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Lignans from *Daphne feddei* Levl. var and Their Cytotoxicity

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A new neolignan, daphnelignan C (**1**), together with four known one, were isolated from the leaves of *Daphne feddei* levl. var. The structures of daphnelignan C (**1**) were elucidated by spectroscopic methods, including extensive 1-D and 2-D NMR techniques. All the five compounds were tested for their cytotoxicity. Compound daphnelignan C (**1**) showed significant potential cytotoxic ability and weak anti HIV-1 activities.

Key Words: *Daphne feddei* levl. var., Lignans, Daphnelignan C, Cytotoxicity.

INTRODUCTION

Daphne feddei levl. var is an evergreen shrub mainly distributed in Yunnan Province, P.R. China. It has been used as a traditional Chinese medicine named Dali Rui Xiang" for the treatment of rheumatoid arthritis, apoplexia, stomach ache[1,2]. The extract of *Daphne feddei* levl. var has also been used as cigarette additivity[1]. Previous phytochemical research on daphne family has revealed that flavanes[3], coumarins[4,5] daphnane diterpenes[6-8], as well as lignans[9-11] are major principles isolated from the plant of this family.

As a part of our search for bioactive materials, the phytochemical study of an EtOAc extract of *Daphne feddei* levl. var led to the isolation of one neolignan (**1**), along with four known compounds (**2-5**). The structural elucidation of compound **1** and the evaluation of the cytotoxicity of **1-5** in several cancer cell lines are reported in the present study.

EXPERIMENTAL

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1-D and 2-D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively.

Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 mm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 mm) column. Column chromatography was performed with silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 5 % H_2SO_4 in EtOH.

Plant material: The leave of *Daphne feddei* levl. var were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in September 2007. The identification of plant material was verified by Prof. Yuan Ning. A voucher specimen (YNNI-07-09-12) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaves of *Daphne feddei* levl. var (4 kg) were extracted four times with 70 % aqueous Me_2CO (4.0 L × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure and partitioned with EtOAc (3 L × 4 L). The EtOAc partition (125 g) was applied to silica gel (200-300 mesh) column chromatography eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give five fractions A-E. The separation of fraction B (14.7 g) by silica gel column chromatography eluted with petroleum ether-acetone (20:1-1:2) yielded mixtures B1-B7. Fraction B2 (6.25 g) was subjected to silica gel column chromatography using petroleum ether-acetone and preparative HPLC (68 % MeOH-H_2O, flow rate 12 mL/min) to give compounds **1** (12.5 mg), **3** (32.8 mg). Fraction B3 (2.22 g) was subjected to silica gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (63 % MeOH-H_2O, flow rate 12 mL/min) to yield compounds **2** (15.6 mg), **5** (19.5 mg). Fraction B4 (1.86 g) was subjected to silica gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (58 % MeOH-H_2O, flow rate 12 mL/min) to give compounds **4** (13.6 mg).

Cytotoxicity assays: The cytotoxicity tests for the isolates were performed using a previously reported procedure[12]. All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50 % reduction of absorbance compared with untreated cells. The cytotoxic ability against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) was shown in Table-1.

Schilancifolignans D: Compound **1**: obtained as pale yellow amorphous solid; $[\alpha]_D^{22.6}$ + 11.5 (c 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 322 (2.47), 278 (4.12), 205 (4.92) nm; IR (KBr, ν_{max}, cm^{-1}): 3450, 2958, 2925, 2882, 2830, 1612, 1594, 1520, 1458, 1418, 1368, 1325, 1274, 1230, 1182, 1140, 1028, 970, 821; ^1H and ^{13}C NMR data, Table-2; positive ESIMS m/z 353 $[M + Na]^+$; HRESIMS m/z 353.1720 $[M + Na]^+$ (calcd. (%) for $C_{20}H_{26}O_4$, 353.1729).

TABLE-1
CYTOTOXICITIES OF CHILANCIFOLIGNANS D AND CHILANCIFOLIGNANS E

Compounds	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	4.02	8.16	3.18	5.22
2	5.08	25.20	15.30	25.60
3	22.40	31.60	17.20	14.30
4	8.27	18.40	33.60	11.40
5	11.80	16.20	8.67	9.22
Camptothecin	1.34	0.83	1.51	1.68

Data are IC_{50} values in μmol/L. For a compound to be deemed effective, an IC_{50} value < 100 μmol/L is required. Camptothecin was used as a positive control. HL-60, human acute promyelocytic leukemia; Hep-G2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells.

