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Hispidanins A–D: Four New Asymmetric Dimeric Diterpenoids from the Rhizomes of *Isodon hispida*

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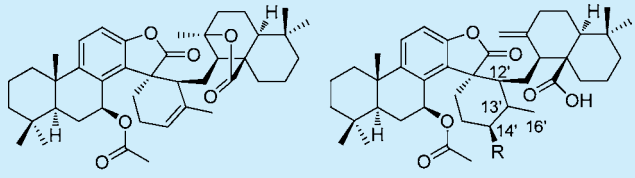
Supporting Information

ABSTRACT: Hispidanins A–D (**1**–**4**), four unprecedented asymmetric dimeric diterpenoids, were obtained from the rhizomes of *Isodon hispida*. Their structures were elucidated by extensive spectroscopic analysis (1D and 2D NMR, MS, UV, IR), as well as single-crystal X-ray diffraction analysis. Hispidanin B showed significant cytotoxicities against tumor cell lines SGC7901, SMMC7721, and K562, with IC_{50} values of 10.7, 9.8, and 13.7 μM, respectively.



Plants from the genus *Isodon* (*Rabdosia*) grow mainly in East Asia, and many of them are used as traditional medicine in China to treat various diseases such as cancers, inflammatory, and respiratory problems. Usually the stems and leaves of *Isodon* plants are utilized for traditional uses, and therefore, chemical investigations have focused on the aerial parts.[1] However, some *Isodon* species have swollen rhizomes which also have medicinal properties.[1b] As an extreme example, the swollen rhizome of *I. yuennanensis* (Bu Yu Hong) is the only plant part used for medicinal purposes instead of the aerial part. Therefore, it is definitely necessary to pay more attention to the underground parts of those *Isodon* plants that have swollen rhizomes. *I. hispida* is a medicinal plant distributed in southwest China only. It has swollen rhizomes which have not been studied,[1a] and its constituent natural products are the subject of this chemical and bioactivity investigation. *I. hispida* was collected in Dali, Yunnan, P.R. China, in 2010, and phytochemical research led to the isolation of 30 compounds from the rhizomes of this plant. Classes of compounds found include diterpenoids, triterpenoids, steroids, phenols, and lipids. Among the major constituents, four new diterpenoids, hispidanins A–D (**1**–**4**, Figure 1), revealed unique asymmetric structures formed by the bonding of totarane and labdane diterpenoids. Herein we describe structural elucidation and bioactivities of these interesting diterpenoid dimers.

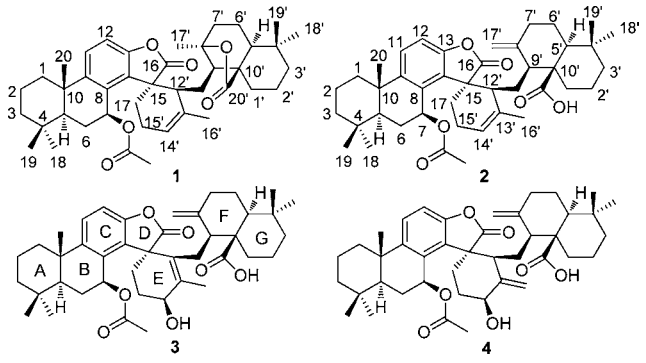


Figure 1. Structures of compounds **1**–**4**.

