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UV-Guided Isolation of Polyynes and Polyenes from the Roots of *Codonopsis pilosula*†

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ABSTRACT

UV-guided isolation of polyacetylenes from the crude extract of *Codonopsis pilosula* has successfully led to the characterization of five new polyynes, pilosulynes A–E (**1**–**5**), and two new polyenes, pilosulynes F and G (**6** and **7**), as well as five known analogues (**8**–**12**). Their structures were determined by means of spectroscopic methods, including that of ICD and 1D/2D NMR experiments. The absolute configurations of the 6,7-diol moiety of the isolates were determined by Snatzke's method, observing the induced circular dichroism after addition of dimolybdenum tetraacetate in DMSO. Compound **6** exhibited anti-HCV activity in the HCVcc infection assay with EC_{50} value of 47.2 μM.

Keywords: *Codonopsis pilosula*; polyynes; polyenes; HCVcc

Introduction

Codonopsis pilosula (Franch.) Nannf. belongs to the family of Campanulaceae and its root usually applies to traditional Chinese medicine (TCM). It has been used commonly in China to strengthen the middle warmer, invigorate the spleen, and nourish lung, and is sometimes used as a substitute of the much more costly *Panax ginseng*.[1] The roots of *C. pilosula* have a mild, sweet taste and are used in some Chinese food therapy recipes; for example, chicken soup may be prepared with it and other mild herbs to make a good flavor tonic.

Water-soluble polysaccharides from Radix Codonopsis Pilosulae, the roots of *Codonopsis pilosula*, were reported to exhibit immunoregulatory activity. The healthy supplementary products of Radix Codonopsis Pilosulae have been developed in the market due to their potent immunological activity, similar to Lucid Ganoderma (*Ganoderma lucidum*) polysaccharide. The polyacetylene (polyyne) glycosides, such as lobetyolin (**10**) and lobetyolinin (**11**),[2,3] are also water-soluble constituents in the water extract of *C. pilosula*. Thus, it is important to clarify the biological activity of the polyynes from *C. pilosula*. Its main constituents include sterols, triterpenes, glycosides, alkaloids, polyyne, and polysaccharides.[4–7] Recently, lobetyolin, a polyyne glycoside, was demonstrated to significantly stimulate the Nuclear factor-kappa B (NF-κB),[8] a transcriptional factor found in almost all animal cell types, playing an important role in regulating the immune response to infection and participates in the inflammatory response, cancer, autoimmune disease, and synaptic

plasticity.[9] However, little is known about the biological properties and absolute configurations of polyynes in *C. pilosula*. In an attempt to disclose the bioactive polyynes from the water extract, a series of extraction, isolation, and structural elucidation experiments were undertaken, which have resulted in the characterization of five new polyynes (**1**–**5**) and two new polyenes (**6** and **7**) as well as six known analogues (**8**–**12**).

Results and discussion

The H_2O extract of the roots of *C. pilosula* was subjected to Diaion HP-20 column chromatography. The fractions containing polyynes or polyenes were selected based on characteristic UV spectra which exhibited a palm-like (or sawtooth-like) shape between 225 to 300 nm with a maximum absorption around 270 nm.[10] The UV-guided fractionation successfully led to the isolation of five new polyynes (**1**–**5**) and two new polyenes (**6** and **7**) as well as six known analogues (**8**–**12**). The known compounds were identified as lobetyol (**8**),[3] tetradeca-4*E*,8*E*,12*E*-triene-10-yne-1,6,7-triol (**9**),[11] lobetyolin (**10**),[3] lobetyolinin (**11**),[2] and cordifolioidyne B (**12**)[12] by comparison of their physical and spectroscopic data with those reported in literature.

The HRESIMS spectrum of pilosulyne A (**1**) exhibited a pseudomolecular ion peak at *m/z* 275.1261 $[M + Na]^+$, consistent with a molecular formula of $C_{14}H_{20}O_4$, appropriate for 5 degrees of unsaturation. The ^{13}C NMR and DEPT spectroscopic data (Table 1) displayed 14 carbon signals, including 6 methylenes, 4 methines, and 4 quaternary carbons. The UV (283, 267, 253, 240 nm) and

IR (2164 cm^{-1}) spectrum were closely correlated to those of known polyacetylenes, lobetyol (**8**), lobetyolin (**10**), and lobetyolinin (**11**).[2,3] In addition, proton resonances at δ 6.41 (1H, dt, J = 15.5, 4.6 Hz), 5.81 (1H, dd, J = 15.5, 2.1 Hz) (Table 2) and carbon resonances at δ 82.5 (C), 70.7 (C), 74.2 (C), 77.4 (C), 108.5 (CH), and 148.2 (CH) (Table 1) corroborated the presence of two C≡C bonds and one C=C bond in conjugation.[2,3] This double bond was assigned as *E* owing to a large vicinal coupling constant (J = 15.5 Hz). The $^{1}H–^{1}H$ COSY correlations between H-12/H-13, H-13/H_2-14 and HMBC correlations from H-13 to C-11 and H-12 to C-10 indicated the attachment of hydroxymethyl group at C-13 (Figure 1). Two mutually coupled protons due to the hydroxyl-containing methines at δ 3.52 (1H, m) and 4.24 (1H, d, J = 6.2 Hz) were assigned at C-6 and C-7, respectively. This was corroborated by the presence of HMBC correlations from H-7 to C-8, C-9, and C-6 as well as from H-6 to C-8 (Figure 1). Considering the molecular formula and degrees of unsaturation, the remaining five sp^3 carbons composed of four aliphatic carbons (δ 33.4, CH_2; 26.9, CH_2; 26.6, CH_2; 33.6, CH_2) and one hydroxyl-containing methylene were assigned to be a hydroxypentyl functionality attached at C-6. The relevant NMR data in literature suggested that *threo* and *erythro vic*-diols with similar partial structures were found to have the coupling constants of 6.0 – 7.0 Hz for *threo* diols and 3.0 – 4.0 Hz for *erythro* diols.[13–15] Accordingly, the *vic*-diol group of **1** was concluded to be *threo*-configuration by the coupling constant (J = 6.2 Hz) between H-6 and H-7.

