The Chemical Constituents from the Callus Culture of *Trewia nudiflora*

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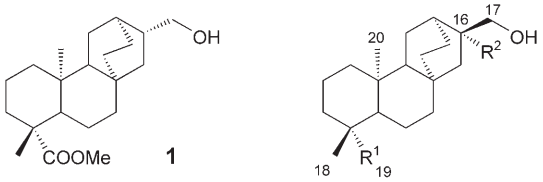
Five *ent*-atisane diterpenoids, including three new ones, *i.e.*, (16α)-17-hydroxy-*ent*-atisan-19-oic acid methyl ester (**1**), (16α)-17-dihydroxy-*ent*-atisan-19-oic acid methyl ester (**2**), (16α)-*ent*-atisan-16,17,19-triol (**3**), and two known compounds, *i.e.*, 17-hydroxy-*ent*-atisan-19-oic acid (**4**), (16α)-16,17-dihydroxy-*ent*-atisan-19-al (**5**), together with one known diterpene glycoside, *i.e.*, sumogaside, two known triterpenes, *i.e.*, germanicone and (3β)-β-amyrin, three known phenolic compounds, *i.e.*, (+)-gallocatechin, (+)-catechin, and gallic acid, and two known sterols, *i.e.*, β-sitosterol and daucosterol, were isolated from the callus cultures of *Trewia nudiflora*. Their structures were elucidated by spectroscopic analysis including 1D- and 2D-NMR experiments. No maytansinoids were isolated or detected by LC-ESI-MS in the extracts of the calli, which suggests that the callus cultures can not produce maytansinoids under these conditions.

Introduction. – *Trewia nudiflora* L. (Euphorbiaceae) is a tropical plant mainly distributed in India, Malaysia, and the south of China [1]. The seed of *T. nudiflora* contains highly unusual glyceride oil [2], several novel pyridinone alkaloids [3], and an inhibitor of protein synthesis [4]. The seed is also a rich source of maytansinoids [5–7]. Our previous studies have revealed that maytansinoids were prominent antifungal constituents in the seed and pericarp of *T. nudiflora* [8]. However, the contents of maytansinoids are very low in this plant. To access new sources of maytansinoids and investigate the actual biosynthetic origin of those compounds, callus cultures of *T. nudiflora* were established and successively subcultured for 14 generations. Studies on the chemical constituents of the callus cultures yielded 13 compounds, including three new atisane-type diterpenoids. But no maytansinoids were isolated or detected by LC-ESI-MS in the extracts of the calli, which means that the callus cultures can not produce maytansinoids under the experimental conditions used by us. Herein, we report the isolation and structure elucidation of compounds from the callus cultures of *T. nudiflora*.

Results and Discussion. – Callus tissues of *T. nudiflora* were induced from axenic seedlings on *Murashige* and *Skoog* (*MS*) [9] agar media, supplemented with 6-benayladenine and naphthalene-1-acetic acid, and maintained for over one year by subculturing at one-month intervals at 26° in the dark on *MS* medium containing the above plant hormones and kinetin. The calli were collected each month and stored in the refrigerator.

Lyophilized calli were extracted with 95% EtOH and the residue of the crude extract was further extracted with $CHCl_3$ and MeOH. The MeOH-soluble part was successively chromatographed over macroporous resin, silica gel, and *Sephadex LH-20* to afford compounds **1**, **2**, sumogaside, (+)-gallocatechin, (+)-catechin, gallic acid

(= 3,4,5-trihydroxybenzoic acid), and daucosterol. The $CHCl_3$-soluble part was subjected to repeated chromatrography to yield compounds **3**–**5**, germanicone (= olean-18-en-3-one), (3β)-β-amyrin, and β-sitosterol. But no maytansinoids were isolated or detected by LC-ESI-MS in the extracts of the calli.



2 R^1 = COOMe, R^2 = OH
3 R^1 = $HOCH_2$, R^2 = OH
4 R^1 = COOH, R^2 = H
5 R^1 = CHO, R^2 = OH

Compound **1** was obtained as colorless needles. Its EI-MS showed a weak molecular-ion peak at *m/z* 334, and a fragment peak at *m/z* 316 ($[M - H_2O]^+$). HR-ESI-MS established the molecular formula $C_{21}H_{34}O_3$ (*m/z* 317.2481, $[M - H_2O + H]^+$), indicating five degrees of unsaturation in the molecule.