TABLE-2
^1H AND ^{13}C NMR DATA OF COMPOUNDS **1** (δ IN ppm, DATA OBTAINED IN PYRIDINE-d_5)

No.	^{13}C	^1H	No.	^{13}C	^1H
1	137.5 s	–	3'	146.6 s	–
2	108.5 d	6.92, s	4'	143.2 s	–
3	146.9 s	–	5'	116.1 d	7.28-7.34 overlop
4	143.4 s	–	6'	120.1 d	7.28-7.34 overlop
5	116.4 d	7.28-7.34 overlop	7'	19.7 q	0.96, d, *J* = 6.4 Hz
6	120.3 d	7.28-7.34 overlop	8'	28.8 d	2.34-2.38 m
7	57.5 d	4.14, d, *J* = 11.6 Hz	9'	19.5 q	1.01, d, *J* = 6.5 Hz
8	39.7 d	3. 29-3.34, m	OMe-3	55.9 q	3.89 q
9	14.3 q	1.28, d, *J* = 6.7 Hz	OMe-3'	55.9 q	3.87 q
1'	136.8 s	–	OH-4	–	11.11 s
2'	108.4 d	6.89, s	OH-4'	–	11.14 s

RESULTS AND DISCUSSION

A 70 % aqueous acetone extract prepared from the leaves of *Daphne feddei* levl. var was partitioned between EtOAc and H_2O. The EtOAc layer was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-5** (Fig. 1), including one neolignans named daphnelignan C (**1**), together with 4 known lignans, 4,4-di-(4-hydroxy-3-methoxy-phenly)-2,3-dimethylbutanol (**2**)[13], austrobailignan-7 (**3**)[14], prinsepiol (**4**)[15], dehydro-diconiferyl alcohol (**6**)[16].

Compound **1** was obtained as yellow gum. Its molecular formula was determined as $C_{20}H_{26}O_4$ by HRESIMS m/z 353.1720 $[M + Na]^+$ calcd. (%) for 353.1729. Its ^1H and ^{13}C NMR spectra (Table-2) showed signals to 26 hydrogens and 20 carbons, respectively, corresponding to two aromatic rings with six aromatic protons, three methyl groups, two methoxy groups, two phenolic hydroxy groups and three methane