The absolute configuration of the *vic*-diol moiety in **1** was assigned using the in situ dimolybdenum CD method developed by Snatzke and Frelek.[16,17] Briefly, this method involves the

formation of chiral complexes in situ by mixing the chiral *vic*-diol with $[Mo_2(OAc)_4]$, which generates a significant induced circular dichroism spectrum (ICD). The sign of the Cotton effect around 310–320 nm in the ICD is related to the chirality of the diol moiety, expressed by the sign of the O-C-C-O torsion angle. The negative Cotton effect at 317 nm in the ICD of **1** led to the assignment of a 6*R*,7*R* configuration on the basis of the empirical rule (Figure 2A).

HRESIMS analysis of pilosulyne B (**2**) provided a molecular formula of $C_{14}H_{18}O_4$, the same as that of a known compound, *threo*-tetradeca-2,10-diene-4,6-diyne-1,8,9,14-tetrol.[12] Their NMR spectroscopic data were quite similar, with differences in the coupling patterns of the C-12/C-13 double bond. The differences in NMR data were ascribed to the *Z* geometry of C-12/C-13 double bond, which was corroborated by an 11.0 Hz coupling constant between H-12 and H-13 and an NOE correlation between these two protons. The coupling constant (J = 6.6 Hz) between H-6 and H-7 suggested *threo*-configuration for the *vic*-diol group of **2**. Similarly, the negative Cotton effect at 320 nm observed in the ICD of **2** confirmed the 6*R*,7*R* configuration according to the Snatzke's empirical rule.

Analysis of the HREIMS and ^{13}C NMR spectroscopic data of pilosulyne C (**3**) suggested a molecular formula of $C_{14}H_{20}O_3$. The NMR spectroscopic data of **3** were similar to those of the aglycone moiety of cordifolioidyne B (**12**),[12] except that the NMR signals of the C-12/C-13 double bond in **12** disappeared and were replaced by a single bond in **3**. This was confirmed by the detailed inspection of the 2D NMR experiments. The absolute configuration at C-5 was determined by the

application of Mosher's method.[18] The (*S*)- and (*R*)-MTPA esters of **3** (**3a** and **3b**, respectively) were prepared using the corresponding (*R*)- and (*S*)-MTPA chlorides, respectively. The determination of chemical shift differences for the protons neighboring C-5 led to the assignment of 5*S* configuration in **3** (Figure 2B).

Pilosulyne D (**4**) gave the same molecular formula as that of a known polyyne, lobetyolin (**10**),[3] based on the interpretation of the HRESIMS and ^{13}C NMR spectroscopic data (Table 3). The ^{1}H NMR spectrum of **4** showed one anomeric proton at δ 4.35 (1H, d, J = 8.0 Hz), which was suggested to arise from one β-glucopyranose moiety resonating at δ_C 100.8 (CH, C-1'), 75.0 (CH, C-2'), 78.1 (CH, C-3'), 71.7 (CH, C-4'), 78.1 (CH, C-5'), and 62.8 (CH_2, C-6').[3,12] The NMR spectra of the aglycone moiety of **4** were similar to those of **10**, except for the coupling constants between H-12 and H-13. An 11.6 Hz coupling constant between H-12 and H-13 suggested the *Z*-configuration of C-12/C-13 double bond. This was confirmed by an NOE correlation between H-12 and H-13. The absolute configuration of the sugar moiety in **4** was determined by reversed phase HPLC analysis of its *o*-tolylthiocarbamate.[19] The liberated glucose from acid hydrolysis of **4** was treated with L-cysteine methyl ester followed by reaction with *o*-tolylisothiocyanate to afford the corresponding *o*-tolylthiocarbamate derivative. The retention time of the sugar derivative by HPLC analysis was found to be consistent with that of standard D-glucose derivative. On the basis of the biogenetic considerations, the absolute configurations at C-6 and C-7 were suggested to be the same as those of **1** and **2**.

The molecular formula of pilosulyne E (**5**) was found to be $C_{28}H_{36}O_5$, as deduced from HRESIMS and ^{13}C NMR data (Table 3). The ^{1}H NMR spectrum of **5** showed signals for two anomeric protons at δ 4.37 (1H, d, J = 7.6 Hz) and 4.60 (1H, d, J = 7.6 Hz), suggesting the presence of two sugar moieties. Except for the sugar residues, the NMR spectroscopic data of the aglycone moiety of **5** was found to be quite similar to those of a known monoglucoside (**12**),[12] suggesting that they shared the same aglycone moiety. By excluding the aglycone moiety, the remaining twelve oxygenated carbons in **5** were ascribed to the presence of two glucose residues (Table 3). The D-glucose was deduced as the only sugar residue in **5** according to RP HPLC analysis of the corresponding *o*-tolylthiocarbamate as described above. The carbon resonances assignable to C-2′ (83.2, CH) showed significant downfield shift, which indicated clearly the attachment of another glucose residue at this position. This was further confirmed by the HMBC correlations from H-1″ to C-2′ and H-5 to C-1′. The large coupling constant (J = 7.6 Hz) of H-1′ and H-1″ revealed the β-configuration of these two glucose residues. Biogenetically, the absolute configuration for the aglycone moiety of **5** was suggested to be the same as that of **3**.

