Minor Furofurano Lignans from the Tibetan Herb, *Lancea tibetica*

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Key words
- *Lancea tibetica*
- Phrymaceae
- cytotoxicity
- furofurano lignin
- lantibeside
- lantibetin

Abstract

Four new furofurano lignans, lantibesides B – D (**1 – 3**) and lantibetin (**4**), along with nine known phenolic compounds were isolated from the EtOH extract of the traditional Tibetan medicinal plant, *Lancea tibetica*. By means of spectroscopic and chemical methods, the structures of the new compounds were elucidated as (1*R*,2*S*,5*R*,6*S*)-2-(3,4-methylenedioxyphenyl)-6-[(3-methoxy-4-*β*-D-xylopyranosyloxy(1→6)-*β*-D-glucopyranosyloxy)phenyl]-3,7-dioxabicyclo[3.3.0]octane (**1**), (1*R*,2*R*,5*R*,6*S*)-2-(3,4-methylenedioxyphenyl)-6-[(3-methoxy-4-*β*-D-xylopyranosyloxy(1→6)-*β*-D-glucopyranosyloxy)phenyl]-3,7-dioxabicyclo[3.3.0]octane (**2**), (1*R*,2*R*,5*R*,6*S*)-2-(3,4-methylenedioxyphenyl)-6-(3-methoxy-4-*β*-D-glucopyranosyloxy)phenyl-3,7-dioxabicyclo[3.3.0]octane (**3**), and (1*R*,2*R*,5*R*,6*S*)-2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (**4**). Compounds **2** and **3** showed weak cytotoxicity against the HL-60 cell line with IC_{50} values of 61 and 99 μM, respectively.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

The traditional Tibetan medicinal herbs are distributed in the pollution-free snowfields and plateaus, and are believed to be effective in the treatment of a number of disease. *Lancea tibetica* Hook. f. et Thoms (Phrymaceae), a perennial herb, is a traditional Tibetan medicinal plant used for the prevention and treatment of leukemia, intestinal angina, heart disease and cough by Zang people [1]. In our screening for the novel anti-cancer agents from Tibetan medicinal herbs, the ethanol extract of *L. tibetica* exhibited cytotoxicity against P388 cells. Although 12 compounds were previously isolated and their structures were elucidated from this plant, including sylvatesmin which showed anti-tumor and antioxidant activities *in vitro* [2], [3], [4], [5], [6], [7], further chemical investigation on this plant are still worthwhile. Bioassay-guided fractionation resulted in the isolation and identification of 4 new furofurano lignans **1 – 4**, and 9 known phenolic compounds, phillyrin (**5**) [8], simplexoside (**6**) [9], sesaminol 2′-*O*-*β*-D-glucoside (**7**) [10], phillygenol (**8**) [11], lantibeside (**9**) [7], tibeticoside A (**10**) [5], 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-*α*-L-rhamnopyranosyl-4-*O*-[(*E*)-3-(3,4-dihydroxyphenyl)propenoyl]-*β*-D-glucopyranoside (**11**) [12], apigenin (**12**) [13] and 5,4′-dihydroxyflavone (**13**) [14] (**Fig. 1**). The new compounds **1** and **3** showed weak cytotoxicity against HL-60 cells, with IC_{50} values of 61 and 99 μM. Since there was no report in the literature, NMR data of lantibeside (**9**) are also reported in this paper.

Materials and Methods

Equipment

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU® 640 spectrophotometer. CD spectra were recorded on a Jasco J-180 spectrometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr discs. 1D- and 2D-NMR spectra were recorded on a Jeol JNM-ECP 600 spectrometer using TMS as internal standard. ESI-MS were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. GC-EI-MS (70eV) data were obtained using an Agilent instrument (HP-5 MS column 30 m × 0.32 mm × 0.25 μm; He, 1 mL/min; detec-