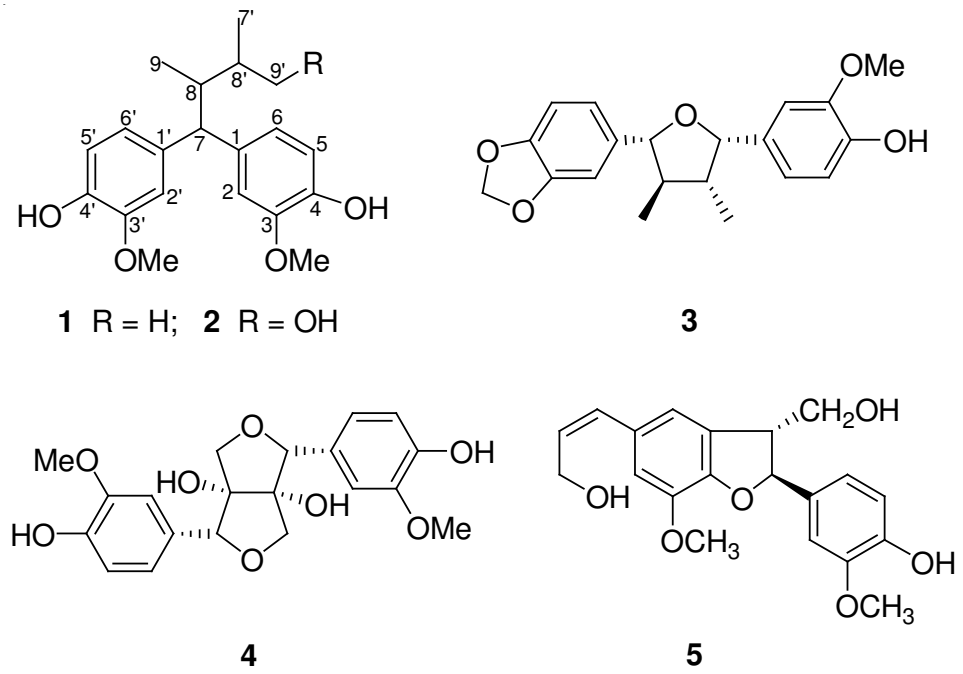

Fig. 1. Structure of compound **1-5**

signals. Strong absorption bands accounting for hydroxy (3450 cm^{-1}) and aromatic groups (1612, 1594, 1520, 1458 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed maximum absorption at 278 and 205 nm which confirmed the existence of the aromatic functions. The HMBC of compound **1** showed cross-peaks between H-7 (δ_H 4.14, d, 11.6) and carbons of both aromatic rings, C-2 (δ_C 108.5 d), C-6 (δ_C 120.3 d), C-2' (δ_C 108.4 d), C-6' (δ_C 120.1 d), whereas there was not correlations between H-8, H-8' and the aromatic carbon, which indicated that the two aromatic rings were linked to C-7. The ^1H-^1H COSY correlations of H-7/H-8/H-8'/H-9', H-8/H-9 and H-8'/H-7, together with HMBC correlations (Fig. 2) of H-7 (δ_H 4.14, d, 11.6) with C-8 (δ_C 39.7 d), C-9 (δ_C 14.3 q) and C-8' (δ_C 28.8 d) and of H-7' (δ_H 0.96, d, 6.4) with C-8' (δ_C 28.8 d), C-9' (δ_C 19.5 q), C-8 (δ_C 39.7 d), suggested the existence of a CH_3-$CH(CH_3)$-$CH(CH_3)$-CH structural unit in **1**. The ^1H and ^{13}C NMR spectra of **1** are similar to those of 4,4-di(4-hydroxy-3-methoxyphenly)-2,3-dimethylbutanol (**2**)[13]. Analysis of the ^1H and ^{13}C NMR data of **2** with those of **1** suggested that the difference was due to an oxidated methylene group (C-9) in **2** was substituted by a methyl groups in **1**, which was supported by the disappearance of singal of an oxidated methylene group and appearance of a methyl groups in **1**. Since the C-C bonds can rotate randomly, the relative configuration of compound **1** could not be determined on the basis of ROESY spectra. Thus, the structure of compound **1** was determined as shown and this compound given the name as daphnelignan C.

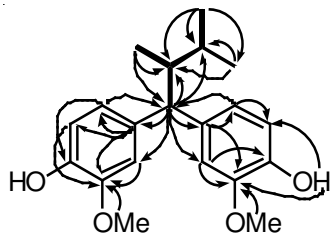

Fig. 2. Selected HMBC (→) and ^{1}H-^{1}H COSY (–) correlations of **1**

REFERENCES

1. Pharmacopoeia of the People's Republic of China (Part 1), Pharmacopoeia Commission of the Ministry of Public Health of PRC, Beijing, China, p. 621 (1990).
2. X.Y. Xiao, J. Shen, S.M. Wang, H.F. Xiao and G.B. Tong, *Palaeogeog. Palaeoclim. Palaeoecol.*, **287**, 45 (2010).
3. W. Zhang, Y.H. Shen, Z.Y. Lou, R.H. Liu, C. Zhang, P. Fu, L. Shan and W.D. Zhang, *Nat. Prod. Res.*, **21**, 1021 (2007).
4. J. Su, Z.J. Wu, W.D. Zhang, C. Zhang, H.L. Li, R.H. Liu and Y.H. Shen, *Chem. Pharm. Bull.*, **56**, 589 (2008).
5. J. Su, Z.J. Wu, R.H. Liu, Y.H. Shen, C. Zhang, H.L. Li, W. Zhang and W.D. Zhang, *Chin. Chem. Lett.*, **18**, 835 (2007).
6. L. Pan, X.F. Zhang, Y. Deng, Y. Zhou, H. Wang and L.S. Ding, *Fitoterapia*, **81**, 38 (2010).
7. M. Mahdavi and R. Yazdanparast, *Arch. Pharm. Res.*, **30**, 177 (2007).
8. Z.J. Zhan, C.Q. Fan, J. Ding and J.M. Yue, *Bioorg. Med. Chem.*, **13**, 645 (2005).
9. Y.C. Shen, Y.C. Lin, Y.B. Cheng, M.Y. Chiang, S.S. Liou and A.T. Khalil, *Phytochemistry*, **70**, 114 (2009).
10. S. Chandra, C. Guin, R.K. Kumar and G. Maiti, *Tetrahedron*, **58**, 2435 (2002).
11. L.G. Zhuang, O. Seligmann, H. Lotter and H. Wagner, *Phytochemistry*, **22**, 265 (1983).
12. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
13. L.J. Xu, F. Huang, S.B. Chen, L.N. Li, S.L. Chen and P.G. Xiao, *J. Integr. Plant Biol.*, **48**, 1493 (2006).
14. D. Takaoka, K. Watanabe and M. Hiroi, *Bull. Chem. Soc. (Japan)*, **49**, 3564 (1976).
15. S.B. Kilidhar, M.R. Parthasarathy and P. Sharma, *Phytochemistry*, **21**, 796 (1982).
16. H. Kasahara, Y. Jiao, D.L. Bedgar, S.J. Kim, A.M. Patten, Z.Q. Xia, L.B. Davin and N.G. Lewis, *Phytochemistry*, **67**, 1765 (2006).

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