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CYTOTOXIC NEOLIGNANS FROM *PERSEA OBOVATIFOLIA*

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Key Word Index—*Persea obovatifolia*; Lauraceae; leaves; neolignans; obovatinal; perseal A; perseal B; cytotoxicity.

Abstract—Three new formyl neolignans, including obovatinal [(2*S*,3*S*)-2,3-dihydro-2-(4,5-dimethoxy-3-hydroxyphenyl)-5-formyl-7-methoxy-3-methyl benzofuran], perseal A and perseal B (erythro- and threo- 1-(4-hydroxy-3-methoxyphenyl)-2-(4-formyl-2-methoxyphenoxy) propan-1-ol) were isolated and characterized from the leaves of *Persea obovatifolia*. The structures of these compounds were elucidated from spectral evidence. The new neolignans all showed significant cytotoxicity against P-388, KB16, A549 and HT-29 cancer cell lines. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Persea obovatifolia is a small evergreen tree, endemic in Taiwan but only found in the Hengchun Peninsula in Taiwan [1]. In a series of studies on the anticancer constituents of Formosan plants, we have screened more than 250 species for *in vitro* cytotoxicity against tumour cell lines. *Persea obovatifolia* was one of the active species and its leaves showed significant cytotoxic activity against P-388, KB16, A549 and HT-29. A previous study reported the isolation of three benzylisoquinoline alkaloids from its root [2]. In the present investigation, bioassay-guided fractionation led to the isolation and characterization of three new cytotoxic formyl neolignans, obovatinal (**1**), perseal A (**2**) and perseal B (**3**) from the chloroform soluble fractions of the leaves. The isolation and structural elucidation of the active principles and their cytotoxic activities are described.

RESULTS AND DISCUSSION

The molecular formula, $C_{19}H_{20}O_6$, of obovatinal (**1**), was determined by EI ($[M]^+$, *m/z* 344) and high-resolution mass spectrometry. The UV absorptions indicated the presence of a benzenoid moiety and the IR spectrum showed formyl absorption at 1680 cm^{-1} and a hydroxyl group at 3375 cm^{-1}. The ^1H NMR features of compound **1** were similar to those of licarin A in the 7-aryl-3′-methoxy-8-methyl-7,8-dihydrobenzofuran unit [3, 4]. However, compound **1** showed the presence of a C-1′ formyl group on the dihydrobenzofuran at δ 9.84 in ^1H NMR spectrum and δ 190.6 in ^{13}C NMR spectrum, instead of propenyl signals. Three methoxyl singlets were assigned to C-5, C-4 and C-3′, and one hydroxyl singlet to C-3. The aromatic regions indicated the presence of two pairs of *meta*-coupled protons, one at δ 7.33 and δ 7.37 (each 1 H, *d*, J = 1.2 Hz) assigned to H-6′ and H-2′, respectively, and another at δ 6.54 and δ 6.65 (each 1 H, *d*, J = 2.0 Hz) assigned to H-6 and H-2, respectively. The coupling constant (J = 8.8 Hz) between H-7 (δ 5.22) and H-8 (δ 3.54) indicated the relative *trans*-vicinal coupling of the dihydrofuran ring [5]. The absolute configuration of compound **1** was proposed as 7*S*-aryl, 8*S*-methyl-substituted dihydrobenzofuran [6] by comparing its specific rotation ($[\alpha]_D$ −37°) with machilin B ($[\alpha]_D$ −40.1°) [7] and licarin B ($[\alpha]_D$ −44°) [3]. From NOESY experiments (Fig. 1), the C-5 methoxyl group was correlated with H-6 but the C-4 methoxyl group was not correlated wtih H-2. Furthermore, compound **1** gave a positive Gibb's test so it is reasonable to locate the two methoxyl groups at the C-4, C-5 positions and one hydroxyl group at the C-3 position on the 7-aryl ring. From the above data, structure **1** was determined and also confirmed by COSY, DEPT and HETCOR experiments.

