 297 (8) 170 (16). HRMS: $C_{20}H_{20}O_5$. Found: 340.1313, calc. 340.1311. ^{1}H NMR: δ 1.91 (3H, *dd*, J = 6.4, 1.6 Hz, Me-8′), 2.41 (3H, *s*, Me-8), 3.97 (3H, *s*, OMe-5), 4.04 (3H, *s*, OMe-3′), 5.46 (1, *br s*, OH-3 or 4, D_2O-exchangeable), 5.58 (1H, *br s*, OH-4 or 3, D_2O-exchangeable), 6.22 (1H, *dq*, J = 16.0, 6.4 Hz, H-8′), 6.49 (1H, *dd*, J = 16.0, 1.6 Hz, H-7′), 6.83 (1H, *s*, H-2), 6.97 (1H, *s*, H-6), 7.03 (1H, *s*, H-2), 7.04 (1H, *s*, H-6′). ^{13}C NMR: δ 9.6 (C-9), 18.4 (C-9′), 56.1 (OMe-5), 56.4 (OMe-3′), 102.3 (C-6), 104.7 (C-2′), 107.9 (C-2), 109.2 (C-6′), 110.6 (C-8), 123.3 (C-1), 124.4 (C-8′), 131.5 (C-7′), 132.6 (C-1′), 133.1 (C-5′), 133.6 (C-4′), 142.1 (C-3′), 143.8 (C-5), 144.8 (C-3), 147.0 (C-4), 151.2 (C-7).

*Perseal C (**3**)*. Oil. $[\alpha]_D^{25}$ −20° ($CHCl_3$, *c* 0.03). IR ν_{max} (log ε): 207 (4.42), 236 (4.34), 290 (4.15), 300 (4.13). UV λ_{max} (KOH) nm (log ε): 211 (4.66), 240 sh (4.39), 305 (4.15). UV ν_{max} (neat) cm^{-1}: 3450 (OH), 2920, 2850, 1680 (CHO), 1600, 1500 (aromatic ring), 1040, 940 (OCH_2O). EIMS *m/z* (rel. int.): 328 [M]$^+$ (85), 310 (100), 298 (47), 280 (30), 252 (24). HRMS: $C_{18}H_{16}O_6$. Found: 328.0959, calc. 328.0947. ^{1}H NMR: δ 3.66 (1H, *dd*, J = 6.4, 6.0 Hz, H-8), 3.88 (1H, *dd*, J = 14.8, 6.0 Hz, H-9a), 3.90 (1H, *br s*, OH-9, D_2O-exchangeable), 3.96 (3H, *s*, OMe-3′), 3.97 (1H, *dd*, J = 14.8, 6.0 Hz, H-9b), 5.69 (1H, *d*, J = 6.4 Hz, H-7), 5.95 (2H, *s*, OCH_2O), 6.78 (1H, *dd*, J = 8.0, 1.2 Hz, H-6), 6.87 (1H, *d*, J = 8.0 Hz, H-5), 6.88 (1H, *d*, J = 1.2 Hz, H-2), 7.40 (1H, *d*, J = 1.0 Hz, H-6′, 7.41 (1H, *d*, J = 1.0 Hz, H-2′), 9.84 (1H, *s*, CHO).

*Perseal D (**4**)*. Oil. $[\alpha]_D^{25}$ ±0° ($CHCl_3$, *c* 0.05). IR ν_{max} (neat) cm^{-1}: 3350 (OH), 2950, 2850, 1680 (CHO), 1600, 1510 (aromatic ring). UV λ_{max} nm (log ε): 205 (4.46), 237 (4.47), 295 (4.52). UV λ_{max} (KOH) nm (log ε): 205 (4.62), 243 (4.46), 303 (4.35). EIMS *m/z* (rel. int.): 328 [M]$^+$ (100), 313 (8), 285 (9). HRMS: $C_{18}H_{16}O_6$. Found: 328.0939, calc. 328.0947. ^{1}H NMR: δ 2.49 (3H, *s*, Me-8), 3.99 (3H, *s*, OMe-5), 4.09 (3H, *s*, OMe-3′), 5.45 (2H, *br s*, OH-3 and 4, D_2O-exchangeable), 6.98 (1H, *d*, J = 1.8 Hz, H-6), 7.05 (1H, *d*, J = 1.8 Hz, H-2), 7.37 (1H, *d*, J = 1.2 Hz, H-2′), 7.67 (1H, *d*, J = 1.2 Hz, H-6′), 10.03 (1H, *s*, CHO).

Cytotoxicity assay

Activities against P-388 (mouse lymphocytic leukemia), KB16 (human mouth epidermoid carcinoma), A549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) cells were assayed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] colorimetric method [10, 11].

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