The IR spectra of **1** revealed the presence of OH groups (3449, 2945 cm^{-1}) and a carboxylic ester (1721 cm^{-1}). The carboxylic ester signal was also present in the ^{13}C-NMR spectrum (δ(C) 180.2 (*s*)). The remaining four degrees of unsaturation indicated that **1** was a tetracyclic diterpene. Further spectral data and their comparison with those of known *ent*-artisane diterpenoids [8] established that **1** is a diastereoisomer of 17-hydroxy-*ent*-atisan-19-oic acid methyl ester [8].

The ^{13}C-NMR spectra and DEPT (*Table 1*) of **1** showed 21 resonances for four quaternary C-atoms and four CH, ten CH_2, and three Me groups. In the ^{1}H-NMR spectra, two tertiary-Me signals appeared at δ(H) 1.15 and 0.86 and a MeO signal at δ(H) 3.65. The HMQC, HMBC, and ^{1}H,^{1}H-COSY experiments defined the intramolecular connectivities of all C-atom and related protons. From the HMBC experiment (*Table 2*), the ^{1}H,^{13}C-NMR long-range correlations of the Me group at δ(H) 1.15 (δ(C) 29.5) and the C-atoms at δ(C) 39.6 (*t*), 45.5 (*s*), 58.7 (*d*) and 20.7 (*t*, weak), and between the Me group at δ(H) 0.86 (δ(C) 16.5) and the C-atoms at δ(C) 58.7 (*d*), 41.0 (*s*) and 20.7 (*t*, weak), and the ^{13}C,^{1}H-NMR long-range correlations between the quaternary C-atom at δ(C) 180.2 and the protons at δ(H) 3.65 (δ(C) 52.1), 1.15 (δ(C) 29.5), 1.08 (δ(C) 58.7), and 1.05 (δ(C) 39.6) afforded the fragment **1a** (*Fig. 2*). The ^{13}C-NMR chemical shifts were similar to those of the known *ent*-atisane diterpenoids in the A ring [8]. The analysis of the ^{1}H,^{1}H-COSY plot revealed that the protons of CH_2OH at δ 3.82 (CH_2(17)) were correlated to the protons at δ 2.09 (H–C(16)), which, together with the HMBC data, led to the determination of fragment **1b** (*Figure*). The HMBC experiments also showed long-range correlations between the protons at δ 1.02 (H–C(9)) in the fragment **1b** and the C-atom at δ 58.7 (C(5)) in fragment **1a**, and between protons at δ 1.86 (H–C(6)) in the fragment **1b** and the C-atom at δ 41.0 (C(10)) in fragment **1a**, which determined the connection of fragments **1b** and **1a** and gave the full molecular connectivity. The structure of **1** was very similar to that of 17-hydroxy-*ent*-atisan-19-oic acid methyl ester [8]. Comparison of the ^{1}H- and ^{13}C-NMR spectra of the two compounds revealed that they were very similar, except for the δ(C) of C(16) and C(17), *i.e.*, δ 39.9 (C(16)) and δ 72.6 (C(17)) in **1** *vs.* δ 43.3 (C(16)) and δ 67.6 (C(17)) in the known ester. Therefore, CH_2(17)OH of **1** was in the α-position, which was also evidenced by an NOE experiment.

Compound **2** was isolated as colorless crystals and had the molecular formula $C_{21}H_{34}O_4$ based on the HR-ESI-MS (*m/z* 373.2351, $[M + Na]^+$). The spectroscopic data

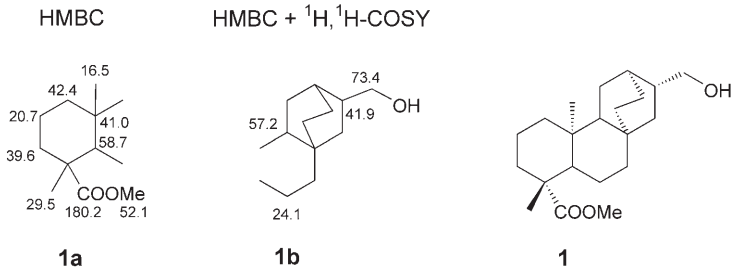


Figure. *^{1}H- and ^{13}C-NMR Chemical shifts of substructures of compound* **1** *as determined by HMBC and COSY experiments*

Table 1. *^{1}H- and ^{13}C-NMR Data for Compounds* **1**–**3**. δ in ppm, J in Hz.