The HRESIMS spectrum of pilosulyne F (**6**) exhibited a pseudomolecular ion peak at *m/z* 277.1414 $[M + Na]^+$, consistent with a molecular formula of $C_{14}H_{22}O_4$. Two pairs of *trans* double bonds [δ 6.19 (1H, dt, J = 16.0, 4.9 Hz), 5.87 (1H, dd, J = 15.5, 1.6 Hz); 6.13 (1H, dd, J = 15.6, 6.0 Hz), 5.84 (1H, d, J = 15.6 Hz)] and carbon resonances at δ 88.8 (C), 88.9 (C), 110.7 (CH), 112.1 (CH), 112.1 (CH), and 143.4 (CH) (Tables 1 and 2) revealed the presence of one C≡C bonds and two

C=C bond in conjugation, which was confirmed by the UV absorption bands at 264 and 279 nm. The hydroxymethyl group (δ_H 4.12, 2H) was assigned at C-13 due to the presence of allylic coupling ($J_{12,14}$ = 1.6 Hz) and HMBC correlations from H_2-14 to C-12 and C-13 as well as H-13 to C-11. Two mutually coupled hydroxyl-containing methine protons at δ 3.45 (1H, m) and 4.00 (1H, t, J = 6.0 Hz) was assigned at C-6 and C-7, respectively, due to the presence of HMBC correlations from H-7 to C-8, C-9, and C-6 as well as H-8 to C-10. The remaining five sp^3 carbons (δ 33.6, CH_2; 26.8, CH_2; 26.9, CH_2; 33.6, CH_2; 62.9, CH_2) were assigned to be a hydroxypentyl functionality attached at C-6. Similarly, the *vic*-diol group of **6** was suggested to be *threo*-configuration by the coupling constant (J = 6.0 Hz) between H-6 and H-7. According to the Snatzke's empirical rule, the negative Cotton effect at 331 nm observed in the ICD of **6** confirmed the 6*R*,7*R* configuration.

The formula of pilosulyne G (**7**) was found to be $C_{14}H_{20}O_4$, 2 mass units less than that of **6**, as deduced from HRESIMS and NMR spectroscopic data. By comparison of ^1H and ^{13}C NMR spectroscopic data of **7** (Tables 1 and 2) with those of **6**, it was found that **7** is the *trans* C-4/C-5 double bond (5.49, 1H, dd, J = 15.4, 7.0 Hz; 5.73, dt, J = 15.4, 7.0 Hz) derivative of **6**. The coupling constant (J = 6.6 Hz) between H-6 and H-7 suggested the *threo*-configuration for the *vic*-diol group in **7**. Similarly, the negative Cotton effect at 329 nm observed in the ICD of **7** confirmed the 6*R*,7*R* configuration.

Lobetyol (**8**), possessing the 6*R*,7*R* configuration, was the only polyyne of which the absolute configuration had been established in prior study.[3,20] Except for **8**, the absolute configurations of the

other polyynes isolated from the genus *Codonopsis* have not been established before. Thus, we applied the aforementioned dimolybdenum CD method on compounds **7** and **9**, and the resulting CD spectra showed negative Cotton effects at 323 and 315 nm, respectively, suggesting the 6*R*,7*R* configurations for both compounds. Similarly, compound **8** also showed negative Cotton effect at 317 nm, confirming the same 6*R*,7*R* configuration with the prior work that established by a serious of chemical reactions.[20]

Hepatitis C is an infectious disease affecting primarily the liver, caused by the hepatitis C virus (HCV). Interferon alone or pegylated interferon combined with ribavirin is the current anti-HCV therapy that usually accompanies with strong side effects and moderate successful rate.[21] Therefore, discovery of small molecular inhibitors of HCV replication would meet an urgent need. Some polyynes were reported to exhibit anti-virus activities, such as polyacetyleneginsenoside-Ro[22] and minquartynoic acid.[23] Thus, the HCVcc infection assay was used to evaluate the anti-HCV activity of compounds **1**–**12**. The result showed that compound **6** exhibited anti-HCV activity with EC_{50} value of 47.2 μM, whereas the other compounds were found to be inactive in the HCVcc infection assay. In addition, **6** was found to be non-toxic toward the tested Huh7.5 cell lines with an IC_{50} value for more than 100μM. Compounds **3**, **4** and **9**–**12** were also evaluated for their inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB. However, they were not observed to exhibit inhibition effect in the above two assays.

Conclusion

Phytochemical study of *C. pilosula* has resulted in the characterization of five new polyynes (**1**–**5**) and two new polyenes (**6** and **7**) as well as five known analogues (**8**–**12**). As far as we know, this is the first report of determination of absolute configurations of *vic*-diols in polyyne and polyene with compounds available in trace amount. Considering its medicinal use and being a potential healthy supplementary product in the future, our work is clearly important, revealing its chemical constituents in the water extract. In this study we have also shown that compound **6** exhibited marginal anti-HCV activity in the HCVcc infection assay.