Hispidanin A (**1**)[2] was assigned the molecular formula $C_{42}H_{56}O_6$ [15 double bond equivalents (DBEs)] on the basis of its HREIMS (*m*/*z* 656.4077 $[M]^+$) and NMR data. The ^1H NMR spectrum of **1** [Table S1, Supporting Information (SI)] showed the signals of eight methyl singlets at δ_H 2.14 (3H, s, −OAc), 1.79 (3H, s, Me-16′), 1.42 (3H, s, Me-17′), 1.30 (3H, s, Me-20), 0.97 (3H, s, Me-18), 0.95 (3H, s, Me-19), 0.84 (3H, s, Me-18′) and 0.82 (3H, s, Me-19′), a set of *ortho*-coupled aromatic protons at δ_H 7.39 (1H, d, J = 8.6 Hz, H-11) and 7.07 (1H, d, J = 8.6 Hz, H-12), an oxygenated methine at δ_H 6.08 (1H, t, J = 8.0 Hz, H-7α), and a trisubstituted ethylene structural unit at δ_H 5.73 (1H, brd, J = 4.8 Hz, H-14′). Analyses of ^{13}C NMR and DEPT spectra revealed the existence of 42 carbons (Table S1, SI) including 8 methyls, 12 sp^3 methylenes, 5 sp^3 methines (containing one oxygenated methine at δ_C 71.7), 3 sp^2 methines (δ_C 125.8, 125.4, and 110.9), 8 sp^2 quaternary carbons (δ_C 179.7, 175.9, 170.0, 151.2, 149.3, 131.7, 131.6, and 130.6), and 6 sp^3 quaternary carbons (δ_C 83.7, 52.2, 49.7, 38.5, 33.9, and 33.3). Careful analyses of NMR spectra of **1** indicated that hispidanin A must contain an acetyl group because of the existence of signals at δ_C 170.0 and 21.6, and δ_H 2.14 (3H, s, −OAc) in the NMR spectra. Therefore, there were in total 40 carbons remaining for the skeleton of **1** in the ^{13}C NMR spectrum, which implied the presence of two diterpenoid units.

Analyses of 2D NMR data (^1H–^1H COSY, Figure 2) revealed that **1** possessed seven spin coupling systems: H_2-1/H_2-2/H_2-3, H-5/H_2-6/H-7, H-11/H-12, H_2-17/H_2-15′/H-14′, H-9′/H_2-11′/H-12′, H-5′/H_2-6′/H_2-7′ and H_2-1′/H_2-2′/H_2-3′. The key correlations were observed from HMBC spectrum of **1** (Figure

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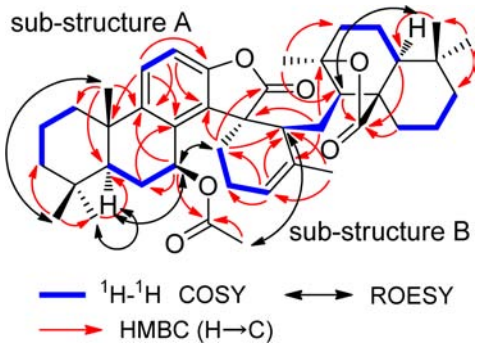

Figure 2. Key 2D NMR correlations of **1**.

2) including H-11 (δ_H 7.39, d, J = 8.6 Hz) to C-8 (δ_C 130.6), C-10 (δ_C 38.5) and C-13 (δ_C 151.2); H-12 (δ_H 7.07, d, J = 8.6 Hz) to C-9 (δ_C 149.3) and C-14 (δ_C 131.7); H_2-17 (δ_H 1.69, overlap and 2.17, m) to C-14 and C-16 (δ_C 175.9); Me-20 (δ_H 1.30, s) to C-1 (δ_C 39.6), C-5 (δ_C 47.7) and C-9; Me-18 (δ_H 0.97, s) and Me-19 (δ_H 0.95, s) to C-3 (δ_C 41.0) and C-5; H-7 (δ_H 6.08, t, J = 8.0 Hz) to C-6 (δ_C 26.6), C-9, C-14 and −OAc; and −OCOCH_3 (δ_H 2.14, s) to −O$COCH_3$ (δ_C 170.0). Compound **1** was further deduced to contain a totara-8,11,13-triene diterpenoid substructure (substructure A, Figures 1 and 2) with a carbonyl group at C-16 and an acetoxyl group at C-7. Analogously, a labdane-style diterpenoid moiety (labd-13′Z-en-8′β-ol with carbonyl at position 20′; substructure B, Figures 1 and 2) was assigned to **1** by the HMBC correlations of Me-16′ (δ_H 1.79, s) with C-12′ (δ_C 42.0) and C-14′ (δ_C 125.4); H-14′ (δ_H 5.73, brd, J = 4.8 Hz) to C-12′ and C-15′ (δ_C 20.0); H_2-11′ (δ_H 1.44, overlap and 1.78, overlap) to C-8′ (δ_C 83.7) and C-13′ (δ_C 131.6); Me-17′ (δ_H 1.42, s) to C-7′ (δ_C 37.0) and C-9′ (δ_C 54.9); H_2-1′ (δ_H 1.18, dd, J = 13.0, 8.6, 1.77 Hz, overlap), H-5′ (δ_H 0.77, overlap) and H-9′ (δ_H 1.05, brd, J = 11.2 Hz) to C-20′ (δ_C 179.7); and Me-18′ (δ_H 0.84, s) and Me-19′ (δ_H 0.82, s) to C-3′ (δ_C 41.4) and C-5′ (δ_C 50.6) (Figure 2). Therefore, compound **1** was considered to be formed by a totarane diterpene and a labdane diterpene. Careful comparison of the NMR spectroscopic data of totara-8,11,13-triene-7β,13-diol[3] and labd-13Z-ene-8β,15-diol[4] (Table S2, SI) with that of **1** indicated structural similarity of the two compounds except for the following fragments. In substructure A, C-15 and C-17 were a quaternary carbon and methylene compared with an sp^3 tertiary carbon and methyl in totara-8,11,13-triene-7β,13-diol,[3] respectively. In substructure B, comparison with labd-13Z-ene-8β,15-diol[4] (Table S2, SI), C-12′ changed from methylene into tertiary carbon while C-15′ showed an upfield chemical shift (δ_H 2.03 and 2.67, $\Delta\delta$ −2.10 and −1.46; δ_C 20.0, $\Delta\delta$ −39.3) due to deoxygenation. These changes implied that the two substructures may be linked by cyclization via C-17/15′ and C-15/12′, and this assumption unambiguously verified by ^{1}H−^{1}H COSY data [correlation between H_2-17 (δ_H 1.69, overlap and 2.17, m) and H_2-15′ (δ_H 2.03, td, J = 13.4, 3.3, 2.67 Hz, brt, J = 12.4)] (Figure 2) and HMBC correlations of H_2-17 with C-12′ (δ_C 42.0) and C-15′ (δ_C 20.0), H_2-11′ (δ_H 1.44, overlap and 1.78, overlap) with C-15 (δ_C 52.2) (Figure 2). Thus, the structural units mentioned above covered 13 unsaturation degrees, and the remaining 2 DBEs were assigned to two lactone rings (13 → 16 and 8′ → 20′ lactones). Thus, the planar structure of **1** was determined as shown in Figure 1.