Position	**1** (CD_3OD)		**1** ($CDCl_3$)	**2** ($CDCl_3$)		**3** (C_5D_5N)	
	δ(H)	δ (C)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.85–1.89 (*m*)	42.4 (*t*)	40.8 (*t*)	1.76–1.80 (*m*) (α) 0.72–0.76 (*m*) (β)	40.6 (*t*)	1.65–1.70 (*m*) (α) 0.65–0.71 (*m*) (β)	40.6 (*t*)
2	1.40–1.42 (*m*) (α) 1.83–1.85 (*m*) (β)	20.7 (*t*)	19.1 (*t*)	1.39–1.42 (*m*) (α) 1.80–1.83 (*m*) (β)	19.0 (*t*)	1.26–1.33 (*m*)	30.0 (*t*)
3	0.99–1.05 (*m*)	39.6 (*t*)	38.1 (*t*)	2.12–2.15 (*m*) (α) 0.94–0.97 (*m*) (β)	38.0 (*t*)	2.14–2.17 (*m*) (α) 0.93–0.95 (*m*) (β)	36.4 (*t*)
4	–	45.5 (*s*)	43.7 (*s*)	–	43.7 (*s*)	–	37.7 (*s*)
5	1.05–1.08 (*m*) (β)	58.7 (*d*)	57.0 (*d*)	1.00–1.02 (*m*) (β)	56.8 (*d*)	0.85–0.88 (*m*) (β)	57.1 (*d*)
6	1.75–1.76 (*m*) (α) 1.83–1.86 (*m*) (β)	24.1 (*t*)	22.4 (*t*)	1.69–1.72 (*m*) (α) 1.80–1.86 (*m*) (β)	22.1 (*t*)	1.55–1.61 (*m*) (α) 1.27–1.30 (*m*) (β)	21.0 (*t*)
7	1.44–1.46 (*m*) 0.83–0.85 (*m*)	43.1 (*t*)	41.5 (*t*)	1.62–1.66 (*m*) 1.39–1.44 (*m*)	41.9 (*t*)	1.55–1.58 (*m*) 1.39–1.40 (*m*)	42.9 (*t*)
8	–	46.4 (*s*)	44.8 (*s*)	–	44.6 (*s*)	–	44.9 (*s*)
9	1.00–1.02 (*m*)	57.2 (*d*)	55.9 (*d*)	0.92–0.96 (*m*)	55.7 (*d*)	0.93–0.95 (*m*)	57.3 (*d*)
10	–	41.0 (*s*)	39.3 (*s*)	–	39.4 (*s*)	–	39.5 (*s*)
11	1.61–1.63 (*m*)	20.2 (*t*)	18.8 (*t*)	1.58–1.61 (*m*) 1.48–1.54 (*m*)	18.5 (*t*)	1.81–1.83 (*m*) 1.47–1.53 (*m*)	18.7 (*t*)
12	2.12–2.14 (*m*)	40.1 (*d*)	38.1 (*d*)	2.00 (br. *s*)	45.2 (*d*)	2.39–2.41 (*m*)	46.1 (*d*)
13	1.83–1.84 (*m*) 1.07–1.08 (*m*)	38.4 (*t*)	37.0 (*t*)	1.89–1.92 (*m*) 1.59–1.60 (*m*)	37.2 (*t*)	1.92–1.95 (*m*) 1.83–1.86 (*m*)	37.9 (*t*)
14	1.52–1.54 (*m*) 1.42–1.44 (*m*)	32.7 (*t*)	31.1 (*t*)	1.51–1.58 (*m*)	26.1 (*t*)	1.80–1.83 (*m*) 1.53–1.55 (*m*)	26.8 (*t*)
15	1.56–1.58 (*m*) 0.99 (*dd*, J = 5.6, 13.6)	46.4 (*t*)	44.8 (*t*)	1.56–1.58 (*m*) 1.39–1.42 (*m*)	53.0 (*t*)	1.80–1.83 (*m*) 1.68–1.70 (*m*)	54.0 (*t*)
16	2.09–2.11 (*m*)	41.9 (*d*)	39.9 (*d*)	–	81.9 (*s*)	–	81.7 (*s*)
17	3.76 (*d*, J = 7.6)	73.4 (*t*)	72.6 (*t*)	3.76 (*d*, J = 10.9) 3.65 (*d*, J = 11.0)	66.3 (*t*)	4.03 (*d*, J = 10.9) 4.11 (*d*, J = 10.8)	66.5 (*t*)
18	1.15 (*s*)	29.5 (*q*)	28.8 (*q*)	1.14 (*s*)	28.7 (*q*)	1.15 (*s*)	28.1 (*q*)
19		180.2 (*s*)	178.1 (*s*)		178.0 (*s*)	4.70 (*d*, J = 9.5) 4.24 (*d*, J = 9.4)	70.1 (*t*)
20	0.86 (*s*, 3 H)	16.5 (*q*)	15.3 (*q*)	0.80 (*s*, 3 H)	15.3 (*q*)	0.99 (*s*)	18.6 (*q*)
MeO	3.65 (*s*)	52.1 (*q*)	51.1 (*q*)	3.62 (*s*)	51.1 (*q*)	–	–