Experimental section

General experimental procedures

Melting points were determined on a Yanagimoto MP-S3 micromelting point apparatus. IR spectra were recorded on a Shimadzu FTIR spectrometer Prestige-21. Optical rotations were measured using a Jasco JASCO P-1020 polarimeter. UV spectra were obtained on a Hitachi UV-3210 spectrophotometer. ESI and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. The NMR spectra, including ^{1}H NMR, ^{13}C NMR, COSY, NOESY, HMBC, HMQC, and 1D-TOCSY experiments, were recorded on Bruker AVANCE-500 and AMX-400. Silica gel (E. Merck 70–230, 230–400 mesh) and RP-18 gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) and precoated

RP-18 F_{254S} plates (Merck) were used for TLC analysis. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT_{VP} series pumping system equipped with a Shimadzu SPD-M10A_{VP} diode array detector and a semipreparative reversed-phase column (Merck, Hibar Purospher RP-18e, 5 μm, 250 × 10 mm).

Plant material

The roots of *Codonopsis pilosula* (Franch.) Nannf. were collected by Kaiser pharmaceutical company from Wen County, Gansu Province, People's Republic of China, in April, 2008 and subsequently air-dried at room temperature without any heat-processing. We purchased this material from the company in August, 2008, in Tainan, Taiwan, and authenticated by Prof. C. S. Kuoh (Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan). The material A voucher specimen (CP-Wu 2008005) has been deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and isolation

The roots of *C. pilosula* (5Kg, dry weight) were minced and extracted four times with H_2O under reflux for 5 h. The combined extract was concentrated and subjected to reversed-phase Diaion HP-20 column chromatography eluting with H_2O, H_2O–MeOH (1:1), and MeOH in sequence to yield three subfractions, CPW, CPWM, and CPM, respectively. The CPM fraction was selected for further

purification owing to its characteristic UV spectra possessing a palm-like shape between 225 to 300 nM, which revealed the presence of polyynes or polyynes.[10] The CPM fraction (90 g) was fractionated by open column chromatography on silica gel using $CHCl_3$–MeOH mixtures of increasing polarity to yield 8 fractions (CPM1 to CPM8). Among the eight fractions, CPM2–CPM5, were found to contain polyynes or polyynes according to the UV spectrum. CPM2 was separated by silica gel column chromatography with CH_2Cl_2–MeOH (9:1) to yield two subfractions (CPM2A and CPM2B). CPM2A was chromatographed on silica gel using $CHCl_3$–MeOH (8:1), followed by RP-18 HPLC (CH_3CN–H_2O, 30%) to obtain compounds **8** (1.0 mg) and **9** (1.6 mg). CPM2B was separated by RP-18 HPLC (CH_3CN–H_2O, 21%) to yield compounds **2** (0.4 mg) and **3** (1.7 mg). CPM3 was chromatographed on silica gel using EtOAc–MeOH (14:1), followed by column chromatography on RP-18 gel (MeOH-H_2O, 33 %) to afford two subfractions CPM3A and CPM3B. Compound **7** (0.8 mg) was obtained from subfraction CPM3A by RP-18 HPLC (CH_3CN–H_2O, 13%). CPM3B was separated by RP-18 HPLC (CH_3CN–H_2O, 15%) to obtain compounds **1** (0.8 mg) and **6** (0.4 mg). CPM4 was separated by repeated column chromatography over RP-18 gel (MeOH–H_2O, 30%), silica gel ($CHCl_3$–MeOH, 5:1), followed by RP-18 HPLC (CH_3CN–H_2O, 18%) to yield compounds **10** (37.0 mg) and **12** (6.0 mg). CPM5 was fractionated by RP-18 column chromatography (MeOH–H_2O, 30%) to yield two subfractions (CPM5A and CPM5B). Compound **4** (2.5 mg) was obtained from subfraction CPM5A by RP-18 HPLC (MeOH–H_2O, 39 %). CPM5B was separated by repeated column chromatography over silica gel ($CHCl_3$–MeOH, 3:1), RP-18 gel (MeOH–H_2O, 33%),

followed by RP-18 HPLC (CH_3CN–H_2O, 15%) to yield compounds **5** (18 mg) and **11** (5.0 mg).

Pilosulyne A (1). Colorless oil; $[\alpha]^{25}_D$ +34.6 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.37), 253 (3.58), 267 (3.73), 283 (3.64) nm; IR (KBr) ν_{max} 3305, 2924, 2854, 2164, 1629, 1458, 1257, 1095, 1033 cm^{-1}; ^{13}C NMR and ^1H NMR data, see Tables 1 and 2; ESIMS *m/z* 275 $[M+Na]^+$; HRESIMS *m/z* 275.1261 $[M+Na]^+$ (calcd for $C_{14}H_{20}O_4Na$, 275.1259).

Pilosulyne B (2). Colorless oil; $[\alpha]^{25}_D$ +20.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 241 (3.24), 254 (3.42), 268 (3.57), 284 (3.47) nm; IR (KBr) ν_{max} 3313, 2924, 2854, 2164, 1678, 1632, 1261, 1026 cm^{-1}; ^{13}C NMR and ^1H NMR data, see Tables 1 and 2; ESIMS *m/z* 273 $[M+Na]^+$; HRESIMS *m/z* 273.1102 $[M+Na]^+$ (calcd for $C_{14}H_{18}O_4Na$, 273.1103).

Pilosulyne C (3). Colorless oil; $[\alpha]^{25}_D$ +26.3 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.49), 252 (2.69), 267 (3.86), 283 (3.76), 293 (3.03), 313 (2.84) nm; IR (KBr) ν_{max} 3302, 2927, 2858, 2233, 2137, 1423, 1330, 1300, 1056, 952 cm^{-1}; ^{13}C NMR and ^1H NMR data, see Tables 1 and 2; ESIMS *m/z* 259 $[M+Na]^+$; HRESIMS *m/z* 259.1309 $[M+Na]^+$ (calcd for $C_{14}H_{20}O_3Na$, 259.1310).