Perseal A (**2**) and perseal B (**3**) have the same molecular formula of $C_{18}H_{20}O_6$ as determined by EI ($[M]^+$, *m/z* 332) and high-resolution mass spectrometry. Both UV absorptions suggested the presence of a benzenoid moiety and the IR spectrum exhibited formyl and a hydroxyl absorptions. The ^1H and ^{13}C NMR spectral data were similar to those of the neolignan, machilin C [7], and revealed the planar structure of compounds **2** and **3** with two adjacent chiral centres on the β-aryloxyarylpropane skeleton. However, the major

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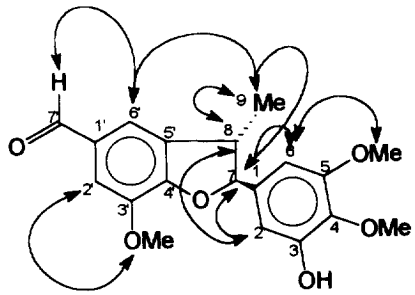
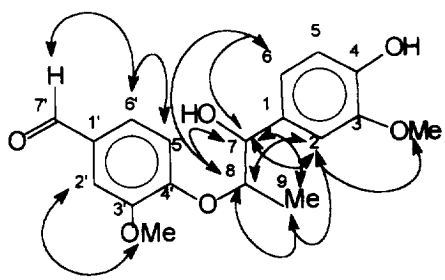
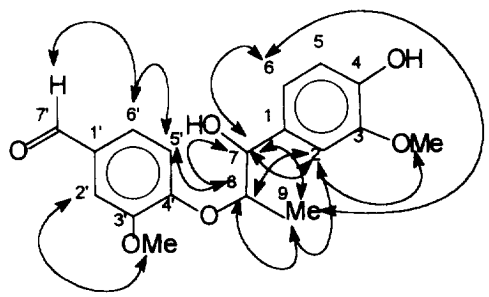


1



2 (*erythro*)



3 (*threo*)

Fig. 1. NOESY correlations of compounds 1–3.

difference between compounds, 2 and 3 and machilin C, was the presence of a C-1′ formyl group at δ 9.86 (*s*) in the ^{1}H NMR spectrum and δ 190.8 in the ^{13}C NMR spectrum, instead of a propenyl group. There were two pairs of 1,3,4-trisubstituted aromatic protons that were assigned to H-2, H-5, H-6, and H-2′, H-5′, H-6′. Comparison of IR, UV, ^{1}H NMR and ^{13}C NMR spectral data for compounds 2 and 3 indicated that both have the same planar structure. However, comparison of the chemical shifts of C-9 (δ 13.4) and C-7 (δ 74.7), and the coupling constants (J = 3.6 Hz) of H-7 (δ 4.90) and H-8 (δ 4.61), with the reported data, indicated that 2 had the erythro-configuration [7–11]. Moreover, the chemical shifts of C-9 (δ 16.4) and C-7 (δ 78.0), and the larger coupling constants (J = 8.0 Hz) of H-7 (δ 4.71) and H-8 (δ 4.40) of compound 3, were different from those of 2. Thus, compound 3 was deduced to be the threo-diasteroisomer of compound 2 [7–11]. From HETCOR experiments were observed C-7 (δ 74.7 and δ 78.0) corresponding to H-7 (δ 4.90 and δ 4.71) and C-8 (δ 80.5 and δ 82.1) to H-8 (δ 4.61 and δ 4.40) of compounds 2 and 3 that were different from those reported [7–11]. The proposed structures were also corroborated by COSY, DEPT, HETCOR and NOESY (Fig. 1) experiments. Neolignans bearing a C-1′ formyl group instead of a C-1′-propanoid group are rare in nature.

Table 1. Cytotoxicity of compounds 1–3

Compound	ED_{50} (μg ml^{-1})			
	P-388	KB16	A549	HT-29
Obovatinol (1)	0.487	0.149	0.705	0.614
Perseal A (2)	0.552	0.266	0.290	0.708
Perseal B (3)	0.745	0.225	1.493	0.794
Mithramycin*	0.061	0.084	0.076	0.082

*Positive control.

Compounds 1–3 showed significant cytotoxic activities against P-388, KB16, A549 and HT-29 cancer cell lines (Table 1); mithramycin was used as a positive control. Their cytotoxic activities were not as potent as mithramycin. It should be noted that the erythro compound (2) showed a fivefold more potent cytotoxicity against A549 adenocarcinoma than that of the corresponding threo isomer (3).

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR (400 MHz) and ^{13}C NMR (50 and 100 MHz): in $CDCl_3$. Chemical shifts are given in δ with TMS as int. standard. Mass spectra were measured using a direct inlet system. UV spectra were determined in EtOH. IR recorded neat, unless specified. CC: silica gel (Merck, 70-230 and 230-400 mesh); TLC on silica gel plates (Merck, 60 GF-254).