Table 2. *HMBC Data of Compounds* **1**–**3**. *w* = weak.

	1	**2**	**3**
H_α–C(1)	C(2), C(5), C(9), C(20)	C(2), C(20)	C(3)
H_β–C(1)	–	C(2), C(3)	C(10), C(20)
H_α–C(2)	C(5) (*w*)	C(3), C(18)	C(10)
H_β–C(2)	–	C(1)	–
H_α–C(3)	C(18)	C(4), C(5), C(19)	C(5)
H_β–C(3)		C(18)	C(18)
C(4)	CH_2(18)	CH_2(3), CH_3(18)	CH_3(18)
H_β–C(5)	C(6), C(18), C(20)	C(1), C(3), C(6), C(9), C(18), C(20)	C(1), C(3), C(7), C(18), C(20)
H_α–C(6)	C(5), C(7), C(10)	C(5), C(15)	C(10)
H_β–C(6)	C(8)	C(5)	C(5), C(7)
H–C(7)	C(6), C(9)	C(6)	–
H′–C(7)	–		C(5), C(6), C(14), C(15)
C(8)	*H*–C(9), CH_2(11), CH_2(14)	CH_2(13), CH_2(15)	*H*–C(9), CH_2(13), CH_2(15)
H–C(9)	C(5), C(11), C(15)	C(5), C(11), C(14)	C(5), C(8), C(11), C(15), C(20)
C(10)	CH_2(6), *H*–C(9), CH_2(11)	CH_2(2), CH_2(11)	CH_2(1), CH_2(2), *H*–C(5), CH_2(6), CH_2(7) (*w*), CH_2(11)
H–C(11)	C(9), C(10), C(12),	C(10), C(12), C(14) (*w*)	C(8), C(10), C(12)
H′–C(11)	C(14) (*w*)	C(10), C(14) (*w*)	C(8), C(9)
H–C(12)	C(11), C(14), C(15)	C(11)	C(11), C(13)
H–C(13)	C(8), C(16)	C(14), C(15), C(16)	C(8), C(12), C(14)
H′–C(13)	C(8), C(14), C(16)	C(14)	–
H–C(14)	C(9), C(12)	C(11), C(13)	C(7), C(10)
H′–C(14)	C(13), C(15)		
H–C(15)	C(9), C(13)	C(13)	C(7), C(8), C(9)
H′–C(15)	C(13), C(16), C(17)	C(9), C(12)	C(8)
H–C(16)	C(13), C(17)	C(13), C(15)	C(13), C(15), C(17)
H–C(17)	C(12), C(15), C(16)	C(15)	C(15)
H′–C(17)	–	–	–
Me(18)	C(3), C(4), C(5), C(2) (*w*)	C(2), C(3), C(4), C(5), C(19)	C(4), C(5), C(19)
C(19)	CH_2(3), *H*–C(5), CH_3(18), CH_3O	CH_2(3), CH_3(18), CH_3O	*H*–C(5), CH_2(18)
Me(20)	C(5), C(10), C(2) (*w*)	C(1), C(5)	C(1), C(5), C(10), C(19) (*w*)
MeO	C(19)	C(4), C(19)	–

