New Benzofuranylpropanoids from the Roots of *Codonopsis lanceolata*

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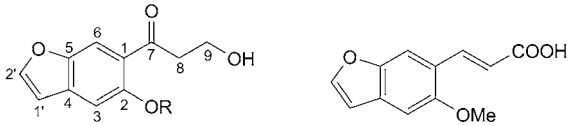
Three new benzofuranylpropanoids, lanceolunes A–C (**1**–**3**), were isolated from the roots of *Codonopsis lanceolata*. Their structures were determined by means of HR-ESI-MS, extensive 1D- and 2D-NMR spectroscopic, and chemical evidence.

Introduction. – The genus *Codonopsis* (Campanulaceae) is represented in China by 39 species. The roots of some *Codonopsis* species, such as *C. pilosula*, *C. tangshen*, *C. lanceolata*, *C. cordifolioidea*, *C. bulleyana*, *C. micrantha*, and *C. subglobosa* are commonly used as herbal medicine or food in China and Japan [1–4]. Among them, *C. lanceolata* has obvious pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory activities [5–8]. The previous work has also revealed that the roots of *C. lanceolata* contain various biologically active compounds, including polyphenols, saponins, tannins, triterpenes, alkaloids, steroids [9–13], and the like.

Motivated by the search for bioactive metabolites from this plant, the phytochemical investigation on *C. lanceolata* was carried out. As a result, the three new benzofuranylpropanoids **1**–**3** were isolated from this plant (*Fig. 1*), and the compounds were screened for anti-HIV activity and cytotoxicity.



1[1]) R = H
2[1]) R = Me

3

Fig. 1. *Compounds* **1**–**3***, isolated from* Codonopsis lanceolata

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Results and Discussion. – A 70% aqueous MeOH extract prepared from the roots of *C. lanceolata* was subjected repeatedly to column chromatography and prep. HPLC to afford the new compounds **1**–**3**. Their ^{1}H- and ^{13}C-NMR spectroscopic data are listed in the *Table*.

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; C_5D_5N) *of Compounds* **1**–**3**[1]). δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		121.9		120.7		110.7
C(2)		155.1		152.5		152.7
H–C(3)	7.08 (*s*)	105.1	7.10 (*s*)	103.5	6.95 (*s*)	104.8
C(4)		133.2		131.8		128.7
C(5)		148.5		148.3		149.0
H–C(6)	7.82 (*s*)	115.0	7.89 (*s*)	104.4	7.34 (*s*)	108.8
C(7) or H–C(7)		198.0		198.2	8.11 (*d*, J = 15.9)	145.0
CH_2(8) or H–C(8)	3.40 (*t*, J = 6.2)	42.6	3.34 (*t*, J = 6.3)	43.0	6.75 (*d*, J = 16.0)	116.9
CH_2(9) or COOH	4.33 (*t*, J = 6.2)	59.1	4.32 (*t*, J = 6.3)	59.1	13.49 (br. *s*)	169.7
OH- or MeO–C(2)	10.90 (br. *s*)		3.89 (*s*)	55.9	3.77 (*s*)	55.9
H–C(1′)	6.83 (*d*, J = 2.5)	106.9	6.85 (*d*, J = 2.6)	106.8	6.84 (*d*, J = 2.5)	106.2
H–C(2′)	7.64 (*d*, J = 2.5)	146.8	7.64 (*d*, J = 2.5)	146.3	7.67 (*d*, J = 2.5)	146.8

