Two New Pyrrolidine Alkaloids, Codonopsinol C and Codonopiloside A, Isolated from *Codonopsis pilosula*

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A new pyrrolidine alkaloid codonopsinol C (1), and pyrrolidine alkaloidal glycoside, codonopiloside A (2), were isolated from the roots of *Codonopsis pilosula*, along with four known pyrrolidine alkaloids, codonopsinol A (3), codonopsinol B (4), codonopyrrolidium B (5), and radicamine A (6). The structures of the new compounds were established by acid hydrolysis and spectroscopic methods. We describe those structures in this paper.

Key words *Codonopsis pilosula*; pyrrolidine alkaloid; Japanese Pharmacopoeia; NMR; Campanulaceae

The crude drug *Tojin* consists of the roots of *Codonopsis pilosula* (Franch.) Nannf. and *C. tangshen* Oliv. In Japan, *Tojin* has been used as a component of Kampo foumulae such as "Umpito" and as an ingredient in several over the counter (OTC) crude drug products. It is categorized as a raw material used exclusively as in pharmaceuticals. Since the crude drug is recognized to be an essential raw material for public health and medical treatment in Japan, the Japanese Pharmacopoeia (JP) committee is discussing its standardization for the purpose of adopting into JP.

In our previous paper, we started to investigate the chemical constituents of crude drugs purchased in Japan to obtain information about secondary metabolites. We isolated three new triterpenyl esters, codonopilates A–C.[1] In order to discuss its standardization and regulation, alkaloidal components are very important. In 1988, Wang *et al.* and Liu isolated pyridine alkaloid, nicotinic acid and 5-hydroxy-2-pyridinemethanol,[2] and *β*-carboline alkaloid perlolyrine[3] from *C. pilosula*, respectively. Tsai and Lin identified pyrrolidine alkaloids codonopyrrolidiums A and B (**5**)[4] from *C. tangshen*. Ishida *et al.* reported codonopsinol (**7**) isolated from *C. clematidea*.[5] The absolute stereochemistry of **7** was revised by comparison to the optical rotation of synthetic compound.[6,7] Li *et al.* isolated quaternary ammonium alkaloids codotubulosines A and B[8] from *C. tubulosa*. In this paper, we deal with the isolation and structural elucidation of the new pyrrolidine alkaloids, codonopsinol C (**1**) and codonopiloside A (**2**).

Results and Discussion

The alkaline BuOH extract of Codonopsis Radix was purified by various chromatographic techniques to give two new pyrrolidine alkaloids codonopsinol C (**1**) and codonopiloside A (**2**), along with four known compounds, (2*R*,3*R*,4*R*,5*R*)-2-(3-hydroxy-4-methoxyphenyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol (**3**)[6] (2*R*,3*R*,4*R*,5*R*)-2-(hydroxymethyl)-5-(4-methoxyphenyl)-1-methylpyrrolidine-3,4-diol (**4**),[6] codonopyrrolidium B (**5**),[4] and radicamine A (**6**)[9] (Fig. 1). Compounds **3** and **4** were new compounds as natural products, therefore we named **3** and **4** as codonopsinol A and B, respectively.

The molecular formula of codonopsinol C (**1**) was confirmed as $C_{12}H_{17}NO_4$ by direct analysis in real-time-time-of-flight-mass spectra (DART-TOF-MS). The ^{1}H-NMR spectrum of **1** showed 1,4-substituted phenyl protons [*δ* 6.75d (8.5, 2H), *δ* 7.17d (8.5, 2H)], an *N*-methyl proton (*δ* 2.16s, 3H), four methine protons (*δ* 3.04, *δ* 3.61, *δ* 3.92, *δ* 3.99) and oxygenated methylene protons [*δ* 3.80dd (4.3, 11.6), *δ* 3.84dd (4.3, 11.6)]. This result was very similar to that of **4**, except for the *O*-methyl group. Therefore, **3** was suggested to be the *O*-demethyl compound of **4**. The planar structure was elucidated by two dimensional (2D)-NMR spectra (Fig. 2). The detailed analysis of the ^{1}H–^{1}H correlation spectroscopy (COSY) spec-

