Isolation and Biomimetic Synthesis of Anti-inflammatory Stilbenolignans from *Gnetum cleistostachyum*

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One new stilbenolignan, gnetucleistol F (1), and four known stilbenolignans, gnetofuran A (2), lehmbachol D (3), gnetifolin F (4) and gnetumontanin C (5) were isolated from the lianas of *Gnetum cleistostachyum* C. Y. CHENG (Gnetaceae). Their structures and relative configurations were determined by means of spectroscopic evidence. Compounds 1, 2, 3 and 4 were synthesized for the first time on the basis of their biogenetic pathway, and their possible biomimetical synthetic mechanisms were discussed. The pharmacological activities of all stilbenolignans have been tested. Among them, 1, 2, 3, 4 and 5 showed moderate inhibitory activities on TNF-α and 1 also showed potent inhibitory activity on malondialdehyde.

Key words *Gnetum cleistostachyum*; Gnetaceae; gnetucleistol F; TNF-α activity; stilbenolignan

To seek for more potent anti-inflammatory active components, we investigated the chemical constituents of *Gnetum cleistostachyum* C. Y. CHENG (Gnetaceae). One new stilbenolignan named gnetucleistol F (**1**), four known stilbenolignans, gnetofuran A (**2**), lehmbachol D (**3**), gnetifolin F (**4**) and gnetumontanin C (**5**), together with shegansu B (**6**), gnetupendin B, gnetol and daucosterol were isolated from this plant. In order to confirm the structures and obtain enough samples for pharmacological test, a biomimetically synthetic route for the stilbenolignans was designed and compounds **1**, **2**, **3** and **4** were synthesized successfully by oxidative coupling reaction. On the basis of the biogenetic pathway, their possible mechanisms were discussed. Pharmacological tests for **1**, **2**, **3**, **4** and **5** indicated that **1**—**5** showed moderate anti-inflammatory activities, and compound **1** also showed potent anti-oxidant activity.

Results and Discussion

Gnetucleistol F (**1**) was obtained as a pale white amorphous powder. Its HR-EI-MS *m/z* 466.1601 [M^+] ($C_{26}H_{26}O_8$ requires 466.1628) suggested a molecular formula of $C_{26}H_{26}O_8$. The characteristic absorption bands of hydroxyl (3350 cm^{-1}, broad) and aromatic moieties (1608, 1518, 1464 cm^{-1}) were observed in the IR spectrum. The UV spectrum [λ_{max}=310 (sh) (4.24), 327 (4.29) nm] revealed the presence of a strong conjugated system in the structure. The ^{1}H-NMR spectrum (Table 1) exhibited two signals of three methoxyls, two signals of a dihydrobenzofuran moiety; a signal of an oxymethylene, and two *trans* olefinic protons. The ^{1}H-NMR spectrum also showed one set of signals of AB_2 system for ring D, two *meta*-coupled signals for ring C; a signal of two symmetric protons for ring A. Its ^{13}C-NMR spectrum (Table 1) revealed the presence of three aliphatic carbons and three methoxyl carbons besides 20 aromatic and olefinic carbons. These evidence suggested that the skeleton of **1** was similar to that of **2**.[1)] In the HMBC spectrum (Fig. 2a), the significant long-range correlations between H-7b/C-4a, C-5a, C-1b, C-2(6)b, C-8b, C-9b; H-8b/C-4a, C-5a, C-1b, C-7b, C-9b; H-7a/C-1a, C-2a, C-6a, C-8a indicated the planar structure of **1** as illustrated in Fig. 1. The relative stereochemistry of **1** was established on the basis of NOESY spectrum (Fig. 2b). The key correlations between H-7b/H-2(6)b, H-9b; H-8b/H-2(6)b, H-9b suggested a *trans* orientation of H-7b and H-8b.[1)] The correlations between the methoxyl signal at δ 3.90 and H-2a, the signal at δ 3.82 and H-2(6)b indicated that the methoxyls should be located at C-3a, C-3b and C-5b respectively. Accordingly, the relative stereochemistry of **1** was elucidated as depicted in **1** (Fig. 1).