Compound **1** was obtained as a pale yellow gum. Its molecular formula was determined as $C_{11}H_{10}O_4$ by HR-ESI-MS (*m*/*z* 229.0482 ($[M+Na]^+$)). Its ^{1}H- and ^{13}C-NMR spectra showed signals of ten H- and eleven C-atoms, respectively, corresponding to a 5,6-disubstituted benzofuran ring system [14] (δ(C) 121.9, 155.1, 105.1, 133.2, 148.5, 115.0, 106.9, and 146.8; δ(H) 7.08 (*s*), 7.82 (*s*), 6.83 (*d*, J = 2.5 Hz), and 7.64 (*d*, J = 2.5 Hz), one CH_2 group (δ(C) 42.6; δ(H) 3.40 (*t*, J = 6.2 Hz)), one O-bearing CH_2 group (δ(C) 59.1; δ(H) 4.33 (*t*, J = 6.2 Hz)), one C=O group (δ(C) 198.0), and a phenol-like OH group (δ(H) 10.90). Strong absorption bands accounting for OH (3368 cm^{-1}), C=O (1715 cm^{-1}), and aromatic groups (1642, 1516, and 1454 cm^{-1}) could be observed in the IR spectrum. The UV spectrum of **1** showing absorption maxima at 298 and 270 nm also confirmed the existence of the aromatic function. The ^{1}H,^{1}H-COSY cross-peak CH_2(8)/CH_2(9); together with the HMBCs (*Fig. 2*) of H–C(6) (δ(H) 7.82) with C(7) (δ(C) 198.0), of CH_2(8) (δ(H) 3.40) with C(1) (δ(C) 121.9), of CH_2(9) (δ(H) 4.33) with C(7) (δ(C) 198.0) and C(8) (δ(C) 42.6) suggested the presence of a 3-hydroxypropan-1-one moiety, and this structure unit was attached to C(1). The HMBCs of the aromatic OH (δ(H) 10.90) with C(1) (δ(C) 121.9), C(2) (δ(C) 155.1), and C(3) (δ(C) 105.1) indicated that this OH group should be located at C(2). Thus, the structure of **1** was established and given the name lanceolune A.

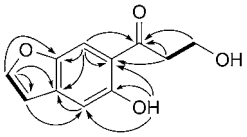


Fig. 2. *Selected HMBC* (H → C) *and ^{1}H,^{1}H-COSY* (—) *features of* **1**

Compound **2** was obtained as a pale yellow gum and showed a sodiated molecular ion at *m/z* 243.0630 ($[M+Na]^+$) in the HR-ESI-MS, corresponding to the molecular formula $C_{12}H_{12}O_4$. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**, the only difference being the presence of a MeO group in **2** instead of OH–C(2) in **1**, as supported by the disappearance of the aromatic-OH H-atom signal (δ(H) 10.90 (br. *s*)) and appearance of MeO signals (δ(C) 55.9 (*qs*); δ(H) 3.89 (*s*)) in **2**. Thus, the structure of **2** was established and given the trivial name lanceolune B.

Compound **3** was obtained as a pale yellow gum and was assigned the molecular formula $C_{12}H_{10}O_4$ by HR-ESI-MS (*m/z* 241.0471 ($[M+Na]^+$)). The ^{1}H- and ^{13}C-NMR spectra showed signals of ten H- and twelve C-atoms, respectively. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **2** revealed a very close resemblance to **2** concerning the 5,6-disubstituted benzofuran moiety. The main differences arose from the prop-2-enoic acid side chain of **3** which replaced the 3-hydroxypropan-1-one side chain of **2**, as supported by the disappearance of the 3-hydroxylpropan-1-one signals and appearance of the prop-2-enoic acid signals (δ(C) 145.0, 116.9, and 169.7; δ(H) 8.11 (*d*, $J=15.9$ Hz), 6.75 (*d*, $J=16.0$ Hz), and 13.49 (br. *s*)) in **3**. Thus, the structure of **3** was established as and given the trivial name lanceolune C.

The compounds were screened for anti-HIV activity and cytotoxicity. They all showed weak anti-HIV-1 activities and cytotoxicities. The results were of low interest.