Pilosulyne D (4). Amorphous powder; $[\alpha]^{25}_D$ +23.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.72), 253 (3.78), 267 (3.89), 283 (3.80) nm; IR (KBr) ν_{max} 3321, 2927, 2877, 2229, 1654, 1573, 1415, 1076, 1045 cm^{-1}; ^{13}C NMR and ^1H NMR data, see Table 3; ESIMS *m/z* 419 $[M+Na]^+$; HRESIMS *m/z* 419.1680 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_8Na$, 419.1682).

Pilosulyne E (5). Amorphous powder; $[\alpha]^{25}_D$ –100.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 247 (4.29), 262 (3.89), 277 (4.13), 294 (4.27), 314 (4.18) nm; IR (KBr) ν_{max} 3356, 2927, 2870, 2206,

1631, 1408, 1072, 1033 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Table 3; ESIMS *m/z* 581 $[M+Na]^+$; HRESIMS *m/z* 581.2208 $[M+Na]^+$ (calcd for $C_{26}H_{38}O_{13}Na$, 581.2210).

Pilosulyne F (6).Colorless oil; $[\alpha]^{25}_D$ +11.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 264 (3.93), 279 (3.83) nm; IR (KBr) ν_{max} 3290, 2924, 2854, 2268, 1675, 1551, 1261, 1095, 1029, 952 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 277 $[M+Na]^+$; HRESIMS *m/z* 277.1414 $[M+Na]^+$ (calcd for $C_{14}H_{22}O_4Na$, 277.1416).

Pilosulyne G (7). Colorless oil; $[\alpha]^{25}_D$ +13.2 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 265 (3.71), 279 (3.62) nm; IR (KBr) ν_{max} 3309, 2924, 2854, 2276, 1676, 1550, 1261, 1095, 1029, 952 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 275 $[M+Na]^+$; HRESIMS *m/z* 275.1257 $[M+Na]^+$ (calcd for $C_{14}H_{20}O_4Na$, 275.1259).

Determination of absolute configuration of the 6,7-diol moieties in compounds 1, 2, and 6–9 by Snatzke's method

Dimolybdenum tetracetate and DMSO of spectroscopy grade (dried with 4 Å molecular sieves) were purchased from Acros. Following the reported procedure,[16,17] a solution of 6,7-diol in dry DMSO (5×10^{-4} M, 3 mL) was mixed with dimolybdenum tetraacetate (0.8 mg) in a ratio of ca. 1:1.2 diol/dimolybdenum tetraacetate. The first CD of the mixture was recorded immediately after mixing, and its time evolution was monitored until stationary (about 30 min after mixing). The inherent CD was subtracted. The observed sign of the diagnostic band at around 310−320 nm in the induced CD

spectrum was correlated to the absolute configuration of the 6,7-diol moiety.

Preparation of (S)-and (R)-MTPA esters of 3

To a solution of **3** (0.4 mg) in pyridine (0.4 mL) was added (*R*)-MTPA chloride (25 μL), and the mixture was allowed to stand for 3 h at room temperature. The reaction was quenched by the addition of H_2O (1.0 mL), and the mixture was subsequently extracted with EtOAc (3 × 1.0 mL). The EtOAc-soluble layers were combined, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to silica gel column chromatography using *n*-hexane−EtOAc (5:1) to yield the (*S*)-MTPA ester, **3a** (0.3 mg). The same procedure was used to prepare the (*R*)-MTPA ester, **3b** (0.4 mg from 0.4 mg of **3**), with (*S*)-MTPA chloride. Selected ^{1}H NMR ($CDCl_3$, 300 MHz) of **3a**: δ 7.400−7.500 (5H, m, Ph), 6.10 (1H, dd, J = 16.0, 7.1 Hz, H-6), 5.74 (1H, J = 16.0 Hz, H-7), 5.42 (1H, q, J = 7.0 Hz, H-5), 4.42 (2H, m, H_2-14), 4.20 (2H, m, H_2-1), 3.54 (3H, s, O*Me*), 3.53 (3H, s, O*Me*), 3.51 (3H, s, O*Me*), 2.38 (2H, t, J = 6.7 Hz, H_2-12), 1.94 (2H, m, H_2-13), 1.61 (2H, m, H_2-4), 1.58 (2H, m, H_2-2), 1.23 (2H, m, H_2-3). ^{1}H NMR ($CDCl_3$, 300 MHz) of **3b**: δ 7.40−7.49 (5H, m, Ph), 6.01 (1H, dd, J = 16.0, 6.7 Hz, H-6), 5.63 (1H, J = 16.0 Hz, H-7), 5.40 (1H, q, J = 6.7 Hz, H-5), 4.42 (2H, m, H_2-14), 4.27 (2H, m, H_2-1), 3.54 (3H, s, O*Me*), 3.53 (3H, s, O*Me*), 3.51 (3H, s, O*Me*), 2.38 (2H, t, J = 6.7 Hz, H_2-12), 1.94 (2H, m, H_2-13), 1.69 (2H, m, H_2-4), 1.63 (2H, m, H_2-2), 1.33 (2H, m, H_2-3).