Lehmbachol D (**3**) was isolated as a pale white amorphous powder, its planar structure was reported previously.[2)] In order to clarify its relative stereochemistry, the NOESY experiment was carried out (Fig. 3). The NOE interactions be-

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** (δ in ppm and *J* in Hz)[a)]

Position	^{1}H	^{13}C
1a		140.2
2a	7.11 s	111.3
3a		144.6
4a		148.7
5a		129.9
6a	7.12 s	115.8
7a	7.07 d, 16.2	128.9
8a	6.95 d, 16.2	126.6
9a		131.5
10a	6.54 d, 2.4	105.0
11a		158.9
12a	6.27 t, 2.4	102.3
13a		158.9
14a	6.54 d, 2.4	105.1
1b		132.6
2b	6.75 d, s	103.8
3b		148.1
4b		136.0
5b		148.1
6b	6.75 d, s	103.8
7b	5.58 d, 6.9	88.3
8b	3.58 d, 6.9	54.2
9b	3.90 m	63.8
OCH_3	3.82 s	56.0
OCH_3	3.82 s	56.0
OCH_3	3.90 s	55.8

a) Measured in CD_3COCD_3 at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR.

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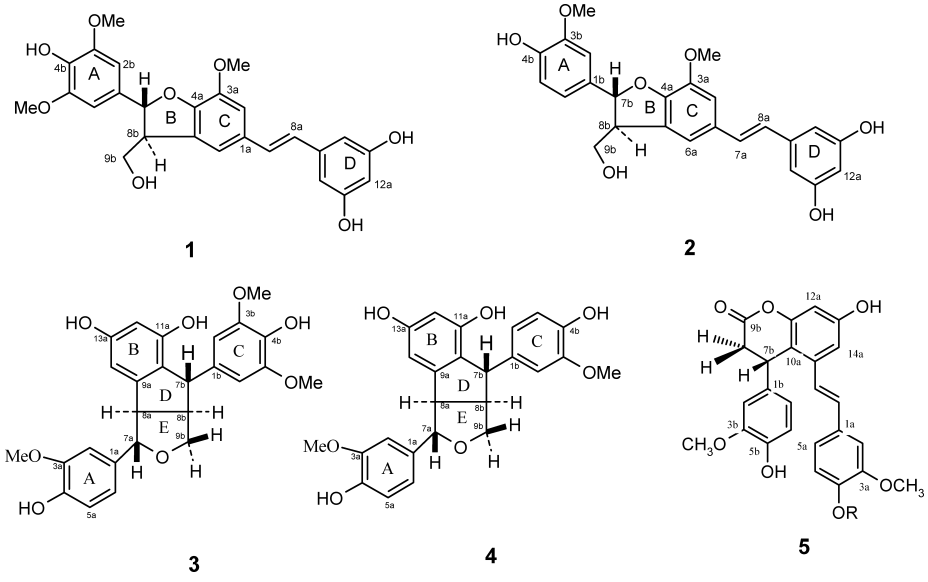

Fig. 1. Stilbenolignans from *Gnetum cleistostachyum*

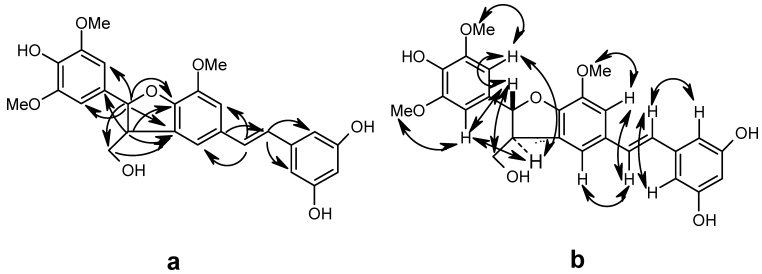

Fig. 2. Important Correlations of **1** in HMBC (a) and NOESY Spectra (b)

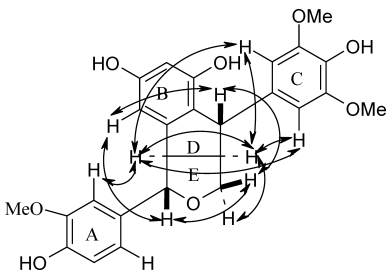

Fig. 3. Important Correlations of **3** in NOESY Spectrum

tween H-8a/H-8b indicated a *cis* orientation of H-8a and H-8b; between H-8b/H-2(6)b, and H-7b/H-2(6)b established a *trans* orientation of H-8b and H-7b; between H-7a/H-2a, H-6a, H-14a and H-8a/H-2a, H-2(6)b revealed a *trans* orientation of H-7a and H-8a. Thus, the relative configuration of **3** was established as depicted in Fig. 1.