The relative configuration of **1** was characterized by interpretation of the ROESY spectrum (Figure 2) and comparisons with model compounds, totara-8,11,13-triene-7β,13-diol[3] and labd-13Z-ene-8β,15-diol.[4] The ROESY correlations of H-7/H-5, H-5/Me-18, and H-5′/H-9′ indicated that these protons were cofacial and arbitrarily assigned as α-orientation and Me-19/Me-20 was assigned as β-orientation. Correlations between H-7 and H_2-17, and H-12′ and Me-OAc suggested that ring E should be perpendicular to the plane of rings C and D, and methylene at position 17 and methine at position 12′ should be located below and above this plane, respectively. For unambiguous confirmation of the stereochemical structure of **1**, a single-crystal X-ray diffraction experiment was performed, and the result is shown in Figure 3.

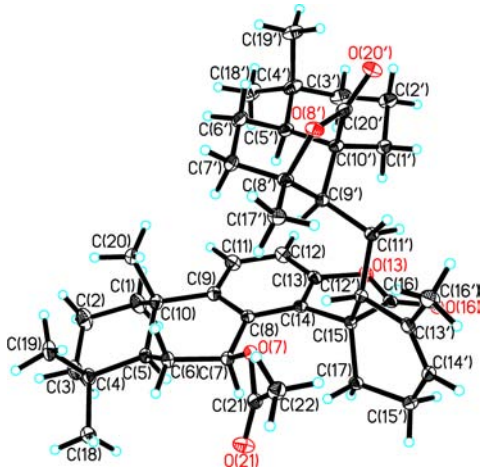

Figure 3. Single-crystal X-ray structure of **1**.

Hispidanin B (**2**)[5] possessed a molecular formula $C_{42}H_{56}O_6$ (15 DBEs) according to its HREIMS (m/z 656.4061 [M]$^+$) and NMR data. Compound **2** was shared the same molecular formula with **1**, suggesting that **2** should be an isomeric analogue of **1**. Analysis of the NMR data (Table S1, SI) indicated that the two compounds had the same partial structure for substructure A. The only difference between the two compounds existed on another part of the structure, substructure B. Compound **2** possessed a terminal alkylene group between C-8′ and C-17′ (δ_C 147.2 and 107.3) instead of the Me-17′ (δ_C 22.8) and oxygenated C-8′ (δ_C 83.7) in **1** (Table S1, SI). The structure was further supported by 2D NMR experiments (Figure 4). Therefore, the structure of **2** was determined as shown in Figure 1.