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Experimental Part

General. Column chromatography (CC): SiO_2 (200–300 mesh, *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China); *Lichroprep RP-18* gel (40–63 μm; *Merck*, Darmstadt, Germany); *MCI* gel (75–150 μm; *Mitsubishi Chemical Corporation*, Tokyo, Japan). Anal. TLC: *HSGF$_{254}$* SiO_2 plates (0.20–0.25 mm; *Yantai Chemical Industrial Institute*, P. R. China); detection by spraying with 5% H_2SO_4 in EtOH followed by heating. Prep. HPLC: *Shimadzu-LC-8A* liquid chromatograph; *Zorbax-PrepHT-GF* (21.2 mm × 25 cm, 7.0 µm) or *Venusil-MP-C18* column (20 mm × 25 cm; 5.0 μm). UV Spectra: *Shimadzu-UV-2401A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Tenor-27* spectrophotometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker-DRX-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *API-QSTAR* time-of-flight (ESI) and *VG-Autospec-3000* spectroscopy (HR-ESI); in *m/z*.

Plant Material. The roots of *C. lanceolata* were collected in Dali Prefecture, Yunnan Province, P. R. China, in September 2009. The identification of the plant material was verified by Prof. *Y. J. Chen* (Yunnan University of Nationalities). A voucher specimen (YNNI 09-9-21) has been deposited with our laboratory.

Extraction and Isolation. The air-dried and powdered roots of *C. lanceolata* (2.2 kg) were extracted four times (81 h each) with 70% MeOH (4 × 2.5 l) at r.t. and filtered. The crude extract (150 g) was applied to CC (SiO_2, $CHCl_3$/acetone 20:1, 9:1, 8:2, 7:3, 6:4, and 5:5): *Fractions A–F.* The further separation of *Fr. C* (with $CHCl_3$/acetone 4:1; 32.5 g) by CC (SiO_2, $CHCl_3$/MeOH 9:1, 8:2, 7:3, 6:4, and 1:1), yielded *Frs. C1–C5*. *Fr. C1* (with $CHCl_3$/MeOH 9:1; 5.16 g) was subjected to prep. HPLC (45% MeOH, flow rate 12 ml/min): **1** (22.8 mg) and **2** (18.4 mg). *Fr. C2* (with $CHCl_3$/MeOH 8:2; 6.42 g) was subjected to prep. HPLC (25% MeOH, flow rate 12 ml/min): **3** (15.2 mg).

Anti-HIV-1 and Cytotoxicity Assays. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method, and anti-HIV-1 activities were evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) [15].

The cytotoxicity tests of the isolates were performed with HL-60, Hep-G2, KB, and MDA-MB-231 tumor cell lines in a MTT assay (with doxorubicin as the positive control) [16].

Lenceolune A (= *3-Hydroxy-1-(5-hydroxybenzofuran-6-yl)propan-1-one*; **1**): Pale yellow gum. UV (MeOH): 298 (4.38), 270 (4.16), 245 (3.87), 210 (4.92). IR (KBr): 3368, 2916, 2852, 1715, 1642, 1516, 1454, 1434, 1276, 1158, 1126, 1048, 964, 769. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 229 ($[M+Na]^+$). HR-ESI-MS: 229.0482 ($[M+Na]^+$, $C_{11}H_{10}NaO_4^+$; calc. 229.0477).

Lanceolune B (= *3-Hydroxy-1-(5-methoxybenzofuran-6-yl)propan-1-one*; **2**): Pale yellow gum. UV (MeOH): 301 (4.32), 272 (4.22), 248 (3.96), 210 (4.98). IR (KBr): 3365, 2912, 2856, 1718, 1647, 1522, 1453, 1430, 1374, 1279, 1163, 1144, 1052, 973, 862, 775. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 243 ($[M+Na]^+$). HR-ESI-MS: 243.0630 ($[M+Na]^+$, $C_{12}H_{12}NaO_4^+$; calc. 243.0633).

Lanceolune C (= *(2*E*)-3-(5-Methoxybenzofuran-6-yl)prop-2-enoic Acid*; **3**): Pale yellow gum. UV (MeOH): 312 (4.42), 258 (4.26), 210 (4.87). IR: 3374, 2915, 2872, 1715, 1647, 1632, 1568, 1529, 1487, 1462, 1429, 1379, 1354, 1273, 1222, 1160, 1149, 1058, 975, 868. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 241 ($[M+Na]^+$). HR-ESI-MS: 241.0471 ($[M+Na]^+$, $C_{12}H_{10}NaO_4^+$; calc. 241.0477).

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