The other seven known compounds, including gnetofuran A (**2**),[1] gnetifolin F (**4**),[3] gnetumontanin C (**5**),[4] gnetupendin B,[5] shegansu B (**6**),[6] gnetol and daucosterol,[7,8] were readily identified by comparison of physical and spectroscopic data with values found in the literature.

From the biogenetic viewpoint, it was presumed that **1**, **3** (or **2**, **4**) should be formed by oxidative coupling of sinapyl alcohol (or coniferol alcohol) and isorhapontigenin. In order to support the hypothesis and obtain these stilbenolignans in sufficient quantities, a route of biomimetic synthesis with ferulic acid (or sinapinic acid) and isorhapontigenin (**10**) as starting materials was designed and four stilbenolignans **1**, **3** and **2**, **4** were obtained, besides the isorhapontigenin dimer **6**. Esterification of ferulic acid (**7a**) afforded methyl ferulate (**8a**) in 96% yield, which was converted to phenylpropenol (**9a**) in 87% yield by reduction with lithium aluminium hydride. Oxidative coupling reaction of **9a** and **10** yielded compounds **2**, **4** and **6**. Similarly, sinapinic acid (**7b**) was converted to phenylpropenol (**9b**) in 29% yield, which afforded compounds **1**, **3** and **6** (Fig. 4) by oxidative coupling with **10**. The properties of the synthetic products **1**—**4** were in all aspects identical to those of the natural products except for the optical rotation.

The possible formation mechanisms of **1**, **2**, **3** and **4** may be rationalized as follows: in the course of oxidative coupling reaction, **10** was presumably converted into the free radicals $\cdot\mathbf{R_4}$, $\cdot\mathbf{R_5}$ and $\cdot\mathbf{R_8}$, while **7a** (or **7b**) provided the radicals $\cdot\mathbf{R'_4}$ (or $\cdot\mathbf{R''_4}$) and $\cdot\mathbf{R'_8}$ (or $\cdot\mathbf{R''_8}$) respectively (as shown in Fig. 5). Coupling of $\cdot\mathbf{R'_8}$ (or $\cdot\mathbf{R''_8}$) and $\cdot\mathbf{R_5}$ produced an unstable bisquinone intermediate **11a** (or **11b**), which generated **2** (or **1**) *via* spontaneous intramolecular cyclization as shown in Fig. 6. A Cβ–Cβ coupling of $\cdot\mathbf{R_8}$ and $\cdot\mathbf{R'_8}$ (or $\cdot\mathbf{R''_8}$) afforded an unstable bisquinone intermediate **12a** (or **12b**), which generated **4** (or **3**) *via* spontaneous intramolecular cyclization. The formation mechanisms were closely in agreement with the assumption on the biogenesis pathway of stilbenolignans.

Pharmacological activities of compounds **1**—**5** have been

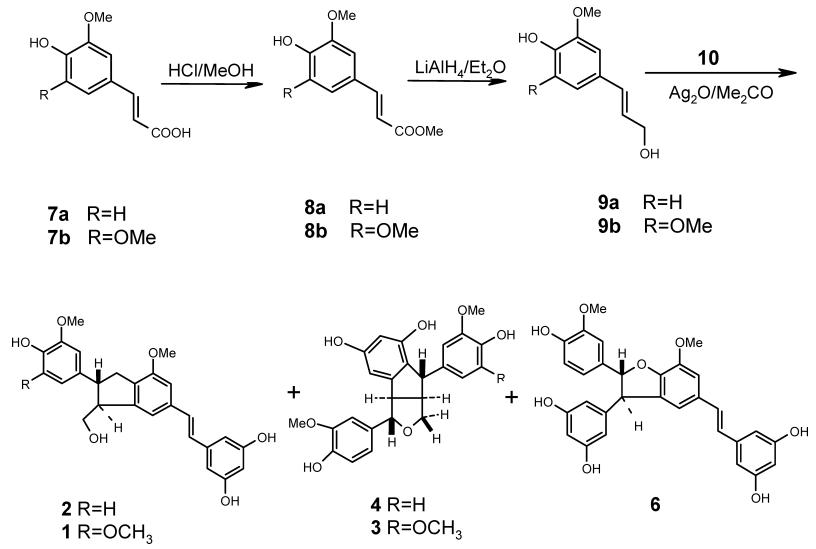

Fig. 4. Biomimetic Synthesis Routes of Compounds **1**, **2**, **3**, **4** and **6**

