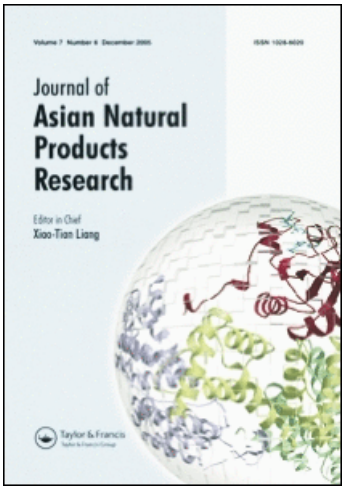

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Terpenoids and bisbibenzyls from Chinese liverworts *Conocephalum conicum* and *Dumortiera hirsuta*

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Four new monoterpene esters, 2α,5β-dihydroxybornane-2-cinnamate (**1**), 2α,5β-dihydroxybornane-5-acetyl-2-cinnamate (**2**), 2α,5β-dihydroxybornane-2-*p*-hydroxycinnamate (**3**) and 2α,5β-dihydroxybornane-2-*cis*-*p*-hydroxycinnamate (**4**), together with a known compound 3,4-dimethoxystyrene (**5**) were isolated from Chinese liverwort *Conocephalum conicum* and six known compounds, 5,7-dihydroxycalamenene (**6**), 7-hydroxycalamenene (**7**), lunularin (**8**), riccardin C (**9**), marchantin C (**10**) and riccardin D (**11**) were isolated from *Dumortiera hirsuta*. Their structures were elucidated by extensive spectral analysis and chemical correlations. Compounds **1** and **8** showed moderate cytotoxicity against human HepG2 cells with IC_{50} 4.5 μg/ml and 7.4 μg/ml, respectively, while compound **8** also showed antimicrobacterial activity against *Pseudomonas aeruginosa* with minimum inhibitory concentration at 64 μg/ml.

Keywords: Liverwort; *Conocephalum conicum*; *Dumortiera hirsuta*; Monoterpenoid; Sesquiterpenoid; Bisbibenzyls

1. Introduction

Liverworts are rich sources of novel terpenoids and often contain pharmacologically active compounds [1,2]. The genus *Conocephalum* (Marchantiales) comprises the thalloid *C. conicum* and *C. japonica*, which grow on wet rock or soil. *Conocephalum conicum* is one of the most widespread liverwort species in the world. Toyota et al. [3] have isolated some new monoterpenic esters such as bornyl ferulate, (+)-borny-*p*-cinnamate, (+)-bornyl-2-methoxy- 4-hydroxycinnamate, (+)-bornyl-*cis*-4-hydroxycinnamate and (+)-bornyl-*cis*-4-hydroxy- 3-methoxycinnamate. The GC-MS spectral analysis of *C. conicum* collected in Japan showed the presence of three brasilane type sesquiterpenes [4]. *Dumortiera hirsuta* grows on wet rocks and is widespread over China. Previous studies on this species led to the isolation of several novel sesquiterpenoids and some bisbibenzyls [5,6]. In this paper, we report the isolation and characterization of four new monoterpene esters (**1**–**4**) from *C. conicum* in addition to seven known compounds including **5** from *C. conicum* and **6**–**11** from *D. hirsute*.