Hispidanin C (**3**)[6] has the molecular formula $C_{42}H_{56}O_7$ (15 DBEs) according to its HREIMS (m/z 672.4009 [M]$^+$) and NMR data. The molecular weight of **3** was 16 mass units higher

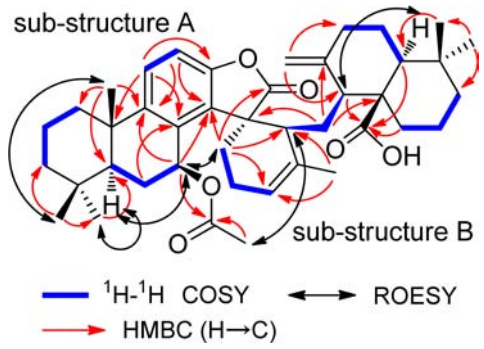

Figure 4. Key 2D NMR correlations of **2**.

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than that of **1** and **2**, suggesting that they were similar dimeric analogues but **3** should have one more oxygen than the other two compounds. Comparison of the NMR spectroscopic data (Table S1, SI) of **3** with those of **1** and **2** indicated that **3** possessed a structure more similar to that of **2**. In fact, most parts of the structure of **3** are consistent with the counterparts of **2** (Table S1, SI). The differences existed in ring E only, and **3** possessed a tetrasubstituted double bond (δ_C 135.4 and 133.2) and an oxygenated methine (δ_C 80.7) instead of the trisubstituted ethylene (δ_C 132.7 and 124.6) and nonoxygenated methine (δ_C 40.9) in **2**. Moreover, compared with **2**, the tetrasubstituted double bond in **3** transferred from C-13′/C-14′ to C-12′/C-13′ and a hydroxyl group laid on C-14′. This partial structure could be verified by the NMR experimental results and the signals for downfield chemical shifts of C-11′ (δ_C 28.6, $\Delta\delta$ +3.9) and C-15′ (δ_C 31.9, $\Delta\delta$ +11.6) were clearly observed. These suppositions were supported by 2D NMR data as well (Figure 5). According

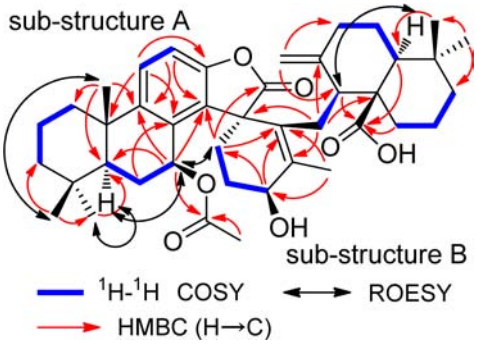


Figure 5. Key 2D NMR correlations of **3**.

to the ^{1}H NMR spectrum, the signal for H-14′ was a broad singlet peak, indicating that the dihedral angles between H-14′ and the two H-15′ should be the same and both equal to about 60°. If the hydroxyl group at C-14′ was of α-orientation, due to the steric effect between the hydroxyl group at C-14′ and carbonyl oxygen at C-16, the same dihedral angles between H-14′ and the two H-15′ could not be formed. Moreover, the assumed β-H at C-14′ (δ_H 4.32) should have cofacial configuration with H-7α (δ_H 6.13) and H-17β (δ_H 2.45) and the correlations in the ROESY spectrum should be able to observe. However, those correlations did not appear in the ROESY spectrum. On the other hand, if the 14′-hydroxyl group was at β-orientation, all phenomenons could be reasonably explained. Thus, the 14′-hydroxyl group was assigned as β-orientation. Structure of **3** is shown in Figure 1.