Determination of sugar configuration

Authentic samples of D-Glc (or L-Glc) and L-cysteine methyl ester hydrochloride (each 0.5 mg) were dissolved in pyridine (0.1 mL) and heated at 60 °C for 1 h. Then the *o*-tolylisothiocyanate (0.5 mg in 0.1 mL pyridine) was added to the mixture and heated at 60 °C for additional 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC (Cosmosil 5C18-AR II column; 4.6 × 250 nm; 25% CH_3CN in 50 mM H_3PO_4; 0.8 mL/min; 25 °C) and detected at 250 nm.[19]

The glycoside (0.3 mg, for each) was hydrolyzed by heating in 0.6 M HCl (0.1 mL) and neutralized with Amberlite IRA-400(OH). After the reaction mixture was dried *in vacuo*, the afforded residue was dissolved in pyridine (0.1 mL) containing L-cysteine methyl ester (0.5 mg) and heated at 60 °C for 1 h. A 0.1 mL solution of *o*-tolylisothiocyanate (0.5 mg) in pyridine was added to the mixture, which was heated at 60 °C for additional 1 h, to yield the corresponding *o*-tolylthiocarbamate derivative. RP HPLC analysis of the *o*-tolylthiocarbamate derivative derived from the hydrolyte of the glycosides **4** and **5** showed peaks at 32.4 and 32.5 min, respectively, while the t_R values for standard L-Glc and D-Glc derivatives were observed at 28.8 and 32.5 min, respectively, suggesting the presence of a D-Glc residue in **4** and **5**.

Measurement of superoxide anion generation

Human neutrophils from the venous blood of healthy, adult volunteers (18–32 years old) were isolated with a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes.[24] Neutrophil superoxide anion generation was

determined using superoxide dismutase (SOD)-inhibitory cytochrome c reduction according to described procedures. Diphenyleneiodonium (DPI, an NADPH oxidase inhibitor) was used as the positive control in the generation of superoxide anion.

Measurement of elastase release

Degranulation of azurophilic granules was determined by elastase release as described previously. Measurement of elastase release was carried out according to such described procedures.[25] The results are expressed as the percentage of elastase release in the (formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B) FMLP/CB-activated, drug-free control system. Phenylmethylsulfonyl fluoride (PMSF, a serine protease inhibitor) was used as the positive control in the release of elastase.

Cell culture and cell viability assay

The human hepatoma Huh7.5 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% non-essential amino acid (NEAA). Viable cells were determined by the CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit.[26]

Infectious HCV particles production and infection inhibition assay

The production of infectious JC1-Luc2A HCV particles (HCVcc) was performed as described previously.[27] For infection inhibition assay, Huh7.5 cells were seeded in 96-well plate at the density of 1×10^4 cells/well. At 24 h after plating, JC1-Luc2A HCV reporter virus (0.1 MOI) was added into each well for 4 h. The virus-containing supernatant was removed and fresh medium with or without the tested compound was added and incubated for a total of 72 h, and the whole system did not keep away from light intentionally. The cell lysates were then collected for luciferase activity and cell viability assay. The luciferase activity assay was performed according to the manufacturer's instructions (Bright-Glo Promega). The relative firefly luciferase versus cell viability activity was reported as the mean plus or minus the standard deviation of three independent reactions. Honokiol, an anti-HCV compound,[28] was used as a positive control with EC_{50}'s of 9.4 μM (anti-HCV activity) and IC_{50}'s of 58.5 μM (cytotoxicity toward Hun 7.5 cells).

Acknowledgements

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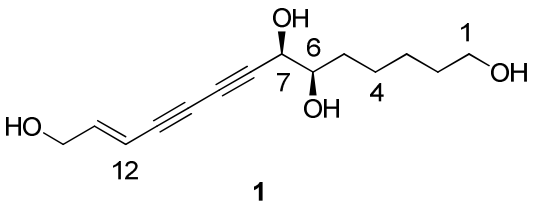

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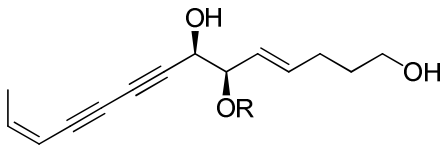
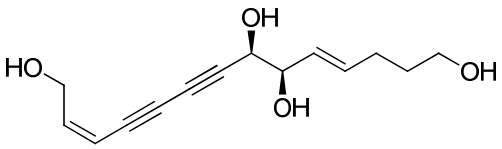


4 R = A



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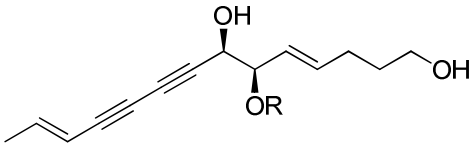
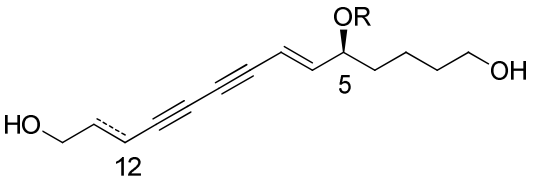


8 R = H

10 R = A

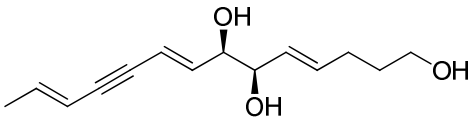
11 R = C



3 R = H, 12,13-dihydro

5 R = B, Δ^{12}

12 R = A, Δ^{12}



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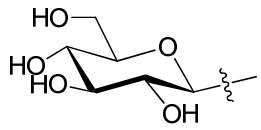
A =