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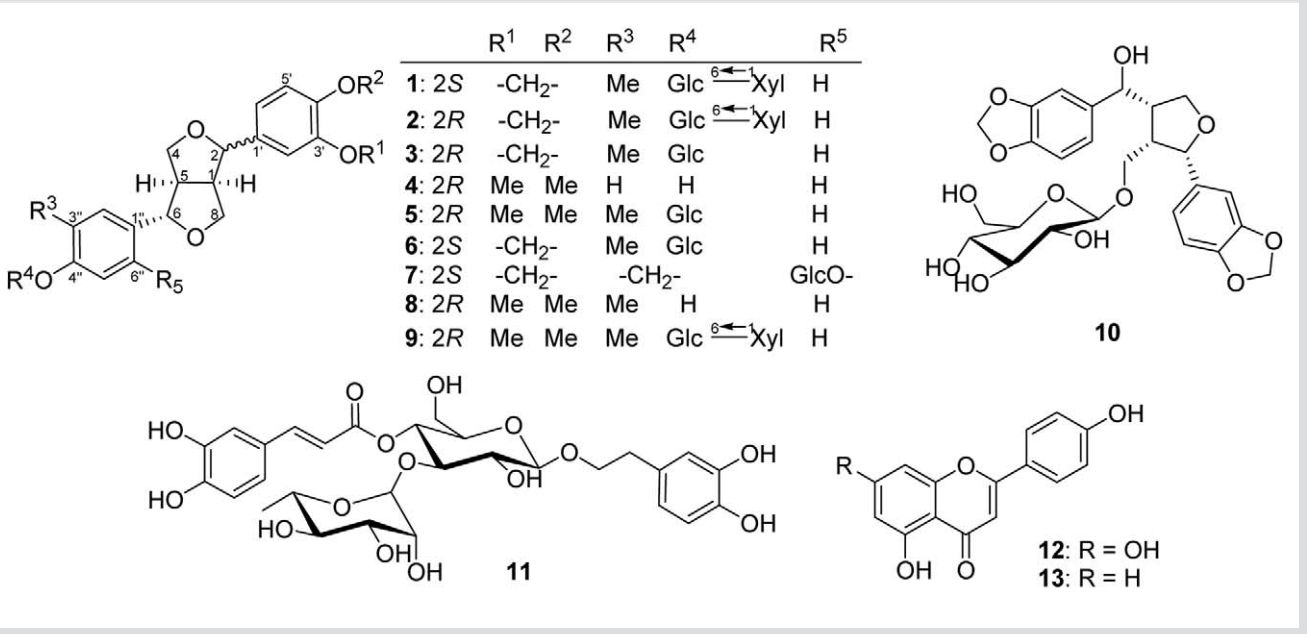

Fig. 1 Chemical structures of compounds **1 – 13**

tor temperature 120 – 250 °C; injection temperature 280 °C). Semipreparative HPLC was performed using an ODS column [YMC pack ODS (A), 20 × 250 mm, 5 μm, 4 mL/min; YMC]. TLC and column chromatography were performed on plates precoated with silica gel GF_{254} (10 – 40 μm) and over silica gel (200 – 300 mesh; Qingdao Marine Chemical Factory), macroporous resin AB-8 (Tianjin Chemical Plant) or Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory), or LiChroprep® RP-18 (25 – 40 μm; Merck KGaA), respectively. Solvents were distilled prior to use.

Plant material

Aerial parts of *L. tibetica* Hook. f. et Thoms were provided by the Pharmaceutical Factory of Tibetan Medicine of the Tibet Autonomous Region in November 2005. The plant material was identified by Prof. Zhandui, and the voucher specimen (Y-083) was deposited in the Tibetan Medicine Hospital.