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2. Results and discussion

Compound **1**, yellowish oil, molecular formula was determined to be $C_{19}H_{24}O_3$ by high-resolution mass spectral data ($[M]^+ m/z$ 300.1684). The α,β-unsaturated carbonyl moiety was confirmed by the signals at δ 7.63 and 6.41 (each 1H, d, $J = 16$ Hz) in ^{1}H NMR and the intense absorption peak at 1708.4 cm^{-1} in IR. The presence of single substituted benzene ring could be deduced from the signals at δ 7.51(2H,m) and δ 7.35 (3H,m) in ^{1}H NMR. The ^{1}H NMR spectrum also displayed signals of three quaternary methyl groups (δ 1.12, δ 0.91 and δ 0.91, 8-CH_3, 9-CH_3, 10-CH_3,), two methenes at δ 2.45 (dd, $J = 13.5$, 8 Hz, H-6β), 1.50 (dd, $J = 13$, 2 Hz, H-6β) and δ 2.41 (dd, $J = 9.5$, 5 Hz, H-3β), 0.87 (dd, $J = 9$, 3 Hz, H-3β), two oxygenated methines at δ 4.90 (1H, m, H-2) and δ 3.91 (1H,m, H-5) which were found to be correlated with the H_β-3 (δ 2.41) and H-6 (δ 2.45) respectively in ^{1}H–^{1}H COSY, H_β-3 was also coupled with the signal at δ 1.77 (H-4). In the ^{1}H–^{1}H COSY spectrum no coupling was observed between H-4 and H-5. A possible explanation could be the dihedral angle of approximately 90°, which made the ^3J-coupling constant minimized. Correlations of H-5 with δ 34.3 and 47.5, H-4 with δ 39.6 and 49.9, 10-CH_3 with δ 39.6, 78.4, 47.5 in HMBC suggested a bridged ring, indicating a bornane moiety. Furthermore, according to the correlation signals between oxygenated methine groups (δ 4.90) and carbonyl carbon (δ 167.1) in HMBC (figure 1), the skeleton structure of **1** could be deduced as depicted in figure 2. The NOESY spectrum of **1** displayed correlations between (i) H-2 and H-3β, (ii) H-2 and 9-CH_3 (or 8-CH_3), (iii) H-5 and H-4, (iv) H-5 and H-3β, (v) H-5 and H-6β (figure 3). These facts indicated the 2α and 5β orientation of the oxygenated groups. Thus the structure of compound **1** was determined to be 2α,5β-dihydroxybornane-2-cinnamate.

Compound **2**, oil, with the molecular formula $C_{21}H_{26}O_4$ (m/z 342.4251). The ^{1}H NMR and ^{13}C NMR spectral data of **2** were similar to those of **1**. The acetyl moiety was confirmed by the methyl signal at δ 2.05 (3H, s) in ^{1}H NMR spectrum and carbonyl signal at δ 170.6 in ^{13}C NMR. The chemical shift of H-5 shifted about 0.8 ppm to low field compared with that of **1** due to esterification. By comparing its NMR spectral data with those of compound **1**, the structure of **2** was identified to be 2α,5β-dihydroxybornane-5-acetyl-2-cinnamate.

Compound **3** has a molecular formula $C_{19}H_{24}O_4$ ($[M]^+ m/z$ 316.3794). The presence of a *p*-coumaroyl moiety was confirmed by the signals at δ 7.45 and 6.79 (each 2H, d, $J = 8.5$ Hz) and δ 7.58 and 6.32 (each 1H, d, $J = 16$ Hz) in ^{1}H NMR spectrum. According to its HMBC and HMQC, compound **3** has also a 2,5-dihydroxybornane moiety. By comparing its spectral data with those of compound **1**, the structure of **3** was determined to be 2α,5β-dihydroxy-bornane-2-*p*-hydroxycinnamate.

Compound **4** has a molecular formula $C_{19}H_{24}O_4$ ($[M]^+ m/z$ 316.3208). The ^{1}H NMR spectrum of **4** was similar to that of **3** except for the replacement of the *trans* olefinic protons ($J = 16$ Hz) of compound **3** by signals at δ 6.86 and 5.76 (each 1H, d, $J = 13$ Hz). Thus the

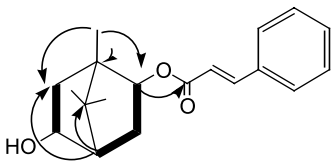

Figure 1. Key ^{1}H–^{1}H correlations (by bold lines) and HMBC correlations (by arrow) of **1**.