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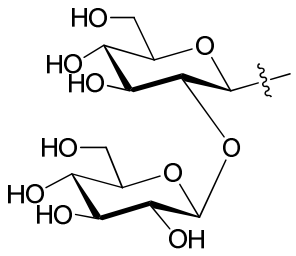
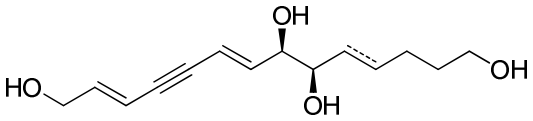




6 4,5-dihydro

7 Δ^4

C =

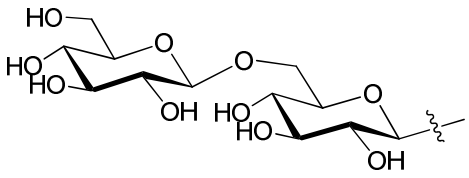


Figure captions

Fig.1 Selected $^{1}H–^{1}H$ COSY and HMBC correlations of compound **1**.

Fig. 2 (A) Projection of the helicity rule for compound **1**. (B) ^{1}H NMR chemical shift differences of MTPA esters of **3**.

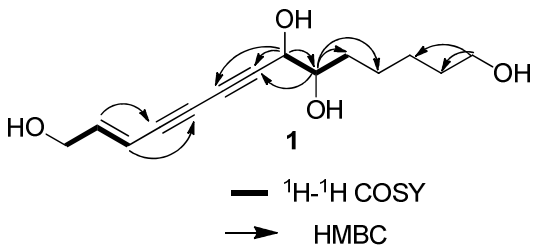
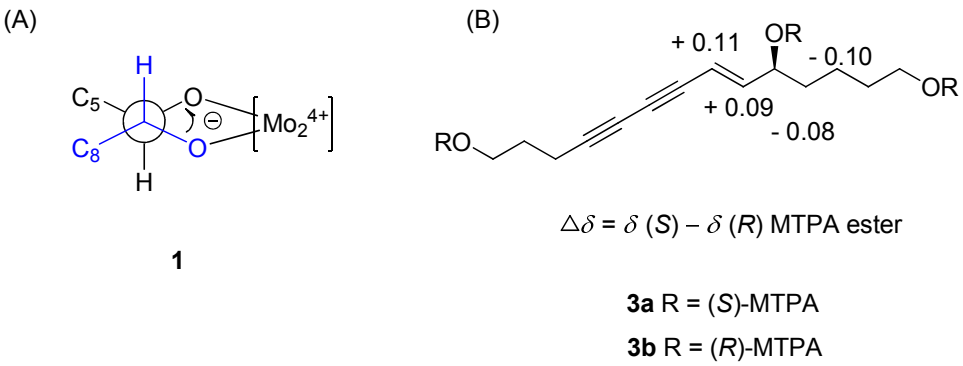


Fig.1 Selected ^{1}H–^{1}H COSY and HMBC correlations of compound **1**.



1

Fig. 2 (A) Projection of the helicity rule for compound **1**. (B) ^{1}H NMR chemical shift differences of MTPA esters of **3**.

Table 1 ^{13}C NMR Spectroscopic Data of Compounds **1–3**, **6**, and **7**

No.	**1**[a], δ_C, mult.	**2**[b], δ_C, mult.	**3**[c], δ_C, mult.	**6**[a], δ_C, mult.	**7**[b], δ_C, mult.
1	62.9, CH_2	62.3, CH_2	62.8, CH_2	62.9, CH_2	62.3, CH_2
2	33.6, CH_2	33.1, CH_2	33.5, CH_2	33.6, CH_2	33.1, CH_2
3	26.6, CH_2	29.7, CH_2	22.8, CH_2	26.9, CH_2	29.7, CH_2
4	26.9, CH_2	135.2, CH	37.7, CH_2	26.8, CH_2	134.5, CH
5	33.4, CH_2	129.6, CH	72.5, CH	33.6, CH_2	130.5, CH
6	75.3, CH	76.6, CH	150.6, CH	75.3, CH	76.7, CH
7	67.7, CH	67.8, CH	109.1, CH	76.2, CH	76.2, CH
8	82.5, C	83.8, C	74.1, C	143.9, CH	143.4, CH
9	70.7, C	70.8, C	75.3, C	110.7, CH	110.7, CH
10	74.2, C	74.7, C	66.1, C	88.9, C	88.9, C
11	77.4, C	79.4, C	84.2, C	88.8, C	88.8, C
12	108.5, CH	109.1, CH	16.6, CH_2	112.1, CH	121.1, CH
13	148.2, CH	147.8, CH	32.3, CH_2	143.4, CH	143.4, CH
14	62.6, CH_2	61.1, CH	61.4, CH_2	63.0, CH_2	62.9, CH_2

[a] Spectra were measured in CD_3OD (125 MHz). [b] Spectra were measured in CD_3OD (100 MHz). [c] Spectra were measured in CD_3OD (75 MHz).