Extraction and isolation

The plant material (8 kg) was extracted with 3 × 25 L of 95 % EtOH once a week at room temperature, and the obtained extract was then concentrated under vacuum to give the crude bisque. The crude bisque was further chromatographed on a macroporous resin column (12 × 110 cm), eluted with a stepwise gradient of H_2O-EtOH (v/v 7 : 3, 10 L; 1 : 1, 10 L; 3 : 7, 10 L; 1 : 9, 10 L) to obtain four fractions. Fraction 3 (68 g, water-EtOH, v/v 3 : 7) was dissolved in MeOH-$CHCl_3$ (v/v 1 : 1, 150 mL), absorbed on silica gel (100 mesh, 230 g), and subjected to VLC (9 × 35 cm, 500 g) of silica gel H, eluting with a gradient of $CHCl_3$-MeOH (v/v 1 : 0, 5 L; 20 : 1, 7 L; 10 : 1, 8 L; 8 : 2, 8 L) to obtain four subfractions, Frc3 – 1, Frc3 – 2 and Frc3 – 3 ($CHCl_3$-MeOH, v/v 10 : 1), and Frc3 – 4 ($CHCl_3$-MeOH, v/v 8 : 2). Frc3 – 1 (13 L–14 L, 2.7 g) was rechromatographyed over Sephadex LH-20 (3 × 85 cm, 100 g), eluting with $CHCl_3$-MeOH (v/v 1 : 1, 1.8 L) to give three subfractions (Frc3 – 1 – 1 - Frc3 – 1 – 3). Compounds **4** (15.3 mg), **12** (5.6 mg), **13** (9.1 mg), and **8** (14.7 mg) were obtained from Frc3 – 1 – 2 (750 mL – 1350 mL, 273 mg) subjected to HPLC separation (MeOH-H_2O, v/v 7 : 3; t_R of **4:** 9.04, **12:** 6.23, **13:** 5.13, **8:** 14.13 min). Frc3 – 2 (15 L – 17 L, 5.1 g) was separated into four parts, Frc3 – 2 – 1 - Frc3 – 2 – 4 by reversed phase VLC (24 × 43 cm, 50 g), eluting with a gradient of MeOH-H_2O (v/v 3 : 7, 500 mL; 1 : 1, 500 mL; 7 : 3, 500 mL; 9 : 1, 500 mL). Compounds **3** (4.0 mg) and **6** (6.3 mg) were isolated from Frc3 – 2 – 2 (69 mg) by HPLC (MeOH-H_2O, v/v 5.5 : 4.5; t_R of **3:** 9.19, **6:** 7.54 min). Compounds **5** (8.3 mg), **7** (9.6 mg) and **10** (20.2 mg) were also obtained from Frc3 – 2 – 3 (217 mg) by HPLC separation over ODS (MeOH-H_2O, v/v 6 : 4; t_R of **5:** 5.69, **7:** 13.64, **10:** 5.34 min). Frc3 – 3 (18 L - 20 L, 870 mg) was also subjected to HPLC purification (MeOH-H_2O, 5.5 : 4.5) to yield **1** (13.6 mg), **2** (6.3 mg) and **9** (9.3 mg) (t_R of **1:** 6.59, **2:** 7.83, **9:** 5.11 min). Frc3 – 4 (4.6 g) was also separated into three parts, Frc3 – 4 – 1 – 3 – 4 – 3, by column chromatography over silica gel (3.5 × 27 cm, 200 – 300 mesh, 70 g) eluted with a gradient of $CHCl_3$-MeOH (v/v 50 : 1, 1 L; 20 : 1, 1 L; 10 : 1, 1.5 L). Compound **11** (14.3 mg) was obtained from the Frc3 – 4 – 3 (57 mg), and purified by HPLC (MeOH-H_2O, v/v 4.5 : 5.5; t_R of **11:** 7.94 min).

Identification of isolated compounds

Lantibeside B (**1**): white amorphous powder (MeOH); R_f 0.42 [silica gel GF_{254}, $CHCl_3$/MeOH (5 : 1)]; R_f 0.50 [RP-18 F_{254s}, MeOH/H_2O (7 : 3)]; $[\alpha]_D^{24}$: –5.15 (*c* 0.13, MeOH); UV (MeOH): λ_{max} (log ε) = 204 (4.483), 231 (3.815), 282 (3.565) nm; CD (*c* 0.04, MeOH): $\Delta\varepsilon_{243}$ + 0.221, $\Delta\varepsilon_{291}$ + 0.209; IR (KBr): ν_{max} = 3445, 2966, 2932, 2861, 1513, 1491, 1454, 1437, 1632, 1416, 1347, 1271, 1243, 1168, 1071, 1044, 916 cm^{-1}; ^{1}H-NMR (DMSO, 600 MHz) and ^{13}C-NMR (DMSO, 150 MHz), see ▶ **Table 1**; HR-ESI-MS: *m/z* = 651.2311 $[M + H]^+$ (calcd. for $C_{31}H_{39}O_{15}$: 651.2289).