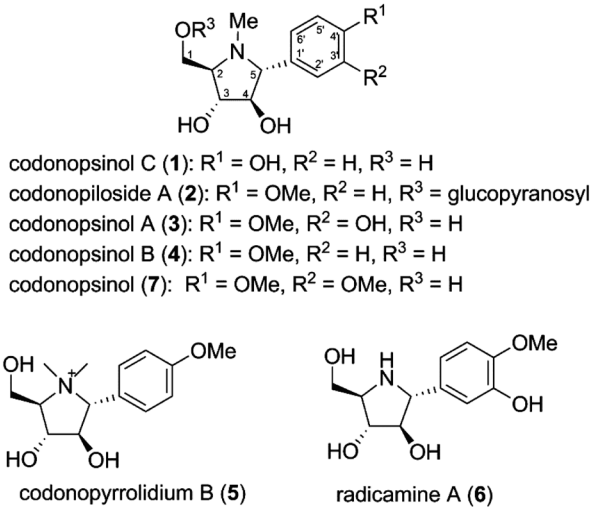

Fig. 1. Structures of Pyrrolidine Alkaloids Isolated from *C. pilosula*

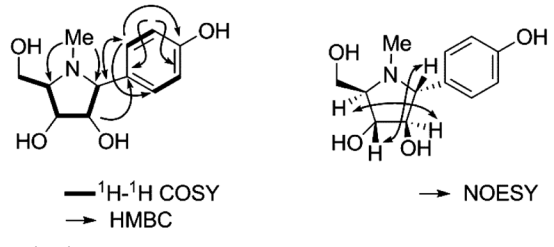

Fig. 2. ^{1}H–^{1}H COSY, HMBC and NOESY Spectra of **1**

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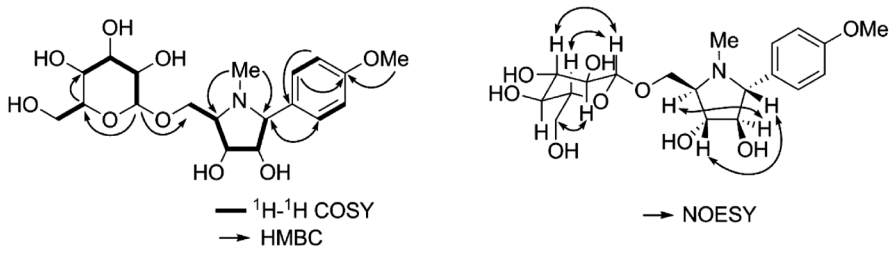

Fig. 3. ^{1}H–^{1}H COSY, HMBC and NOESY Spectra of **2**

trum showed correlations between 1-H_2 [δ 3.80dd (4.3, 11.6), δ 3.84dd (4.3, 11.6)] and 2-H [δ 3.04td (4.3, 4.8)], 2-H and 3-H [δ 3.99t (4.8)], 3-H and 4-H [δ 3.92dd (4.8, 6.5)], 4-H and 5-H [δ 3.61d (6.5)], and the analysis of heteronuclear multiple bond correlation (HMBC) spectra shown from *N*-methyl proton (δ 2.16s) to C-2 (δ 71.0) and C-5 (δ 75.3). These data suggested the presence of *N*-methyl-2-hydroxymethyl-pyrrolidine-3,4-diol moiety. Also, the HMBC correlations from 4-H to C-1′ (δ 132.2) and from 5-H to C-2′/C-6′ (δ 130.7) suggested that the phenyl moiety was connected to the C-5 of the pyrrolidine moiety. The relative stereochemistry was confirmed by the NOESY spectrum that showed 2-H to 4-H and 3-H to 5-H. The absolute stereochemistry was considered to be (2*R*, 3*R*, 4*R*, 5*R*), the same as in **4**, because of the comparison of the CD spectra of **4**[8] with that of **1**.