Plant material. Fresh leaves of *P. obovatifolia* Kost. (*Machilus obovatifolia* Kanehira et Sasaki) were collected from Hengchun Peninsula, Pingtung Hsien, Taiwan, in August 1994. A voucher sample is deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, R.O.C.

Extraction and isolation. Dried leaves (4.9 kg) were extracted with MeOH and concd under red. pres. to a dark residue (0.83 kg). The MeOH extract was partitioned between H_2O–$CHCl_3$ (1:1) and extracted exhaustively with $CHCl_3$ to yield a $CHCl_3$-sol. fr. (0.41 kg). Part of this fr. (0.11 kg) was chromatographed over silica gel and eluted with $CHCl_3$ and $CHCl_3$–MeOH mixts to give 26 frs. Fr. 4 (8.64 g, $CHCl_3$) was rechromatographed on silica gel using *n*-hexane and *n*-hexane–EtOAc mixts to yield 13 frs. Fr. 4-11 (0.58 g, *n*-hexane–EtOAc, 10:3) was rechromatographed on silica gel and eluted with CH_2Cl_2 and CH_2Cl_2–Me_2CO mixts to obtain 7 frs. Fr. 4-11-3 (65.6 mg, *n*-hexane–CH_2Cl_2, 2:3) was purified by prep. TLC (benzene–

CH_2Cl_2–Me_2CO, 7:2:1) to yield compound **1** (6 mg, *Rf* 0.62). Fr 4-11-4 (170.1 mg, *n*-hexane CH_2Cl_2, 1:5) was purified by prep. TLC (benzene–Me_2CO, 5:1) and recrystallized (benzene) to yield compound **2** (5.7 mg, *Rf* 0.46) and **3** (5.4 mg, *Rf* 0.38), respectively.

Obovatinal (**1**). Oil. $[\alpha]_D$ −37° ($CHCl_3$; *c* 0.112). IR ν_{max} cm^{-1}: 3375 (OH), 2930, 2850, 1680 (CHO), 1595, 1500 (aromatic ring). UV λ_{max} nm (log ε): 230 (4.44), 280 (4.22), 310 (4.12). UV λ_{max}^{KOH} nm (log ε): 230 sh (4.38), 280–300 (4.21). EI-MS *m/z* (rel. int.): 344 $[M]^+$ (100), 329 (8.3), 311 (10.8), 283 (9.3), 269 (26.7), 251 (12.1), 241 (10), 221 (7.9), 197 (7.9), 181 (10), 167 (21), 151 (47.6). HR-MS: $C_{19}H_{20}O_6$, Found: 344.1268, calcd.: 344.1260. ^1H NMR (400 MHz): δ 1.46 (3H, *d*, *J* = 7.2 Hz, Me-8), 3.54 (1H, *dq*, *J* = 8.8, 7.2 Hz, H-8), 3.86 (3H, *s*, OMe-5), 3.90 (3H, *s*, OMe-4), 3.95 (3H, *s*, OMe-3′), 5.22 (1H, *d*, *J* = 8.8 Hz, H-7), 5.82 (1H, *s*, OH-3, disappeared after addition of D_2O), 6.54 (1H, *d*, *J* = 2.0 Hz, H-6), 6.65 (1H, *d*, *J* = 2.0 Hz, H-2), 7.33 (1H, *d*, *J* = 1.2, H-6′), 7.37 (1H, *d*, *J* = 1.2 Hz, H-2′), 9.84 (1H, *s*, CHO). ^{13}C NMR (50 MHz): δ 18.2 (Me-9), 45.0 (C-8), 56.0 (OMe-5), 56.1 (OMe-3′), 61.0 (OMe-4), 94.5 (C-7), 102.1 (C-6), 106.2 (C-2), 111.8 (C-2′), 120.1 (C-6′), 131.5 (C-1′), 133.5 (C-5′), 135.6 (C-1), 135.7 (C-4), 145.0 (C-3), 149.4 (C-5), 152.6 (C-3′), 153.1 (C-4′), 190.6 (CHO). Gibb's test: positive.