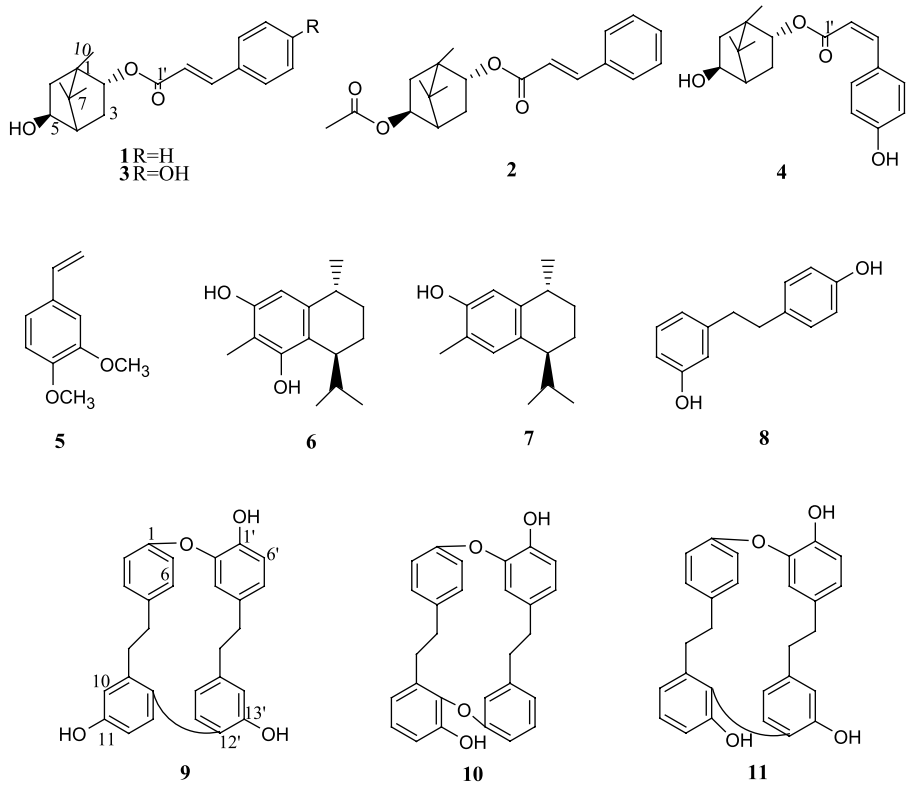

Figure 2. Structures of **1**–**11**.

structure of compound **4** was determined to be 2,5β-dihydroxybornane-2-*cis*-*p*-hydroxycinnamate.

Compounds **5**–**11** were identified to be 3,4-dimethoxystyrene, 5,7-dihydroxycalamenene, 7-hydroxycalamenene, lunularin, riccardin C, marchantin C and riccardin D, respectively, on the basis of their spectral data (^{1}H and ^{13}C NMR) and comparison with those published [7–12]. In the experimental section, the ^{13}C NMR spectral data of riccardin C (**9**) are reported for the first time.

Compound **1** showed some cytotoxicity with an IC_{50} of 4.5 μg/ml against human HepG2 cells. This could be due to the α,β-unsaturated lactone on which Michael addition with biological nucleophile could happen [13]. Lunularin (**8**) also showed moderate cytotoxicity with an IC_{50} of 7.4 μg/ml against HepG2 cells, plus weak antimicrobial activity against *Pseudomonas aeruginosa* with MIC 64 μg/ml.

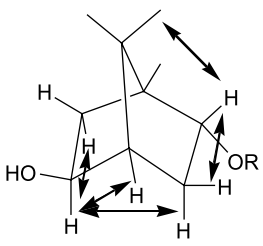

Figure 3. Key NOE correlations of **1**.

3. Experimental

3.1 General experimental procedures

IR spectra were measured on a Nicolet Nexus 470FT-IR spectrometer with KBr pellets. Electrospray MS/MS data were recorded on an API 4000 spectrometer and HR-EIMS on a HP5989A spectrometer. ^{1}H and ^{13}C NMR data were measured on a Bruker DRX-500 (^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz) with a cryo-probe. Silica gel 230–400 meshes (Merck), RP-18 modified silica gel and Sephadex LH-20 were used for column chromatography.