Table 2 ^{1}H NMR Spectroscopic Data of Compounds **1**–**3**, **6**, and **7**

No	**1**, δ_H (J in Hz)[a]	**2**, δ_H (J in Hz)[b]	**3**, δ_H (J in Hz)[c]	**6**, δ_H (J in Hz)[a]	**7**, δ_H (J in Hz)[b]
1	3.56, t (6.6)	3.58, t (6.6)	3.55, t (6.6)	3.55, t (6.5)	3.57, t (6.5)
2	1,56, m	1.65, quin (6.6)	1.53, m	1.56, m	1.62, quin (6.5)
3	1.57, m	2.16, dt (6.9, 6.6)	1.44, m	1.57, m	2.13, dt (7.0, 6.5)
4	1.38, m	5.82, dt (15.4, 6.9)	1.51, m	1.38, m	5.73, dt (15.4, 7.0)
5	1.68, m	5.57, dd (15.4, 6.6)	4.10, m	1.58, m	5.49, dd (15.4, 7.0)
	1.46, m			1.36, m	
6	3.52, m	4.00, t (6.6)	6.25, dd (16.2, 5.7)	3.45, m	3.90, dd (7.0, 6.6)
7	4.24, d (6.2)	4.26, d (6.6)	5.72, d (16.2)	4.00, t (6.0)	4.00, dd (6.6, 6.0)
8				6.13, dd (15.6, 6.0)	6.09, dd (15.5, 6.0)
9				5.84, d (15.6)	5.85, d (15.5)
12	5.81, dd (15.5, 2.1)	5.64, br d (11.0)	2.42, t (7.2)	5.87 dd (16.0, 1.6)	5.85, dd (15.5, 1.6)
13	6.41, dt (15.5, 4.6)	6.24, dt (11.0, 6.4)	1.73, m	6.19, dt (16.0, 4.9)	6.18, dt (15.5, 5.0)
14	4.14, dd (4.6, 2.1)	4.31, dd (6.4, 1.2)	3.63, t (6.3)	4.12 dd (4.9, 1.6)	4.12, dd (5.0, 1.6)

[a] Spectra were measured in CD_3OD (500 MHz). [b] Spectra were measured in CD_3OD (400 MHz). [c] Spectra were measured in CD_3OD (300 MHz).

Table 3 ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **4** and **5**

No.	**4**		**5**	
	δ_H (J in Hz)[a]	δ_C, mult.[b]	δ_H (J in Hz)[a]	δ_C, mult.[b]
1	3.61, t (6.4)	62.3, CH_2	3.55, t (6.5)	62.8, CH_2
2	1.68, m	33.1, CH_2	1.55, m	33.5, CH_2
3	2.22, q (6.8)	29.9, CH_2	1.45, m	22.4, CH_2
4	5.95, dt (15.2, 6.8)	139.0, CH	1.61, m	36.2, CH_2
5	5.50, dd (15.2, 8.4)	126.7, CH	4.41, m	78.7, CH
6	4.31, dd (8.4, 6.0)	81.9, CH	6.27, dd (16.0, 6.0)	147.9, CH
7	4.48, d (6.0)	66.7, CH	6.12, d (16.0)	112.0, CH
8		82.7, C		80.4, C
9		71.1, C		74.8, C
10		78.6, C		75.3, C
11		76.1, C		80.7, C
12	5.57, dd (11.6, 1.6)	109.7, CH	5.85, br d (16.0)	108.9, CH
13	6.23, dq (11.6, 6.8)	144.3, CH	6.40, dt (16.0, 4.8)	147.9, CH
14	1.91, dd (6.8, 1.6)	16.6, CH_3	4.15, dd (4.8, 2.0)	62.7, CH_2
	Glc		Glc	
1′	4.35, d (8.0)	100.8, CH	4.37, d (7.6)	100.6, CH
2′	3.25, m	75.0, CH	3.47, dd (9.2, 7.6)	83.2, CH
3′	3.31, m	78.1, CH	3.55, dd (9.2, 8.6)	78.0, CH
4′	3.27, m	71.7, CH	3.30, m	71.7, CH
5′	3.18, m	78.1, CH	3.22, m	78.1, CH
6′	a:3.88, dd (12.0, 2.4)	62.8, CH_2	3.86, dd (12.0, 2.0)	63.0, CH_2
	b:3.68, dd (12.0, 5.9)		3.65, dd (12.0, 6.0)	
			Glc	
1″			4.60, d (7.6)	105.6, CH
2″			3.26, dd (8.8, 7.6)	76.3, CH
3″			3.39, dd (8.8, 8.8)	77.9, CH
4″			3.34, dd (9.2, 8.8)	71.7, CH
5″			3.31, m	78.4, CH
6″			3.88, dd (12.0, 2.0)	63.0, CH_2
			3.71, dd (12.0, 5.2)	

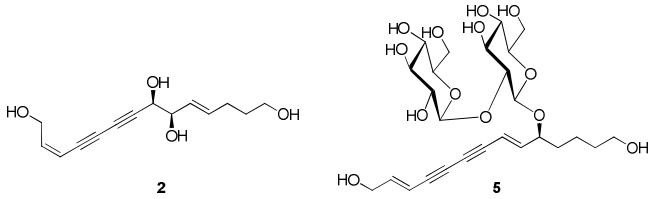
[a] Spectra were measured in CD_3OD (400 MHz). [b] Spectra were measured in CD_3OD (100 MHz).

Graphical abstract

UV-Guided Isolation of Polyynes and Polyenes from the Roots of *Codonopsis pilosula*

Chih-Hua Chao, Shin-Hun Juang, Hsiu-Hui Chan, De-Yang Shen, Yu-Ren Liao, Hung-Cheng Shih, Chieh-Hung Huang, Ju-Chien Cheng, Fu-An Chen, Hsin-Yi Hung and Tian-Shung Wu*

UV-guided isolation of polyacetylenes from *Codonopsis pilosula* has successfully led to the characterization of new polyynes and polyenes. Trace amount of compounds were needed for the determination of absolute configurations of *vic*-diols using the in situ dimolybdenum CD method. The HCVcc infection assay was used to evaluate the anti-HCV activity of compounds **1**–**12**.



2 **5**