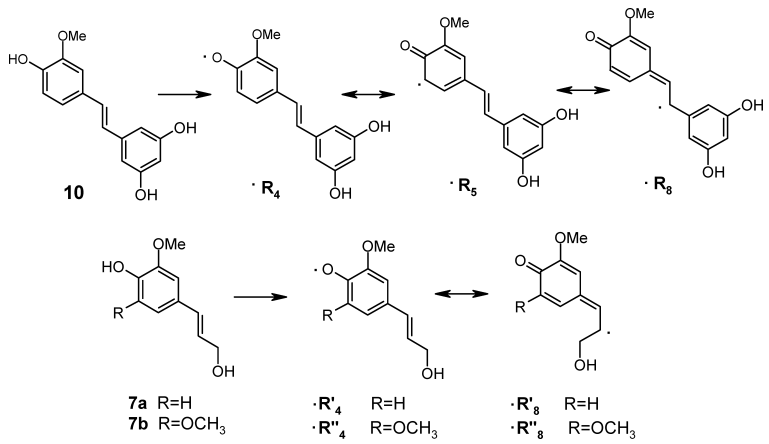

Fig. 5. Possible Free Radicals in the Oxidative Coupling Reaction

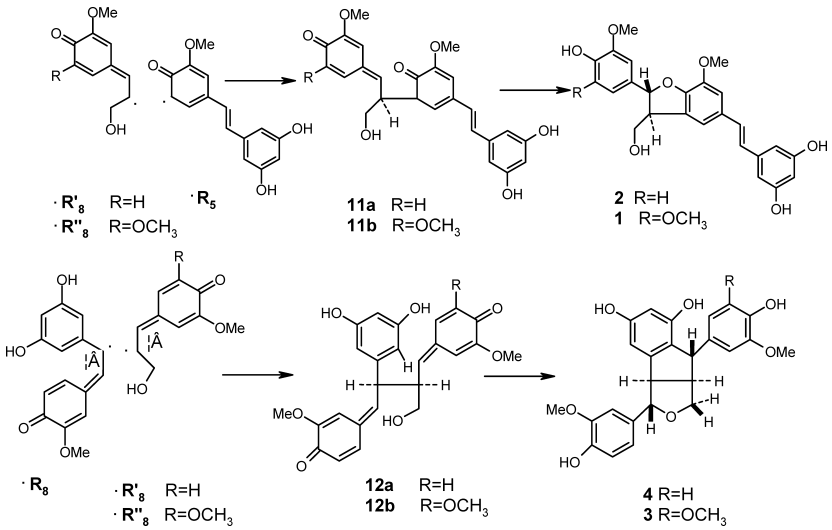

Fig. 6. Postulated Intermediates in the Formation of Compounds **1**, **2**, **3** and **4**

tested (Table 2). Among them, **1**—**5** showed inhibition on TNF-α production by murine peritoneal macrophages with IC_{50} values of 1.03×10^{-5} mol l^{-1}, 1.09×10^{-5} mol l^{-1}, 1.10×10^{-5} mol l^{-1}, 0.92×10^{-5} mol l^{-1} and 2.46×10^{-5} mol l^{-1}. Compound **1** obtained by biomimetic synthesis also exhibited inhibition on malondialdehyde (MDA) with IC_{50}

Table 2. Pharmacological Activities of Compounds **1**—**5**

Compounds	TNF-α inhibitory activity IC_{50} (10^{-5} M)	MDA inhibitory activity IC_{50} (10^{-6} M)
1	1.03	6.36
2	1.09	
3	1.10	
4	0.92	
5	2.46	
Dexamethasone[a)]	0.10	
Vitamin E[a)]		1.0

a) Positive control.

value of 6.36×10^{-6} mol l^{-1}. The results suggested that **1**, **2**, **3**, **4** and **5** has moderate anti-inflammatory activities, and **1** has potent anti-oxidant activity.