and their comparison with those of compounds **1** and **5** revealed the structure of **2** to be (16α)-16,17-dihydroxy-*ent*-atisan-19-oic acid methyl ester.

The IR spectra of **2** revealed the presence of OH (3432, 2939 cm^{-1}) and a carboxylic ester (1726 cm^{-1}). The ^{13}C-NMR spectra and DEPT (*Table 1*) showed 21 resonances for five quaternary C-atoms, three CH, ten CH_2, and three Me groups. The ^{1}H-NMR spectra revealed the presence of two tertiary Me groups (δ(H) 0.86 and 1.14) and a CH_2OH moiety (δ(H) 3.65, 3.76). These data, together with the similarity of the spectral parameters of **2** with those of compounds **1** and **5**, were consistent with the presence of an atisane skeleton. The principal difference between them was that the aldehyde function at C(19) of compound **5** was replaced by a carboxylic ester in **2**. The ^{13}C-NMR spectra showed that the O-atoms arose from a primary alcohol function at C(17) (δ(C) 66.5, δ(H) 3.65, 3.76) and a tertiary alcohol function at C(16) (δ(C) 81.9) in **2**, which were very similar to those of compound **5** (C(17) (δ (C) 66.4, δ(H) 3.66, 3.78) and C(16) (δ(C) 81.8), therefore suggesting that they have the same configurations.

The isolation of a more polar fraction of the extract yielded compound **3,** which was assigned the molecular formula $C_{20}H_{34}O_3$ by EI-MS (*m/z* 322). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** and **5** indicated that the two compounds are very similar. The principal difference in their NMR data was that **3** exhibited signals of an additional CH_2OH moiety (δ(C)70.1, δ(H) 4.70, 4.24) instead of those of the aldehyde signals (δ(H) 3.62, δ(C) 51.1) of **5**. The *ent*-configuration of compound **1 – 3** was assumed from the co-occurrence and close similarity of their structures. Thus, compound **3** was determined to be (16α)-*ent*-atisan-16,17,19-triol.

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Experimental Part

1. *General*. TLC: precoated TLC plates (*Si gel G*) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. Column chromatography (CC): silica gel (200 – 300 and 80 – 100 mesh) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China; silica gel HF_{254} from *Merck*; reversed-phase C_{18} silica gel from Merck; *Sephadex-LH-20* from *Amersham Biosciences*. M.p.: *YuHua X-4* apparatus; uncorrected. Optical rotations: *Jasco DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* IR spectrometer; KBr pellets; in cm^{-1}. NMR-spectra: *Bruker AM-400* or *DRX-500* instruments; $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. MS: *VG Auto Spec-3000*-spectrometer; in *m/z* (rel. %). LC/MS: *Waters HPLC-2695* and *Thermo-Finnigan LCQ-Advantage* spectrometer.

2. *Callus Induction and Subculture Condition.* Seedlings were obtained from surface-sterilized seeds (75% EtOH for 1 min and 0.12% $HgCl_2$ soln. for 10 min.) on sucrose-free *MS* agar medium. The callus tissues were induced by a 1-month incubation after the inoculation of the segments of seedlings at 26° in the dark. The best formation and growth of calli were observed on *MS* agar medium containing 6-benzyladenine (2 mg/l of medium), naphthalene-1-acetic acid (1 mg/l), and kinetin (0.2 mg/l). The callus tissues were subcultured at 1-month intervals at 26° in the dark on *MS* agar medium containing the above plant hormones.