Codonopiloside A (**2**) showed the molecular formula as $C_{19}H_{29}NO_9$ by DART-TOF-MS. The ^{1}H-NMR spectrum of **2** showed 1,4-substituted phenyl protons [δ 6.90d (8.6, 2H), δ 7.28d (8.6, 2H)], a methoxy proton (δ 3.78s, 3H), an *N*-methyl proton (δ 2.16s, 3H), eight methine protons (δ 3.21, δ 3.24, δ 3.29, δ 3.29, δ 3.37, δ 3.69, δ 3.93, δ 4.11), an anomeric proton (δ 4.28) and two methylene protons [δ 3.73dd (4.0, 10.6), δ 4.30dd (4.0, 10.6), δ 3.67dd (4.0, 11.7), δ 3.88bd (11.7)]. Therefore, the structure of **2** suggested the glycosidal compound of **4**. The position of the *N*-methyl group was determined by the HMBC correlations of **2** from the *N*-methyl proton to C-2 (δ 69.6) and C-5 (δ 75.6). Therefore, the aglycone moiety of **2** was determined to be same as that of **4**. To determine the sugar moiety of **2**, it was hydrolyzed by 4 M HCl. In the results, (2*R*,3*R*,4*R*,5*R*)-2-(hydroxymethyl)-5-(4-methoxyphenyl)-pyrrolidine-3,4-diol[6] and D-glucose were afforded. The sugar moiety was identified as D-glucose by the analysis of ^{1}H-NMR spectrum of sugar part in **2** and the comparison of the optical rotation to standard compound (sugar moiety of **2**: $[\alpha]_D$ +49.8°, standard compound: $[\alpha]_D$ +50.7°). The anomeric proton of **2** was confirmed to be β-anomer as a result of the coupling constants of anomeric proton [δ 4.28d (8.0)]. The HMBC correlation from 1″-H to C-1 (δ 69.1) suggested that D-glucose was attached to C-1 of the pyrrolidine moiety. Therefore, **2** was confirmed to be codonopsinol C-1-*O*-β-D-glucopyranosyl and was named Codonopiloside A.

Conclusion

In our search of alkaloid compounds from *C. pilosula*, we found two new pyrrolidine alkaloids, codonopsinol C (**1**) and codonopiloside A (**2**), together with four known pyrrolidine alkaloid codonopsinols A (**3**) and B (**4**), codonopyrrolidium B (**5**) and radicamine A (**6**). Compounds **3** and **4** were already reported for the synthesized compound as (2*R*,3*R*,4*R*,5*R*)-2-(3-hydroxy-4-methoxyphenyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol and (2*R*,3*R*,4*R*,5*R*)-2-(hydroxymethyl)-5-(4-methoxyphenyl)-1-methylpyrrolidine-3,4-diol, respectively, but they were first isolated as natural compounds. Codonopiloside A (**1**), the glucosidal compound of this type of pyrrolidine alkaloids, was first reported in this paper.

Discussion

The pyrrolidine alkaloids isolated from *C. pilosula*, codonopsinol A (**3**), codonopsinol B (**3**), and radicamine A (**6**), were already reported to be α-glucosidase inhibitory.[8,9] In addition, pyrrolidine alkaloids, broussonetinines E and F isolated from the bark of *Broussonetia kazinoki*, were also α-glucosidase inhibitors.[10] Otherwise, some pyrrolidine alkaloids showed toxicity. For example, bgugaine is a hepatotoxin in rat and human liver cells.[11] Therefore, we think that the crude drug *Tojin*, which detects pyrrolidine alkaloids, should be used carefully as a raw material used exclusively as a pharmaceuticals, and its standardization by JP is very important for public health.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO (Tokyo, Japan) DIP-370 digital polarimeter, UV spectra on a Shimadzu (Kyoto, Japan) UV-2550 spectrophotometer, IR spectra on a JASCO FT-IR-5300 spectrophotometer and circular dichroism (CD) spectra on a JASCO J-720 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL ECA-500 spectrometer (500.16 MHz for ^{1}H, 125.77 MHz for ^{13}C) and/or ECA-800 (800.14 MHz for ^{1}H, 201.20 MHz for ^{13}C) spectrometer, using tetramethylsilane as an internal standard. Coupling patterns are indicated as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad. Mass spectra were obtained on a JEOL DART ion source coupled to a AccuTOF JMS-100LC mass spectrometer. Preparative LPLC was performed on an EYELA ceramic pump VSP-3050, using a prepacked glass column, Yamazen SI-40B (26 mm i.d.×300 mm) or ODS-S-40B (26 mm i.d.×300 mm). HPLC was performed on a Shimadzu LC-8A pump with a flow rate of 4 mL/min using a GL Sciences (Tokyo, Japan). Inertsil SIL 100A, NH_2 (10 mm i.d.×250 mm), and/or a Kanto Chemical (Tokyo, Japan) Mightysil RP-18GP (10 mm i.d.×250 mm) prepacked column equipped with a Shimamura RI monitor YRD-883 or a Shimadzu SPD-6AV monitor.