Perseal A (**2**). Oil. $[\alpha]_D$ +26° ($CHCl_3$; *c* 0.05). IR ν_{max} cm^{-1}: 3425 (OH), 2950, 2850, 1675 (CHO), 1600, 1500 (aromatic ring). UV λ_{max} nm log (ε): 230 (4.49), 280 (4.29), 310 (4.18). UV λ_{max}^{KOH} nm (log ε): 231 sh (4.45), 250 sh (4.25), 281 (4.28), 300 (4.25). EI-MS *m/z* (rel. int.): 332 $[M]^+$ (3.8), 180 (91.7), 165 (23), 153 (88.1), 152 (41.3), 151 (100), 136 (29.3), 135 (82). HR-MS: $C_{18}H_{20}O_6$, Found: 332.1259; calcd.: 332.1258. ^1H NMR (400 MHz): δ 1.25 (3H, *d*, *J* = 6.0 Hz, Me-8), 3.01 (1H, *s*, OH-7, disappeared after addition of D_2O), 3.91 (3H, *s*, OMe-3), 3.93 (3H, *s*, OMe-3′), 4.61 (1H, *dq*, *J* = 3.6, 6.0 Hz, H-8), 4.90 (1H, *d*, *J* = 3.6 Hz, H-7), 5.62 (1H, *s*, OH-4, disappeared after addition of D_2O), 6.83 (1H, *dd*, *J* = 8.2, 1.6 Hz, H-6), 6.88 (1H, *d*, *J* = 8.2 Hz, H-5), 7.04 (1H, *d*, *J* = 1.6 Hz, H-2), 7.05 (1H, *d*, *J* = 8.4 Hz, H-5′), 7.44 (1H, *d*, *J* = 1.6 Hz, H-2′), 7.45 (1H, *d*, *J* = 8.4, 1.6 Hz, H-6′), 9.86 (1H, *s*, CHO). ^{13}C NMR (50 MHz): δ 13.4 (Me-9), 55.9 (OMe-3), 56.0 (OMe-3′), 74.7 (C-7), 80.5 (C-8), 109.0 (C-2), 110.0 (C-6′), 114.0 (C-5), 115.6 (C-5′), 119.5 (C-6), 126.3 (C-2′), 131.0 (C-1), 131.5 (C-1′), 145.2 (C-4), 146.5 (C-3), 151.1 (C-3′), 152.6 (C-4′), 190.8 (CHO).

Perseal B (**3**). Amorphous powder. $[\alpha]_D$ +54° ($CHCl_3$; *c* 0.1). IR ν_{max} (film) cm^{-1}: 3420 (OH), 2930, 2850, 1675 (CHO), 1590, 1510 (aromatic ring). UV λ_{max} nm (log ε): 230 (4.51), 280 (4.32), 309 (4.19). UV λ_{max} (KOH) nm (log ε): 230 sh (4.45), 253 (4.12), 282 (4.31), 301 (4.29). EI-MS *m/z* (rel. int.): 332 $[M]^+$ (3.46), 180 (84.7), 153 (83.8), 151 (100), 135 (46). HR-MS: $C_{18}H_{20}O_6$, Found: 332.1254; calcd.: 332.1256. ^1H NMR (400 MHz): δ 1.22 (3H, *d*, *J* = 6.4 Hz, Me-8), 3.42 (1H, *br s*, OH-7, disappeared after addition of D_2O), 3.90 (3H, *s*, OMe-3), 3.96 (3H, *s*, OMe-3′), 4.40 (1H, *dq*, *J* = 8.0, 6.4 Hz, H-8), 4.71 (1H, *d*, *J* = 8.0 Hz, H-7), 5.63 (1H, *s*, OH-4, disappeared after addition of D_2O), 6.88 (1H, *dd*, *J* = 8.0, 1.6 Hz, H-6), 6.90 (1H, *d*, *J* = 8.0 Hz, H-5), 6.94 (1H, *d*, *J* = 1.6 Hz, H-2), 7.05 (1H, *d*, *J* = 8.0 Hz, H-5′), 7.44 (1H, *d*, *J* = 8.0, 1.6 Hz, H-6′), 7.46 (1H, *d*, *J* = 1.6 Hz, H-2′), 9.86 (1H, *s*, CHO). ^{13}C NMR (100 MHz): δ 16.4 (Me-9), 56.0 (OMe-3), 56.0 (OMe-3′), 78.0 (C-7), 82.1 (C-8), 109.3 (C-2), 109.9 (C-6′), 114.2 (C-5), 115.2 (C-5′), 120.6 (C-6), 126.3 (C-2′), 131.0 (C-1), 131.4 (C-1′), 145.8 (C-4), 146.7 (C-3), 150.8 (C-3), 153.2 (C-4′), 190.8 (CHO).

Cytotoxicity assay. Activities against P-388 (mouse lymphocytic leukaemia), KB16 (human nasopharyngeal carcinoma), A549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) cells were assayed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric method [12–13].

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