3. *Extraction and Isolation*. Lyophilized callus tissues (120 g dry weight) were extracted with EtOH (5 × 2 l). The combined EtOH extract was evaporated and the residue extracted successively with $CHCl_3$ and MeOH to give a $CHCl_3$-soluble and a MeOH-soluble extract. The MeOH extract (18 g) was subjected to CC (macroporous resin *D101*, H_2O, then 20% EtOH → 50% EtOH → 75% EtOH → 95% EtOH, then Me_2CO): *Fractions MP1 – 6*. *Fr. MP1* and *Fr. MP2* were not further fractionated because the major components were sugars. *Fr. MP3* (1.27 g) was subjected to CC (silica gel (60 g), $CHCl_3$/MeOH 20 : 1(400 ml) → 9 : 1 (800 ml) → MeOH (200 ml)): *Fr. MP3a – f*). *Fr. MP3a* (25 mg) was further purified by VLC (silica gel HF_{254} (2 g), AcOEt/MeOH 100 : 3): *(+)-catechin* (15 mg). *Fr. MP3b* (130 mg) was purified by repeated CC (silica gel (10 g), AcOEt/MeOH 10 : 1; silica gel (6 g), $CHCl_3$/MeOH 20 : 1 → 9 : 1): *(+)-gallocatechin* (20 mg). *Fr. MP3d* (220 mg) was purified by CC (*Sephadex LH-20*, MeOH): *gallic acid* (10 mg). *Fr. MP4* (340 mg) was subjected to CC (silica gel (30 g), $CHCl_3$/MeOH 20 : 1 → 9 : 1 → 1 : 1): *Fr. MP4a – e*). *Sumogaside* (3 mg) was obtained from *Fr. MP4a* by repeated recrystallization from MeOH. *Fr. MP4e* was subjected to repeated CC (*Sephadex LH-20*, MeOH; *Rp-18*, MeOH/H_2O 3 : 7): **3** (9 mg). *Fr. MP5* (70 mg) was fractionated by CC (silica gel (10 g), $CHCl_3$/MeOH 20 : 1 → 9 : 1) and further purified by CC (*Sephadex LH-20*, MeOH): **1** (10 mg). The $CHCl_3$ extract (1.9 g) was subjected to VLC (silica gel (90 g), $CHCl_3$/Me_2CO 100 : 3 → 9 : 1 → 3 : 1 → 2 : 1): *Fr. CP1 – 11*. *Germanicone* (13 mg) was obtained from *Fr. CP1* after purification by CC (silica gel, petroleum ether/$CHCl_3$ 2 : 1 and 1 : 1). *Fr. CP2* was recrystallized and further purified by CC (*Sephadex LH-20*): (*3β*)-*amyrin* (11 mg). *Fr. CP5* was fractionated by CC (*Sephadex LH-20*) and further purified by VLC (silica gel, $CHCl_3$/Me_2CO 20 : 1): **4** (3 mg). *Fr. CP6* was fractionated by CC (*Sephadex LH-20*): *CP6a – d*. *Fr. CP6d* was subjected to VLC (silica gel HF_{254}, petrleum ether/Me_2CO 10 : 1 and 9 : 1): *Fr. CP6d1 – 3*. Compound **2** (8 mg) was obtained from *Fr. CP6d1* by recrystallization. *Fr. CP6d3* was further purified by CC (*Sephadex LH-20*): **5** (3 mg).

LC-ESI-MS Analysis. HPLC (MeOH/H_2O 63 : 37 (0 – 20 min) → 100 : 0 (21 – 25 min) → 63 : 37 (25 – 30 min), flow rate 0.2 ml/min): The trewiasine standard sample was prepared from the seed of *T. nudiflora*. No trewiasine was detected in the $CHCl_3$ extract from the calli.

(*16α*)-17-*Hydroxy*-ent-*atisan-19-oic Acid Methyl Ester* (**1**): Colorless needles. M.p. 121–123°. $[\alpha]_D^{19} = -61.7$ ($c = 1.2$, $CHCl_3$). IR (KBr): 3449, 2945, 1721, 1632, 1450, 1384, 1230. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 334 (1, M^+), 316 (60, $[M - H_2O]^+$), 274 (12), 257 (60). HR-ESI-MS (pos.): 317.2481 ($[M - H_2O + H]^+$, $C_{21}H_{34}O_3^+$; calc. 317.2480).