Lantibeside C (**2**): white amorphous powder (MeOH); R_f 0.39 [silica gel GF_{254}, $CHCl_3$/MeOH (5 : 1)]; R_f 0.43 [RP-18 F_{254s}, MeOH/H_2O (7 : 3)]; $[\alpha]_D^{24}$: + 13.1 (*c* 0.07, MeOH); UV (MeOH): λ_{max} (log ε) = 204 (4.499), 230 (3.857), 282 (3.578) nm; CD (MeOH, *c* 0.04): $\Delta\varepsilon_{235}$ + 0.283, $\Delta\varepsilon_{281}$ – 0.216; IR (KBr): ν_{max} = 3445, 2970, 2928, 2862, 1515, 1455, 1429, 1394, 1366, 1242, 1335, 1266,

Table 1 ^{1}H- and ^{13}C-NMR data for compounds **1 – 4** and **9** (DMSO-d_6)[a]

	1		2		3		4		9[b]	
Position	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C[b]**	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C**
1	3.00 m	53.7 d	3.37 m	49.2 d	3.36 m	49.8 d	3.35 m	49.4 d	3.38 m	49.2 d
2	4.64 d (5.0)	84.8 d	4.78 d (6.4)	81.2 d	4.78 d (6.4)	81.8 d	4.75 d (6.0)	81.4 d	4.80 d (6.0)	81.2 d
4	3.77 dd (9.2, 4.1), 4.14dd (9.2, 6.9)	71.0 t	4.07 brd (9.2), 3.75dd (9.2, 6.0)[c]	70.3 t	4.08 brd (9.6), 3.74 m[c]	70.9 t	4.04 brd (9.6), 3.73 m	70.2 t	4.09 brd (9.2), 3.7 6 m	70.3 t
5	3.04 m	53.8 d	2.84 m	54.0 d	2.84 m	54.4 d	2.81 m	53.9 d	2.84 m	54.0 d
6	4.65 d (5.0)	85.0 d	4.35 d (6.8)	86.7 d	4.36 d (6.8)	87.1 d	4.30 d (6.9)	86.9 d	4.37 d (6.8)	86.6 d
8	3.76 dd (9.2, 4.1), 4.13 dd (9.2, 6.9)	71.0 t	3.08 brt (9.2), 3.75 dd (9.2, 6.0)[c]	68.9 t	3.08 brt (9.6), 3.74 m[c]	69.3 t	3.08 brt (8.7), 3.72 m	68.8 t	3.08 brt (8.3), 3.76 m	68.0 t
1′		135.5 s		132.6 s		133.1 s		129.6 s		131.2 s
2′	6.93 brs	106.6 d	6.91 d (1.2)	106.2 d	6.91 d (1.4)	106.7 d	6.73 d (1.2)	109.7 d	6.93 d (1.2)	109.5 d
3′		146.5 s		146.0 s		146.5 s		147.3 s		147.6 s
4′		147.4 s		147.1 s		147.6 s		147.5 s		148.5 s
5′	6.87 d (7.2)	108.0 d	6.89 dd (7.2)	108.0 d	6.88 d (7.2)	108.4 d	6.89 d (8.2)	110.3 d	6.92 d (8.2)	110.5 d
6′	6.86 brd (7.2)	119.4 d	6.84 d (7.2,1.2)	118.6 d	6.84 dd (7.2, 1.4)	119.1 d	6.89 dd (8.2, 1.2)	118.6 d	6.87 dd (1.2, 8.2)	118.3 d
1″		135.1 s		135.2 s		135.8 s		132.3 s		135.3 s
2″	6.94 d (1.8)	110.4 d	6.95 d (1.8)	110.3 d	6.95 d (1.8)	111.1 d	6.73 d (1.8)	115.1 d	6.95 d (1.7)	111.6 d
3″		148.8 s		148.8 s		149.5 s		145.2 s		148.8 s
4″		145.8 s		145.8 s		145.8 s		145.9 s		145.9 s
5″	7.10 d (8.2)	115.4 d	7.11 d (7.2)	115.5 d	7.05 d (7.2)	115.9 d	6.72 d (8.2)	115.1 d	7.11 d (8.4)	115.6 d
6″	6.84 dd (8.2, 1.8)	118.4 d	6.87 dd (1.8, 7.2)	118.4 d	6.87 dd (1.8, 7.8)	118.7 d	6.76 dd (1.8, 8.2)	117.9 d	6.88 dd (1.7, 8.4)	117.5 d
1‴	4.83 d (7.2)	100.2 d	4.84 d (7.8)	100.2 d	4.88 d (7.8)	100.8 d			4.83 d (7.4)	100.3 d
2‴	3.24 m[c]	73.4 d	3.26 m[c]	73.4 d	3.26 m[c]	73.8 d			3.25 m[c]	73.3 d
3‴	3.47 d dd (9.6, 6.4, 1.2)	76.5 d	3.48 ddd (9.6, 8.7, 1.2)	76.5 d	3.25 ddd (9.6, 5.5, 1.8)	77.4 d			3.48 m	76.4 d
4‴	3.16 ddd (9.6, 8.7, 4.6)	69.6 d	3.16 ddd (11.0, 8.7,2.3)	69.6 d	3.16 ddd (9.6, 5.0, 2.3)	70.3 d			3.16 m	69.6 d
5‴	3.24 m[c]	76.7 d	3.26 m[c]	76.7 d	3.26 m[c]	77.5 d			3.25 m[c]	76.7 d
6‴	3.91 brd (11.0), 3.56 dd (11.5, 6.4)	68.0 t	3.92 brd (10.6), 3.56 dd (11.5, 6.4)	68.1 t	3.66 ddd (11.8, 6.8, 5.7), 3.45 ddd (11.8, 5.9, 5.7)	61.3 t			3.92 brd (7.4), 3.56 dd (4.6, 7.4)	68.0 t
1⁗	4.16 d (7.3)	103.7 d	4.17 d (7.2)	103.7 d					4.17 d (7.6)	103.7 d
2⁗	2.94 ddd (8.7, 8.3, 5.0)	73.1 d	2.95 ddd (8.7, 7.8, 4.6)	73.1 d					2.94 m	73.1 d
3⁗	3.05 m[c]	76.0 d	3.05 ddd (8.7, 8.7, 4.6)	76.0 d					3.05 m	75.9 d
4⁗	3.24 m[c]	69.6 d	3.26 m[c]	69.6 d					3.25 m[c]	69.5 d
5⁗	2.90 brt (11.0), 3.63 dd (11.5, 5.5)	65.6 t	2.90 brt (11.0), 3.63dd (11.0, 5.0)	65.9 t					2.90 brt (10.6), 3.65 dd (11.3, 5.3)	65.5 t
-OCH_3	3.76 s (3 H)	55.7 q	3.76 s (3 H)	55.6 q	3.76 s (3 H)	56.3 q	3.76 s (6 H)	55.6 q	3.74 s (3 H), 3.75 s (3 H), 3.76 s (3 H)	55.7 q, 55.4 q
-OCH_2O-	5.99 s (2 H)	100.8 t	6.01 s (2 H)	100.9 t	6.01 s (2 H)	101.3 t				