On the basis of the HREIMS experiment (m/z 672.4024 [M]$^+$) and NMR data, hispidanin D (**4**)[7] is proposed to possess the molecular formula $C_{42}H_{56}O_7$ (15 DBEs) which is the same molecular formula as **3**, indicating that the two compounds should be isomers. Inspection of NMR data (Table S1, SI) of **4** confirmed this assumption and the only difference between the two compounds was that the tetrasubstituted double bond between C-12′ and C-13′ in **3** was replaced by a terminal double bond between C-13′ and C-16′ (δ_C 143.4 and 116.0) in **4**. Meanwhile, Me-16′ (δ_C 20.1) was eliminated in **4** and a methine was present at position 12′ in **3**. The structure was further supported by 2D NMR data (Figure 6). Similarly, the configuration of the 14′-hydroxyl group in **4** was determined as β-orientation since the H-14′ (δ_H 4.65) was a singlet and correlations between H-14′, Me-OAc (δ_H 2.25), and H_2-17 (δ_H 1.49 and 2.52) were not observed in the ROESY spectrum as

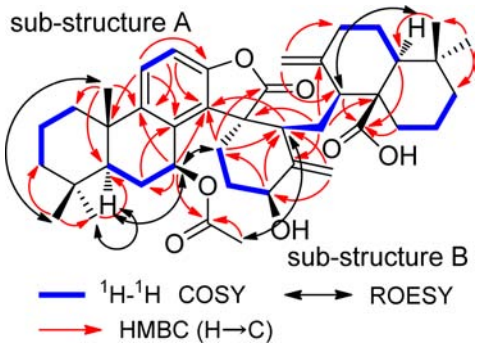


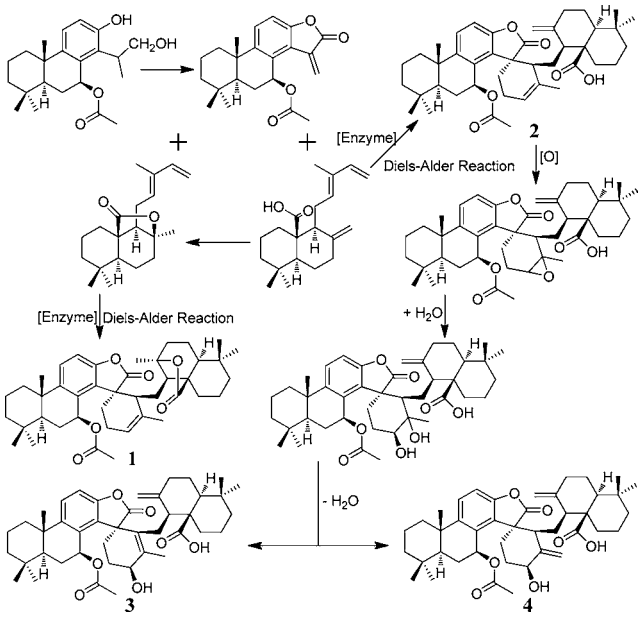
Figure 6. Key 2D NMR correlations of **4**.

well. Compound **4** was finally elucidated as the structure shown in Figure 1.

Dimeric diterpenoids from *Isodon* plants were often formed between similar diterpenoids, but rarely originated from two different types of diterpenoids.[8] Hispidanins A–D (**1**–**4**) were formed by a labdane diterpene and a totarane diterpene. Totarane diterpenoids are not only a type of diterpenoids different from labdane diterpenoids, but also a kind of diterpenoid which is seldom found in nature as well. So far, they were reported in six families including Podocarpaceae, Cupressaceae, Taxodiaceae, Verbenaceae, Celastraceae, and Schistochilaceae. Therefore, this was the first report about totarane diterpenoids from Labiatae family. The biosynthetic pathway of **1**–**4** should be intermolecular Diels–Alder reaction between totarane and labdane derivatives. The biosynthetic pathway of intermolecular Diels–Alder reaction was reported before.[8c,d] A possible biosynthetic pathway for **1**–**4** is proposed as shown in Scheme 1.

Since *Isodon* plants are often used for their antitumor activity, the 70% acetone extract of rhizomes of *I. hispida*, as well as its ethyl acetate and *n*-butanol fractions, and hispidanin B (**2**), were evaluated for the cytotoxic activities against cancer cell lines SGC7901, SMMC7721, and K562. Compound **2** exhibited significant cytotoxic activities against cancer cell lines SGC7901,

Scheme 1. Hypothetical Biosynthetic Pathways for 1–4



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SMMC7721, and K562 with IC_{50} values of 10.7, 9.8, and 13.7 μM, respectively, while mitomycin C was used as a positive control (IC_{50} 10.5, 15.0, and 6.6 μM, respectively) (Table S3, SI). However, none of the raw extracts showed activities (Table S3, SI).