Plant Material *Codonopsis pilosula* (FRANCH.) NANNF. was purchased from Uchida Wakanyaku (Tokyo, Japan). A voucher specimen was deposited at the National Institute of Health Sciences, Japan.

Extraction and Isolation The dried roots of *C. pilosula* (500 g) were extracted with MeOH (2×2 L) at room tempera-

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** and **2** in Methanol-d_4

No.	Codonopsinol C (**1**)		Codonopiloside A (**2**)	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	60.8	3.80 dd (4.3, 11.6)	69.1	3.73 dd (4.0, 10.6)
		3.84 dd (4.3, 11.6)		4.30 dd (4.0, 10.6)
2	71.0	3.04 td (4.3, 4.8)	69.6	3.21 m
3	79.9	3.99 t (4.8)	80.4	4.11 t (4.6)
4	85.5	3.92 dd (4.8, 6.5)	86.1	3.93 dd (4.6, 6.9)
5	75.3	3.61 d (6.5)	75.6	3.69 d (6.9)
1′	132.2	—	133.3	—
2′/6′	130.7	7.17 d (8.5)	130.6	7.28 d (8.6)
3′/5′	116.1	6.75 d (8.5)	114.8	6.90 d (8.6)
4′	158.1	—	160.8	—
N-CH_3	35.0	2.16 s	35.0	2.16 s
O-CH_3			55.7	3.78 s
1″			105.1	4.28 d (8.0)
2″			75.3	3.24 dd (8.0, 9.1)
3″			77.9	3.37 t (9.1)
4″			71.7	3.29 m
5″			78.1	3.29 m
6″			62.8	3.67 dd (4.0, 11.7)
				3.88 brd (11.7)

ture, and the solvent was evaporated *in vacuo*. The residue was suspended in H_2O and extracted with $CHCl_3$. The H_2O layer was adjusted for pH 2 with 4 M HCl, and then extracted with *n*-BuOH. The residual aqueous layer was adjusted for pH 11 with 25% NH_4OH and then extracted with *n*-BuOH. The alkaline BuOH layer (12.0 g) was chromatographed on Sephadex LH-20 [$CHCl_3$–MeOH (1 : 1)] to give six fractions. Fraction 2 was purified by HPLC with 80% CH_3CN to give lobetyolinin (25 mg) and codonopiloside A (**2**: 4 mg). The third fraction was separated by HPLC with 80% CH_3CN to give codonopyrrolidium B (**5**: 15 mg), codonopsinol B (**3**: 3 mg), and codonopsinol A (**3**: 3 mg). The fifth fraction was repeatedly purified by HPLC with 80% CH_3CN and EtOH to give radicamine A (**6**: 11 mg) and codonopsinol C (**1**: 3 mg).

Codonopsinol C (**1**): Colorless amorphous gum: $[\alpha]_D$ −9° (c=0.60, MeOH). IR (ATR) ν_{max} cm^{-1}: 3333 (–OH), 1602, 1512 (phenyl). UV (MeOH) λ_{max}nm (logε): 277 (3.09), 226 (3.86). CD (MeOH) $\Delta\varepsilon$ (nm): +3.88 (278), +5.02 (225). The ^{1}H- and ^{13}C-NMR spectral data are summarized in Table 1. DART-TOF-MS m/z 240.1244 $[M+H]^+$ (Calcd for $C_{12}H_{18}NO_4$: 240.1236).

Codonopiloside A (**2**): Colorless amorphous gum: $[\alpha]_D$ −15° (c=0.57, MeOH). IR (ATR) ν_{max} cm^{-1}: 3354 (–OH), 1508 (phenyl). UV (MeOH) λ_{max}nm (logε): 275 (3.05), 226 (3.81). CD (MeOH) $\Delta\varepsilon$ (nm): +3.61 (275), −3.12 (227), +4.21 (214). The ^{1}H- and ^{13}C-NMR spectral data are summarized in Table 1. DART-TOF-MS m/z 416.1913 $[M+H]^+$ (Calcd for $C_{19}H_{30}NO_9$: 416.1920).