[a] ^{1}H- and ^{13}C-NMR spectra were obtained at 600 and 150 MHz, respectively.

[b] ^{1}H- and ^{13}C-NMR spectra were obtained at 500 and 125 MHz, respectively.

[c] The signals overlap.

1165, 1075, 1042, 924 cm^{-1}; ^{1}H-NMR (DMSO, 600 MHz) and ^{13}C-NMR (DMSO, 150 MHz), see **Table 1**; HR-ESI-MS: *m/z* = 651.2316 $[M + H]^+$ (calcd. for $C_{31}H_{39}O_{15}$: 651.2289).

Lantibeside D (**3**): white amorphous powder (MeOH); R_f 0.62 [silica gel GF_{254}, $CHCl_3$/MeOH (5 : 1)]; R_f 0.25 [RP-18 F_{254s}, MeOH/H_2O (7 : 3)]; $[\alpha]_D^{24}$: + 33.5 (*c* 0.105, MeOH); UV (MeOH): λ_{max} (log ε) = 204 (4.459), 230 (3.857), 282 (3.588) nm; CD (MeOH, *c* 0.033): $\Delta\varepsilon_{235}$ + 0.645, $\Delta\varepsilon_{281}$ – 0.670; IR (KBr): ν_{max} = 3445, 2921, 2869, 1515, 1455, 1427, 1363, 1335, 1316 1273, 1234, 1071, 1039, 932 cm^{-1}; ^{1}H-NMR (DMSO, 600 MHz) and ^{13}C-NMR (DMSO, 125 MHz), see **Table 1**; HR-ESI-MS: *m/z* = 541.1677 $[M + Na]^+$ (calcd. for $C_{26}H_{30}O_{11}Na$: 541.1686).