(*16α*)-*16,17-Dihydroxy*-ent-*atisan-19-oic Acid Methyl Ester* (**2**): Colorless needles. M.p. 68–70°. $[\alpha]_D^{19} = -81.3$ ($c = 0.8$, $CHCl_3$). IR (KBr): 3432(br), 2938, 1726, 1633, 1465, 1448. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 350 (1, M^+), 319 (100 $[M - CH_2OH]^+$), 301 (20), 287 (23), 273 (36), 259 (66). HR-ESI-MS: 373.2351 ($[M + Na]^+$, $C_{21}H_{34}NaO_4^+$; calc: 373.2354).

(*16α*)-ent-*Atisan-16,17,19-triol* (**3**): Colorless needles. $[\alpha]_D^{19} = -33.3$ ($c = 1.2$, MeOH). M.p. 195–197°. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 322 (1, M^+), 316 (60), 273 (5), 174 (100).

17-Hydroxy-ent-*atisan-19-oic Acid* (**4**): Colorless needles. ^{1}H- and ^{13}C-NMR: in agreement with data in [8]. EI-MS: 320 (12, M^+), 302 (60), 274 (78).

(*16α*)-*16,17-Dihydroxy*-ent-*atisan-19-al* (**5**): Colorless needles. ^{1}H- and ^{13}C-NMR: in agreement with data in [8]. EI-MS: 320 (2, M^+), 302 (2), 290 (15), 289 (100), 271 (30).

(*15*R)-*15,16-Dihydroxypimar-9(11)-en-19-oic Acid β-D-Glucopyranosyl Ester (Sumogaside = (1*R,*4a*R,*7*R,*8a*S,*10a*R)-*7-[(1*R)-*1,2-dihydroxyethyl]-1,2,3,4,4a,6,7,8,8a,9,10,10a-dodecahydro-1,4a,7-trimethylphenanthrene-1-carboxylic Acid β-*D-*Glucopyranosyl Ester):* Colorless needles. $[\alpha]_D^{19} = -50.0$ ($c = 0.4$, MeOH). ^{1}H- and ^{13}C-NMR: in agreement with data in [10]. FAB-MS: 497 ($[M - H]^+$, $C_{26}H_{41}O_9^+$), 335 ([aglycon – H]$^+$).

Olean-18-en-3-one (= Germanicone). Colorless needles. ^{1}H- and ^{13}C-NMR: in agreement with data in [11]. EI-MS: 424 (8, M^+, $C_{30}H_{48}O^+$), 409 (10), 218 (56), 204 (56), 203 (42), 189 (76), 177 (100).

3β-β-Amyrin (= (3β)-Olean-12-en-3-ol). Colorless needles. ^{1}H- and ^{13}C-NMR: in agreement with data in [12]. EI-MS: 426 (6, M^+, $C_{30}H_{50}O^+$), 412 (5), 218 (100), 203 (68), 189 (40).

*(+)-Gallocatechin (= (2*R,*3*S*)-3,4-Dihydro-2-(3,4,5-trihydroxyphenyl)-2*H*-1-benzopyran-3,5,7-triol):* Off-white amorphous powder. ^{1}H- and ^{13}C-NMR: in agreement with data in [13]. EI-MS: 306 (10, M^+, $C_{15}H_{14}O_7^+$), 290 (3), 168 (18), 152 (10), 139 (100), 126 (27).

*(+)-Catechin (= (2*R,*3*S*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol):* Off-white amorphous powder. ^{1}H- and ^{13}C-NMR: in agreement with data in [14]. EI-MS: 290 (M^+, $C_{15}H_{14}O_6^+$, 37), 259 (18), 222 (6), 170 (10), 152 (55), 139 (100), 123 (58).

Gallic Acid (= 3,4,5-Trihydroxybenzoic Acid): Colorless needles. ^{1}H- and ^{13}C-NMR: in agreement with data in [8]. EI-MS: 170 (100, M^+), 153(80), 126(92).

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