3.2 Plant material

Conocephalum conicum L. *Dumort* and *Dumortiera hirsuta* SW. *Reinw* were collected from Mount Emei (1500 m), Sichuan Province of China, in October 2002. They were identified by Professor Zhu Ruiliang at the School of Life Sciences, HuaDong Normal University. A voucher specimen is deposited at the College of Pharmacy, Shandong University.

3.3 Extraction and isolation

Air dried, powdered plant materials *C. conicum* (0.9 kg) and *D. hirsuta* (3.0 kg) were sequentially ultrasonic extracted with Et_2O and MeOH. The Et_2O extract (15.0 g) of *C. conicum* was chromatographed on silica gel (Petro–EtOAc, gradient) to yield 7 Frs. Frs. 2 (3.6 g) was repeatedly chromatographed on Sephadex LH-20 and silica gel to afford compound **1** (25 mg) and **2** (8 mg) and Frs. 3 to afford compound **3** (8 mg), **4** (4 mg) and **5** (7 mg). The Et_2O extract of *D. hirsuta* (15 g) was also chromatographed on silica gel to yield 6 Frs. Further purification of Frs. 2 (3.5 g) by Sephadex LH-20 and silica gel afforded compound **8** (8 mg), **9** (7 mg), **10** (9 mg) and **11** (6 mg). The MeOH extract (20 g) of *D. hirsuta* was chromatographed by silica gel ($CHCl_3$–MeOH, gradient) to yield 7 Frs. After being chromatographed on Sephadex LH-20 and silica gel (Petro–$CHCl_3$, 1:1), compound **6** (8 mg) and **7** (9 mg), respectively were obtained from Frs. 1.

3.3.1 2α,5β-Dihydroxybornane-2-cinnamate(1). Yellow oil, HR-EIMS 300.1684 ($[M]^+$) (calcd for $C_{19}H_{24}O_3$, 300.1725); ^{1}H NMR ($CDCl_3$, 500 Hz) δ: 7.63 (1H, d, $J = 16$ Hz, H-3′), 7.51 (2H, m, H-5′ and 9′), 7.35 (3H, m, H-6′, 7′ and 8′), 6.41 (1H, d, $J = 16$ Hz, H-2′), 4.90 (1H, m, H-2), 3.91 (1H, m, H-5), 2.45 (1H, dd, $J = 8$, 13.5 Hz, H-6β), 2.41 (1H, dd, $J = 5$, 9.5 Hz, H-3β), 1.76 (1H, d, $J = 5$ Hz, H-4), 1.50 (1H, d, $J = 13.5$ Hz, H-6α), 1.12 (3H, s, 8-CH_3), 0.91 (3H, s, 9-CH_3), 0.91 (3H, s, 10-CH_3), 0.87 (1H, dd, $J = 3$, 9 Hz, H-3α); ^{13}C NMR ($CDCl_3$, 125 Hz) δ: table 1.

3.3.2 2α,5β-Dihydroxybornane-5-acetyl-2-cinnamate (2). Oil, HR-EIMS: 342.4251 ($[M]^+$); ^{1}H NMR (CD_3OD, 500 Hz) δ: 7.68 (1H, d, $J = 15.6$ Hz, H-3′), 7.56 (2H, m, H-5′ and 9′), 7.40 (3H, m, H-6′, 7′ and 8′), 6.46 (1H, d, $J = 16.2$ Hz, H-2′), 4.98 (1H, d, $J = 9.6$ Hz, H-2), 4.73 (1H, dd, $J = 3.6$, 7.8 Hz, H-5), 2.57 (1H, dd, $J = 7.8$, 13.8 Hz, H-6β), 2.51 (1H, m, H-3β), 2.05 (3H, s, 2″-CH_3), 1.96 (1H, d, $J = 5.4$ Hz, H-4), 1.59 (1H, d, $J = 14.4$ Hz, H-6α), 1.07 (3H, s, 8-CH_3), 1.05 (1H, m, H-3α), 0.96 (3H, s, 9-CH_3), 0.95 (3H, s, 10-CH_3); ^{13}C NMR (CD_3OD, 125 Hz) δ: table 1.

Table 1. ^{13}C NMR spectral data of **1**–**4** (125 MHz).