Lantibetin (**4**): white amorphous powder (MeOH); R_f 0.76 [silica gel GF_{254}, $CHCl_3$/MeOH (5 : 1)]; R_f 0.30 [RP-18 F_{254s}, MeOH/H_2O (7 : 3)]; $[\alpha]_D^{24}$: + 92.5 (*c* 0.19, MeOH); UV (MeOH): λ_{max} (log ε) = 206 (4.377), 231 (3.961), 281 (4.082) nm; CD (MeOH, *c* 0.04): $\Delta\varepsilon_{235}$ + 3.51, $\Delta\varepsilon_{289}$ – 0.331; IR (KBr): ν_{max} = 3384, 2961, 2852, 1605, 1517, 1463, 1429, 1372, 1273 1238, 1158, 1124, 1075, 1032 cm^{-1}; ^{1}H-NMR (DMSO, 600 MHz) and ^{13}C-NMR (DMSO, 150 MHz), see **Table 1**; HR-ESI-MS: *m/z* = 357.1329 $[M - H]^-$ (calcd. for $C_{20}H_{21}O_6$: 357.1338).

Determination of glucose and xylose

1 mg of **1** in 100 μL of DMSO was added to 300 μL of 6 M HCl in a hydrolysis tube and heated at 100 °C for 24 h. The reaction was then quenched with 1 mL of water and extracted twice with 2 mL of EtOAc to remove the aglycone. After evaporation of the aqueous layer, 1 mg of L-cysteine methyl ester hydrochloride in 100 μL of pyridine was added and the resulting mixture was stirred at 60 °C for 1 h. A 3 : 1 mixture of HMDS-TMCS (hexamethyldisilazane-trimethylchlorosilane) was then added (100 μL), and the solution was stirred for 30 min. The solution was then partitioned with hexane, and the hexane layer was directly subjected to GC-MS analysis [15]. The resulting xylose and glucose derivatives coeluted with the derivatized D-xylcose (t_R17.22) and D-glucose standard (t_R 19.42), but not with the derivatized L-xylose (t_R17.55) and L-glucose standard (t_R 19.60) (**Fig. 1S** Supporting Information). By the same procedure, **2** gave D-glucose and D-xylose, and **3** gave d-glucose, respectively (**Figs. 2S, 3S** Supporting Information).

Cytotoxicity assay

The cytotoxicity of **1** – **4** was assayed using HL-60 and MOLT-4 cells (Institute of Biochemistry and Cell Biology, Chinese Academy of Science) with the MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method [22], and BEL-7402 and A-549 cells (ATCC) with the SRB (sulforhodamine B) method [23]. Vp-16 (etoposide; Yadong Pharmaceutical Company) was used as positive control.

Supporting information

GC-MS profiles of the glucose and xylcose derivatives of **1**, **2**, **3** and ^{1}H- and ^{13}C-NMR spectra of compounds **1** – **4** are available as Supporting Information.

Results and Discussion

Lantibeside B (**1**) was obtained as a white amorphous powder, with the molecular formula of $C_{31}H_{38}O_{15}$ deduced from the NMR spectral data (**Table 1**) and the molecular ion peak at *m/z* = 651.2311 $[M + H]^+$ (calcd.: 651.2289) in high-resolution ESI-MS. Compound **1** displayed a similar UV absorption spectrum to that of furofurano ligands at 204 (log ε 4.483), 231 (log ε 3.815) and 282 (log ε 3.565) nm [16]. With the exception of one additional pentose, its 1 D NMR spectra were very similar to those of simplexoside (**6**) (**Table 1**), indicating that **1** and **6** have the same skeleton and the same relative configuration in the furofurano nucleus, suggesting that **1** is probably a pentose-substituted derivative of **6**. Subjected to acidic hydrolysis and then to reaction with L-cysteine methyl ester followed by trimethylsilylation, **1** produced sugars that had identical properties as D-glucose and D-xylose in GC-MS analysis [15] (**Fig. 1S** Supporting Information). Besides, + 0.26, + 0.12 and + 7.1 ppm of downfield shift effects for H-6‴a, H-6‴b and C-6‴, respectively, of the glucose moiety were observed. Furthermore, the key long-range ^{1}H-^{13}C correlations from H-6‴a (δ = 3.91, dd) to C-1‴‴ (δ = 103.7, d) and from H-1‴‴ (δ = 4.16, d) to C-6‴ (δ = 68.0, t) were observed in the HMBC experiment. In the NOE difference experiment of **1**, when H-2 and H-6 were irradiated, only the signals of H-4a and H-8a were enhanced, which revealed the relative configuration of the 3,7-dioxabicyclo[3.3.0]octane system (**Fig. 2**). The CD spectrum of **1** showed positive Cotton effects at 243 and 291 nm similar to those of (1*R*,2*S*,5*R*,6*S*)-(–)-methylpiperitol [17]. In addition, the specific rotation of **1** ($[\alpha]_D^{24}$: –5.2) was near to that of (+)-syringaresinol 4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside ($[\alpha]_D^{24}$: –10.7) [18]. These observations supported that **1** is the 6‴-*O*-*β*-D-xylopyranosyl-substituted derivative of **6**. Thus, the structure of **1** was determined as (1*R*,2*S*,5*R*,6*S*)-2-(3,4-methylenedioxyphenyl)-6-[(3-methoxy-4-*β*-D-xylopyranosyloxy(1→6)-*β*-D-glucopyranosyloxy)phenyl]-3,7-dioxabicyclo [3.3.0]octane.