Experimental

General Procedures Optical rotations were determined on a Perkin-Elmer digital polarimeter. UV spectra were taken on a Shimadzu UV-300 spectrophotometer. IR spectra were run on a Perkin-Elmer 683 infrared spectrometer recorded in KBr pellets. NMR spectra were recorded on a Bruker AM-500 NMR spectrometer and a Varian Mercury-300 NMR spectrometer using TMS as internal standard. EI-MS and HR-EI-MS were obtained using an Autospec-Ulma-Tof mass spectrometer.

Plant Material The lianas of *Gnetum cleistostachyum* C. Y. Cheng (Gnetaceae) were collected in Hekou county of Yunnan province, People's Republic of China, in October 2001. The plant was identified by Dr. Y. M. Shui of Kunming Institute of Botany, Chinese Academy of Sciences, China, where a voucher specimen (No. 39795) was deposited.

Extraction and Isolation The dried and pulverized lianas of *Gnetum cleistostachyum* (35 kg) were extracted with 65% EtOH (3×30 l) under reflux. After removing the solvent under vaccum, the residue (3.0 kg) was extracted with $CHCl_3$ (3×4 l), EtOAc (3×4 l), Me_2CO (3×4 l) and MeOH (3×4 l) successively. The EtOAc soluble fraction (185 g) was subjected to silica gel column chromatography (10×150 cm, 100—200 mesh, 2.5 kg) eluted with a gradient system of $CHCl_3$–MeOH to provide six fractions A—F. Fraction E (62.3 g) was further subjected to silica gel column chromatography (5×150 cm 140—180 mesh, 1 kg) eluted with a gradient system of cyclohexane–acetone to give fractions E1—E7. Fraction E5 (16 g) was then further divided into Er1—Er5 by silica gel chromatography [5×80 cm 140—180 mesh, cyclohexane–acetone (4 : 1—1 : 2)]. Compound **3** (48 mg) and **4** (30 mg) were obtained from fraction Er3 (800 mg) by silica gel column chromatography (3×30 cm 140—180 mesh, 50 g) eluted with $CHCl_3$–MeOH (25 : 1). Gnetol (80 mg) was obtained from fraction Er4 (0.30 g) by MPLC with MeOH–H_2O (3 : 7) as eluent. Er5 (1.6 g) was subjected to a silica gel column chromatography (3×40 cm, 200—300 mesh, 80 g) eluted with cyclohexane–acetone (3 : 1) to provide compounds **1** (34 mg) and **2** (26 mg). E3 (5.10 g) was subjected to column chromatography on Rp-18 (35—75 mm, 350 g) eluted with MeOH–H_2O system (1 : 1, 4 l) to afford compound **5** (60 mg). Fraction D (8.0 g) was applied to a silica gel column chromatography (5×80 cm, 200—300 mesh, 450 g) using cyclohexane–acetone (4 : 1—1 : 1) as eluent to provide fraction D1—D4. Fraction D2 (900 mg) was subjected to silica gel column chromatography (3×30 cm, 200—300 mesh, 50 g) eluted with petrol ether–acetone (3 : 1) to provide gnetupendin B (16 mg) and **6** (200 mg). The MeOH insoluble fraction of D3 (1.5 g) provided daucosterol (500 mg).

Gnetucleistol F (**1**): A pale white amorphous powder. UV λ_{max} (MeOH) nm (log ε): 235 (sh) (4.29), 310 (sh) (4.24), 327 (4.29). IR (KBr) cm^{-1}: 3350, 2954, 2924, 2852, 1608, 1518, 1464, 1377, 1213, 1115, 1009, 957, 837. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) see Table 1. $[\alpha]_D^{20}$ +4.67° (c=0.1, MeOH). EI-MS m/z: 466 (M^+). HR-EI-MS m/z: 466.1601 (Calcd for $C_{26}H_{26}O_8$, 466.1628).