Carbon no.	***1***[a]	***2***[a]	***3***[b]	***4***[b]
1	47.5	47.7	46.6	47.4
2	78.4	77.9	79.8	79.7
3	34.3	34.0	35.3	35.1
4	52.7	49.9	53.5	53.4
5	75.3	75.4	75.6	75.6
6	39.6	37.2	40.3	40.1
7	49.9	49.7	50.8	50.7
8	19.8	19.6	20.2	20.2
9	20.7	20.4	21.1	21.1
10	12.9	12.7	13.3	13.3
1′	167.1	167.1	169.5	168.7
2′	118.4	118.5	115.4	117.5
3′	144.5	144.7	146.3	144.4
4′	134.4	134.4	127.2	128.2
5′,9′	128.0	128.1	131.1	133.1
6′,8′	128.9	128.9	116.8	115.9
7′	130.2	130.2	161.3	159.5
1″	–	170.6	–	–
2″	–	21.4	–	–

a The solvent was $CDCl_3$.
b The solvent was CD_3OD.

3.3.3 2α,5β-Dihydroxybornane-2-*p*-hydroxycinnamate (3). Oil, HR-EIMS: 316.3794 ([M^+]); ^{1}H NMR (CD_3OD, 500 Hz) δ: 7.58 (1H, d, $J = 16$ Hz, H-3′), 7.45 (2H, d, $J = 9$ Hz, H-6′ and 8′), 6.79 (2H, d, $J = 8.5$ Hz, H-5′ and 9′), 6.32 (1H, d, $J = 16$ Hz, H-2′), 4.89 (1H, m, H-2), 3.84 (1H, dd, $J = 3.5$ Hz, 8 Hz, H-5), 2.44 (1H, m, H-6β), 2.37 (1H, m, H-3β), 1.75 (1H, d, $J = 5$ Hz, H-4), 1.60 (1H, d, $J = 16.5$ Hz, H-6α), 1.12 (3H, s, 8-CH_3), 0.93 (3H, s, 9-CH_3), 0.91 (3H, s, 10-CH_3), 0.87(1H, m, H-3α); ^{13}C NMR (CD_3OD, 125 Hz) δ: table 1.

3.3.4 2α,5β-Dihydroxybornane-2-*cis*-*p*-hydroxycinnamate (4). Oil, HR-EIMS: 316.3208 ([M^+]); ^{1}H NMR ($CDCl_3$, 500 Hz) δ: 7.53 (2H, d, $J = 8.5$ Hz, H-6′ and 8′), 6.86 (1H, d, $J = 13$ Hz, H-3′), 6.74 (2H, d, $J = 8.5$ Hz, H-5′ and 9′), 5.76 (1H, d, $J = 13$ Hz, H-2′), 3.73 (1H, dd, $J = 3.5$ Hz, 8 Hz, H-5), 2.18 (2H, m, H-3β, 6β), 1.72 (1H, d, $J = 5$ Hz, H-4), 1.36 (1H, d, $J = 13$ Hz, H-6α), 1.09 (3H, s, 8-CH_3), 0.85 (3H, s), 0.84(1H, m, H-3α), some hydrogen signals were overlapped; ^{13}C NMR ($CDCl_3$, 125 Hz) δ: table 1.

3.3.5 Riccardin C (9). Yellow oil. ESI-MS: 425.30 [M-1]. ^{13}C NMR ($CDCl_3$, 125 Hz): 155.8 (C-11), 152.5 (C-1), 151.8 (C-13′), 146.2 (C-2′) 143.7 (C-9), 143.3 (C-1′), 141.9 (C-9′), 139.8 (C-4), 133.0 (C-4′), 132.8 (C-13), 131.4 (C-3,11′), 129.2 (C-5), 128.2 (C-14), 124.3 (C-12′), 122.3 (C-2), 122.1 (C-5′), 121.6 (C-10′), 117.4 (C-6, 10), 116.0 (C-3′, 14′), 114.9 (C-6′), 114.3 (C-12), 38.1 (C-7), 37.7 (C-8′), 35.0 (C-7′), 29.7 (C-8).

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