The Hydrolysis of 2 Compound **2** (2 mg) was dissolved in 4 M HCl (10 mL), and the solution was refluxed for 3 h. After cooling, the reaction mixture was extracted with AcOEt, and then the aqueous layer was adjusted for pH 11 with 25% NH_4OH, and extracted with AcOEt. The alkaline organic layer was concentrated *in vacuo* to give (2*R*,3*R*,4*R*,5*R*)-2-(hydroxymethyl)-5-(4-methoxyphenyl)-pyrrolidine-3,4-diol (0.5 mg). The residue was purified by HPLC on an amino column with 75%CH_3CN to give D-glucose (0.3 mg). (2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(4-methoxyphenyl)-pyrrolidine-3,4-diol was confirmed by comparison to data in the literature[6] and D-glucose was decided by comparison with the standard compound.

Codonopsinol A (**3**): Colorless amorphous gum: $[\alpha]_D$ +5° (c=0.30, MeOH). IR (ATR) ν_{max} cm^{-1}: 3317 (–OH), 1512 (phenyl). UV (MeOH) λ_{max}nm (logε): 281 (3.35), 227 (3.73). CD (MeOH) $\Delta\varepsilon$ (nm): +2.41 (279), +6.65 (225). ^{1}H-NMR (CD_3OD) δ: 2.22 (3H, s, N-CH_3), 3.10 (1H, td, J=4.2, 4.8 Hz, 2-H), 3.65 (1H, d, J=6.6 Hz, 5-H), 3.82 (1H, d, J=4.2, 11.7 Hz, 1-H), 3.85 (3H, s, O-CH_3), 3.86 (1H, dd, J=4.2, 11.7 Hz, 1-H), 3.96 (1H, dd, J=4.8, 6.6 Hz, 4-H), 4.01 (1H, t, J=4.8 Hz, 3-H), 6.81 (1H, dd, J=2.0, 8.3 Hz, 6′-H), 6.87 (1H, d, J=2.0 Hz, 2′-H), 6.90 (1H, d, J=8.3 Hz, 5′-H). ^{13}C-NMR (CD_3OD) δ: 35.1 (q, N-CH_3), 56.4 (t, C-1), 60.6 (q, O-CH_3), 71.1 (d, C-2), 75.4 (d, C-5), 79.6 (d, C-3), 85.1 (d, C-4), 112.5 (d, C-5′), 116.2 (d, C-2′), 121.3 (d, C-6′), 133.5 (s, C-1′), 147.7 (s, C-3′, 148.8 (s, C-4′). ESI-TOF-MS m/z: 270.1351 $[M+H]^+$ (Calcd for $C_{13}H_{20}NO_5$: 270.1341).

Codonopsinol B (**4**): Colorless amorphous gum: $[\alpha]_D$ −8° (c=0.45, MeOH). IR (ATR) ν_{max} cm^{-1}: 3313 (–OH), 1612, 1514 (phenyl). UV (MeOH) λ_{max}nm (logε): 276 (2.98), 226 (3.83). CD (MeOH) $\Delta\varepsilon$ (nm): +2.66 (277), +4.57 (220). ^{1}H-NMR (CD_3OD) δ: 2.16 (3H, s, N-CH_3), 3.06 (1H, td, J=4.0, 4.6 Hz, 2-H), 3.65 (1H, d, J=6.5 Hz, 5-H), 3.77 (3H, s, O-CH_3), 3.80 (1H, d, J=4.6, 11.5 Hz, 1-H), 3.84 (1H, dd, J=4.0, 11.5 Hz, 1-H), 3.92 (1H, dd, J=4.6, 6.5 Hz, 4-H), 4.00 (1H, t, J=4.6 Hz, 3-H), 6.89 (2H, d, J=8.5 Hz, 3′-H, 5′-H), 7.27 (2H, d, J=8.5 Hz, 2′-H, 6′-H). ^{13}C-NMR (CD_3OD) δ: 35.0 (q, N-CH_3), 55.7 (q, O-CH_3), 60.8 (t, C-1), 71.0 (d, C-2), 75.3 (d, C-5), 79.9 (d, C-3), 85.6 (d, C-4), 114.8 (d, C-3′, C-5′), 130.6 (d, C-2′, C-6′), 133.5 (s, C-1′), 160.7 (s, C-4′). ESI-TOF-MS m/z 254.1401 $[M+H]^+$ (Calcd for $C_{13}H_{20}NO_4$: 254.1392).

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