Lehmbachol D (**3**): A pale white amorphous powder. EI-MS m/z: 466 (46%) (M^+); HR-EI-MS m/z: 466.1610 (Calcd for $C_{26}H_{26}O_8$, 466.1628). Its spectral data were in agreement with those reported in the literature.[2)]

Preparation of Coniferol Alcohol (9a) and Sinapyl Alcohol (9b) **7a** (8 g, 41.24 mmol) was dissolved in methanol saturated with hydrochloric acid (50 ml) and the solution was kept at room temperature for 48 h. Removal of the solvent yielded **8a** (8.2 g, 96%), which was dissolved in anhydrous ether (100 ml) and added to a suspension of lithium aluminium hydride (2.96 g, 77.89 mmol). After stirring for 12 h at room temperature, the mixture was poured into water and extracted with ether. Removing of the solvent yielded **9a** (6.0 g, 87%). **8b** (1.0 g, 94%) and **9b** (0.24 g, 29%) were obtained by the same method using **7b** (1.0 g) as starting material.

Methyl Ferulate **8a**: ^{1}H-NMR (300 MHz in CD_3COCD_3) δ: 8.13 (s, OH), 7.60 (1H, d, J=15.9 Hz), 7.32 (1H, br s), 7.14 (1H, br d, J=8.1 Hz), 6.87 (1H, d, J=8.1 Hz), 6.41 (1H, d, J=15.9 Hz), 3.91 (3H, s, OCH_3), 3.71 (3H, s, $COOCH_3$). EI-MS m/z: 208 (M^+).

Methyl Sinapate **8b**: ^{1}H-NMR (300 MHz in CD_3COCD_3) δ: 7.54 (1H, d, J=15.9 Hz), 7.01 (2H, s), 6.44 (1H, d, J=15.9 Hz), 3.88 (6H, s, OCH_3), 3.71 (3H, s, $COOCH_3$). EI-MS m/z: 238 (M^+).

Coniferol Alcohol (**9a**): ^{1}H-NMR (300 MHz in CD_3COCD_3) δ: 7.60 (s, OH), 7.04 (1H, d, J=1.8 Hz), 6.86 (1H, dd, J=8.4, 1.8 Hz), 6.77 (1H, d, J=8.4 Hz), 6.52 (1H, d, J=15.9 Hz), 6.25 (1H, dt, J=15.9, 5.4 Hz), 4.20 (2H, m), 3.85 (3H, s, OCH_3). EI-MS m/z: 180 (M^+).

Sinapyl Alcohol (**9b**): ^{1}H-NMR (300 MHz in CD_3COCD_3) δ: 7.28 (s, OH), 6.72 (2H, br s, J=15.9 Hz), 6.45 (1H, d, J=15.9 Hz), 6.27 (1H, dt, J=15.9, 5.4 Hz), 4.19 (2H, m), 3.82 (6H, s, OCH_3). EI-MS m/z: 210 (M^+).

Biomimetic Synthesis of Compounds 1, 2, 3, 4 and 6 Silver oxide (2 g, 8.62 mmol) was added to a solution of **9a** (1 g, 5.56 mmol) and **10** (2.3 g, 8.91 mmol) in acetone (50 ml), the mixture was stirred at room temperature for 8 h. After filtration, the filtrate was evaporated to dryness and the residue was chromatographied on silica gel column eluted with hexane–acetone (5 : 1—1 : 1) to afford **2** (250 mg), **4** (19.3 mg) and **6** (44.2 mg), together with the unreacted starting material **10** (100 mg). A solution of **9b** (240 mg, 1.14 mmol) and **10** (300 mg) in acetone (50 ml) was treated in the same way as described above to afford **1** (4.5 mg), **3** (13.5 mg), **6** (11 mg) and the unreacted starting material **10** (20 mg). The spectral data of the synthetic compounds **1**—**4** and **6** were identical to those of natural products in all respects except for the optical rotation.