Lantibeside C (**2**) was obtained as a white amorphous powder, with the same molecular formula as 1 from high-resolution ESI-MS at *m/z* = 651.2316 $[M + H]^+$ (calcd.: 651.2289). Except for the obvious upfield effects of C-1, C-2 and C-1′ (-4.5, -3.6 and -2.9 ppm, respectively), the ^{13}C-NMR data of **2** were similar to those of **1** (**Table 1**), indicating **2** to be the 2-epimer of **1**. In difference NOE experiments of **2**, the Overhauser effects of H-2 and H-5, and no enhancement of H-1 and H-5 after irradiation of H-6 were observed. Considering that the two tetrahydrofuran rings were fused in the *cis*-form for steric reasons [19], the relative configurations of **2** were deduced as 1,2-*cis* and 5,6-*trans*. Acidic hydrolysis followed by acetylation of **2** produced glucose and xylose. In addition, the CD spectrum of **2** showed similar Cotton effects (positive at 235 nm and negative at 281 nm) to that of (1*R*,2*R*,5*R*,6*S*)-(+)-epieudesmin [17]. However, the specific rotation of **2** ($[\alpha]_D^{24}$: + 13.1) was similar to that of phillyrin 6-*O*-*β*-glucoside ($[\alpha]_D^{24}$: + 9.5) [20]. The structure of **2** was thus determined as (1*R*,2*R*,5*R*,6*S*)-2-(3,4-methylenedioxyphenyl)-6-[(3-methoxy-4-*β*-D-xylopyranosyloxy(1→6)-*β*-D-glucopyranosyloxy)phenyl]-3,7-dioxabicyclo[3.3.0]octane.

Lantibeside D (**3**) was obtained as a white amorphous powder, with the molecular formula of $C_{26}H_{30}O_{11}$ deduced from the NMR spectra and the molecular ion peak at *m/z* = 541.1677 $[M + Na]^+$ (calcd.: 541.1686) in high-resolution ESI-MS. Except for the lacking xylose, its 1 D NMR spectra were very similar to those of **2** (**Table 1**), indicating that they have the same skeleton and the same relative configuration in the furofuran ring. Acidic hydrolysis followed by acetylation of **3** only produced glucose. Furthermore, the -6.8 ppm of upfield shift effect for C-6‴ and the similar Cotton effects to those of **2** were observed indicated that **3** is the 6‴-*O*-dexylosyl derivative of **2**. Therefore, the structure of **3** was elucidated as (1*R*,2*R*,5*R*,6*S*)-2-(3,4-methyle-