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures, NMR data tables, NMR, IR, and MS spectra of **1**−**4**, and crystal data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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(2) Hispidanin A (**1**): colorless crystals (in acetone); $[\alpha]^{25}_{D}$ −85.5 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 289.4 (3.45), 209.6 (4.63) nm; IR (KBr) ν_{max} 3020, 2928, 2868, 1800, 1762, 1728, 1634, 1610, 1586, 1458, 1373, 1278, 1218, 1132, 1027, 943, 823, 756 cm^{-1}; ^{1}H and ^{13}C NMR data are listed in Table S1 in the Supporting Information; EIMS *m*/*z* 656 $[M]^+$ (5), 596 (25), 294 (44), 85 (75), 83 (100), 69 (40); HREIMS *m*/*z* 656.4077 $[M]^+$ (calcd for $C_{42}H_{56}O_6$, 656.4077). The crystal data were as follows: crystal size: 0.23 × 0.13 × 0.05 mm; crystal data: a = 12.7581 (10) Å, b = 12.8850 (10) Å, c = 23.6145 (3) Å, $\alpha = \beta = \gamma = 90°$, V = 3881.94 (7) $Å^3$, space group $P2_12_12_1$, Z = 4, D_{calc} = 1.223 Mg/m^3, λ = 1.54178 Å, μ (Cu Kα) = 0.640 mm^{-1}, $F(000)$ = 1552, and T = 100 (2) K; the total of 18175 reflections (6089 independent, R_{int} = 0.0316) were collected in the range of $3.94° \leq \theta \leq 69.68°$ and 479 refined parameters. The final indices were R_1 = 0.0372, wR_2 = 0.0992 for 6013 observed reflections with $I > 2\sigma(I)$ and had a goodness-of-fit = 1.043. Flack parameter = 0.06 (13).

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(5) Hispidanin B (**2**): colorless crystals (in acetone); $[\alpha]^{26}_{D}$ −169.7 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 285.6 (3.45), 208.6 (4.58) nm; IR (KBr) ν_{max} 3432, 3085, 2962, 2929, 2867, 2847, 1803, 1738, 1700, 1647, 1612, 1587, 1461, 1368, 1226, 1127, 1028, 985, 942, 885, 816 cm^{-1}; ^{1}H and ^{13}C NMR data are listed in Table S1 in the Supporting Information; ESIMS *m*/*z* 1336 $[2\ M + Na + H]^+$, 680 $[M + Na + H]^+$, 619 $[M + Na - HOAc]^+$, 551 $[M - HOAc - COOH]^+$; HREIMS *m*/*z* 656.4061 $[M]^+$ (calcd for $C_{42}H_{56}O_6$, 656.4077).

(6) Hispidanin C (**3**): colorless crystals (in acetone); $[\alpha]^{17}_{D}$ −203.40 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 284.1 (3.73), 204.0 (4.90) nm; IR (KBr) ν_{max} 3421, 3081, 2930, 2869, 1800, 1737, 1716, 1647, 1466, 1368, 1231, 1125, 1045, 945, 819, 756 cm^{-1}; ^{1}H and ^{13}C NMR data are listed in Table S1 in the Supporting Information; EIMS *m*/*z* 672 $[M]^+$ (4), 671 (8), 628 (23), 612 (67), 610 (68), 595 (44), 566 (61), 564 (71), 549 (46), 390 (100), 362 (96), 347 (56), 294 (54), 270 (53), 211 (28), 177 (43), 69 (75); HREIMS *m*/*z* 672.4009 $[M]^+$ (calcd for $C_{42}H_{56}O_7$, 672.4026).

(7) Hispidanin D (**4**): colorless crystals (in acetone); $[\alpha]^{17}_{D}$ −64.60 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (logε) 284.2 (3.30), 203.6 (4.48) nm; IR (KBr) ν_{max} 3417, 3081, 2960, 2930, 2868, 2846, 1802, 1737, 1718, 1460, 1376, 1214, 1131, 1025, 982, 942, 908, 848 cm^{-1}; ^{1}H and ^{13}C NMR data are listed in Table S1 in the Supporting Information; EIMS *m*/*z* 672 $[M]^+$ (5), 670 (10), 644 (14), 612 (16), 610 (21), 595 (36), 566 (19), 564 (14), 548 (27), 390 (8), 362 (12), 347 (12), 294 (61), 279 (21), 211 (28), 177 (14), 69 (100); HREIMS *m*/*z* 672.4024 $[M]^+$ (calcd for $C_{42}H_{56}O_7$, 672.4026).

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