Gnetofuran A (**2**): A pale white amorphous powder. ^{1}H-NMR (300 MHz in CD_3COCD_3) δ: 7.10 (1H, d, J=2.0 Hz), 7.12 (1H, br s), 7.02 (1H, d, J=16.5 Hz), 6.94 (1H, d, J=16.5 Hz), 6.53 (2H, d, J=2.0 Hz), 6.26 (1H, t, J=2.0 Hz), 7.04 (1H, d, J=2.0 Hz), 6.82 (1H, d, J=8.0 Hz), 6.89 (1H, dd, J=8.0, 2.0 Hz), 5.59 (1H, d, J=7.0 Hz), 4.16 (1H, t, J=5.5 Hz), 3.86 (1H, t, J=5.5 Hz), 3.56 (1H, q, J=7.0 Hz), 3.88 (3H, s), 3.82 (3H, s). ^{13}C-NMR (300 MHz in CD_3COCD_3) δ: 140.7 (C-1a), 111.9 (C-2a), 145.2 (C-3a), 149.3 (C-4a), 130.5 (C-5a), 116.4 (C-6a), 129.5 (C-7a), 127.1 (C-8a), 131.9 (C-9a), 105.5 (C-10a, 14a), 159.5 (C-11a, 13a), 102.6 (C-12a), 134.2 (C-1b), 110.4 (C-2b), 147.2 (C-3b), 148.3 (C-4b), 115.5 (C-5b), 119.5 (C-6b), 88.6 (C-7b), 54.6 (C-8b), 64.5 (C-9b), 56.3 (OCH_3), 56.2 (OCH_3). IR (KBr) cm^{-1}: 3300, 2954, 2924, 2852, 1603, 1464, 1377, 1271, 1122, 960. UV λ_{max} (MeOH) nm (log ε): 220 (sh) (4.61), 287 (sh) (4.12), 328 (4.26). EI-MS m/z: 436 (M^+); HR-EI-MS m/z: 436.1501 (Calcd for $C_{25}H_{24}O_7$: 436.1522). $[\alpha]_D^{25}$ 0° (c=0.1, MeOH).

Gnetifolin F (**4**): A pale white amorphous powder. mp 144—147 °C; ^{1}H-NMR (300 MHz, in CD_3COCD_3) δ: 7.02 (1H, d, J=1.8 Hz, H-2a), 6.82 (1H, d, J=8.1 Hz, H-5a), 6.89 (1H, dd, J=8.1, 1.8 Hz, H-6a), 4.70 (1H, d, J=4.5 Hz, H-7a), 3.75 (1H, dd, J=8.4, 4.5 Hz, H-8a), 6.26 (1H, d, J=2.1 Hz, H-12a), 6.34 (1H, d, J=2.1 Hz, H-14a), 6.72 (1H, d, J=2.1 Hz, H-2b), 6.66 (1H, d, J=8.1 Hz, H-5b), 6.49 (1H, dd, J=8.1, 2.1 Hz, H-6b), 4.17 (1H, br s, H-7b), 3.04 (1H, q, J=8.4 Hz, H-8b), 3.49 (1H, t, J=8.4 Hz, H-9bα), 4.45 (1H, t, J=8.4 Hz, H-9bβ), 3.73 (3H, s), 3.85 (3H, s). ^{13}C-NMR (300 MHz in CD_3COCD_3) δ: 135.5 (C-1a), 110.4 (C-2a), 148.3 (C-3a), 146.7 (C-4a), 115.3 (C-5a), 119.6 (C-6a), 88.3 (C-7a), 59.6 (C-8a), 147.9 (C-9a), 122.7 (C-10a), 159.7 (C-11a), 102.5 (C-12a), 155.8 (C-13a), 103.1 (C-14a), 138.0 (C-1b), 111.8 (C-2b), 148.3 (C-3b), 145.5 (C-4b), 115.4 (C-5b), 120.2 (C-6b), 50.9 (C-7b), 55.8 (C-8b), 74.4 (C-9b), 56.1 (OCH_3), 56.0 (OCH_3). EI-MS (m/z): 466 (M^+). $[\alpha]_D^{20}$ 0° (c=0.021, MeOH).

Shegansu B (**6**): A yellowish amorphous powder. IR (KBr) cm^{-1}: 3302, 1603, 1514, 1462, 1340, 1279, 1155, 1005, 837. UV λ_{max} (EtOH) nm (log ε): 285 (sh) (4.21), 328 (4.48); FAB-MS m/z: 515 ($M+1$)$^+$. $[\alpha]_D^{20}$ 0° (c=0.048, MeOH). Its ^{1}H-NMR spectral data (300 MHz, in CD_3COCD_3) were in agreement with those repored in the literature.[6)]

Anti-inflammation and anti-oxidant activity tests were carried out in accord with the methods discussed in the literature.[9,10)]

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