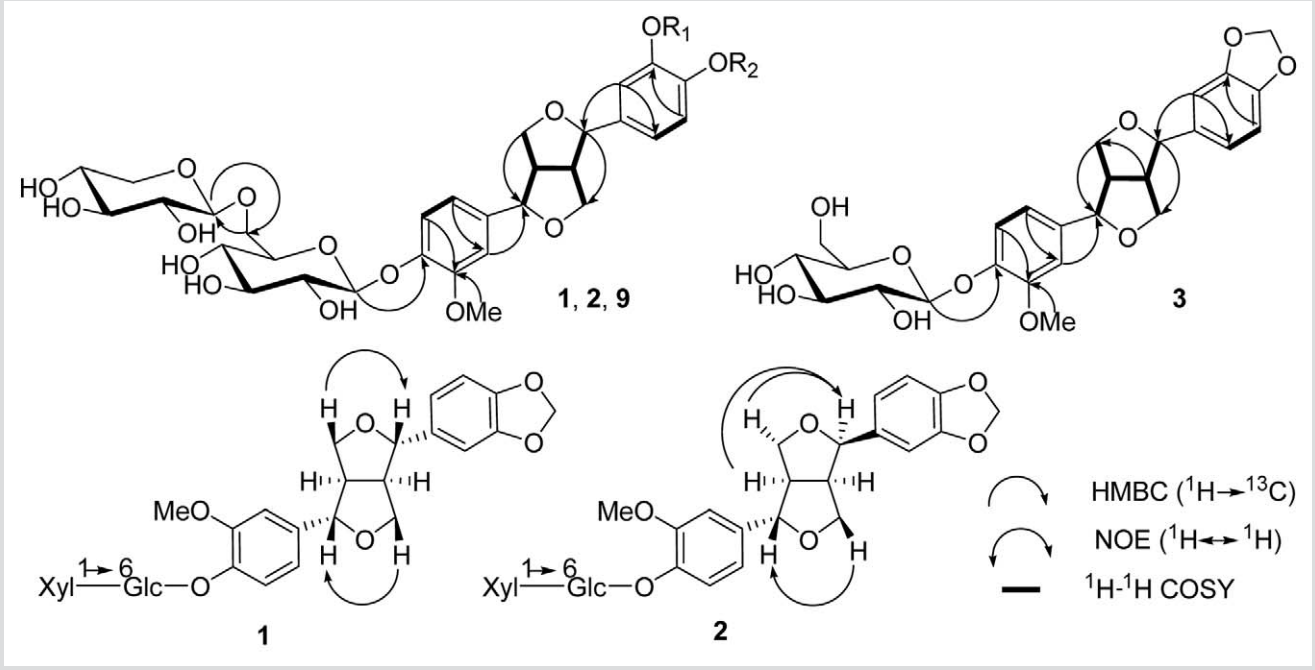

Fig. 2 Key HMBC correlations and NOE effects of compounds **1 – 3** and **9**

nedioxyphenyl)-6-(3-methoxy-4-*β*-D-glucopyranosyloxy)phenyl-3,7-dioxabicyclo[3.3.0]octane.

Lantibetin (**4**) was obtained as a white amorphous powder. Its molecular formula was determined as $C_{20}H_{22}O_6$ according to the NMR spectra and the high-resolution ESI-MS at m/z = 357.1329 $[M - H]^-$ (calcd.: 357.1338). Except for the substitution of hydroxy (δ = 8.90) for methoxy (δ = 3.37/55.9), its 1 D NMR data were very similar to those of phillygenol (**8**) [11], indicating that they have the same skeleton and the same relative configuration. Besides, + 4.8, –3.3 and + 0.61 ppm chemical shift changes for C-2″, C-3″ and 4″-OH were observed in the 1 D NMR spectra of **4**, showing **4** to be the 3″-*O*-demethyl derivative of **8** or *ent*-**8**. Its specific rotation ($[\alpha]_D^{24}$: + 92.5) and CD Cotton effects (positive at 235 and 272 nm, and negative at 286 nm) were similar to those of (1*R*,2*R*,5*R*,6*S*)-epieudesmin [17], [21]. Thus, the structure of **4** was determined as 3″-*O*-demethylsylvatesmin, i. e., (1*R*,2*R*,5*R*,6*S*)-2-(3,4-dimethoxyphenyl)-6-(3,4-dihydroxyphenyl-3,7-dioxabicyclo[3.3.0]octane.

The cytotoxicity of **1** – **4** was assayed using HL-60 and MOLT-4 cells with the MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] method [22], and BEL-7402 and A-549 cells with the SRB (sulforhodamine B) method [23]. Vp-16 (etoposide) was used as the positive control with IC_{50} values of 0.04, 0.001, 1.03, 0.63 and 0.05 μM against HL-60, MOLT-4, BEL-7402, A-549 and P388 cells, respectively. Compounds **2** and **3** exhibited very weak cytotoxicities against HL-60 cells with IC_{50} values of 61 and 99 μM, respectively, while **1** and **4** were inactive. Compounds **1** – **11** were also assayed for their cytotoxicity against P388 cells and antioxidant activities [24], but were inactive (IC_{50} > 100 μM).

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