A Rare New Cleistanthane Diterpene from the Pericarp of *Trewia nudiflora*

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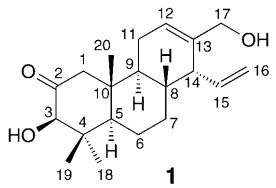
A minor, unprecedented diterpene, 3β,17-dihydroxycleistantha-12,15-dien-2-one (**1**), was isolated from the $CHCl_3$-soluble part of the EtOH extract of the pericarp of *Trewia nudiflora.* The structure of **1** was elucidated by means of 1D- and 2D-NMR spectroscopic analyses as well as by HR-MS. Also isolated were two known triterpenes, glutin-5-en-3-ol and olean-18-en-3-one (germanicone), as well as three known sterols, (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol, (22*E*,24*R*)-5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol, and (22*E*,24*R*)-6-methoxyergosta-7,22-dien-3,5-diol.

Introduction. – *Trewia nudiflora* L. (Euphorbiaceae) is a tropical plant mainly distributed in India, Malaysia, and South China [1]. The seeds of *T. nudiflora* contain highly unusual glyceride oils [2], several novel pyridinone alkaloids [3], and an inhibitor of protein synthesis [4]. The seeds are also a rich source of maytansinoids [5–7]. The EtOH extract of *T. nudiflora* was shown to have antifungal activity against *Penicillium avellaneum* UC-4376.

In our previous work on this extract, we had reported three new *ent*-atisane diterpenes and several known compounds [8]. Now, we report the isolation and structure elucidation of a rare new cleistanthane diterpene (**1**). Compound **1** was isolated together with two known triterpenes, glutin-5-en-3-ol and olean-18-en-3-one (germanicone), and three known sterols, (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol, (22*E*,24*R*)-5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol, and (22*E*,24*R*)-6-methoxyergosta-7,22-diene-3,5-diol.



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Results and Discussion. – Diterpenes with a cleistanthane (=14-ethyl-13-methylpodocarpane) skeleton are uncommon in Nature. The first members of this series were isolated from species of the family Euphorbiaceae, and all known cleistanthane diterpenes from this family reported so far contain an aromatic *C*-ring [9–12]. In our ongoing search for bioactive natural products, we now report the isolation of the rare new

cleistanthane diterpene **1** from the EtOH extract of *T. nudiflora.* Compound **1** was found to have a nonaromatic *C*-ring.

Compound **1**, obtained as a colorless gum, had the molecular formula $C_{20}H_{30}O_3$, based on its HR-EI-MS data (*m*/*z* 318.2192 (M^+; calc. 318.2195)). The IR spectrum showed the presence of OH (3442) and C=C (1636 cm^{-1}) functions. The ^{1}H-NMR Spectrum of **1** (*Table*) showed three Me *singlet*s at δ(H) 0.71, 0.83, and 1.18, and two *doublet*s at δ(H) 3.94 and 4.05 assigned to a CH_2OH group connected to a quaternary C-atom, as well as another *singlet* at δ(H) 3.94 for an oxygenated methine. Further, four olefinic H-atoms at δ(H) 5.17, 5.18, 5.52, and 5.79 were observed.

Table. *^{1}H- and ^{13}C-NMR Data of* **1**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, *J* in Hz.

Position	^{13}C	^{1}H	HMBC (H→C)
1	51.5 (*t*)	2.51 (*d*, *J* = 12.8)	C(2), C(3), C(5), C(10)
		2.22 (*d*, *J* = 12.4)	C(2), C(9), C(10), C(20)
2	211.0 (*s*)	–	–
3	82.6 (*d*)	3.94 (*s*)	C(2), C(4), C(18), C(19)
4	45.6 (*s*)	–	–
5	53.4 (*d*)	1.60 (*dd*, *J* = 2.4, 12.4)	C(4), C(6), C(10), C(19), C(20)
6	21.2 (*t*)	1.78 (*m*), 1.42 (*m*)	C(5), C(7), C(8)
7	32.6 (*t*)	1.05, 2.18 (2*m*)	C(5), C(6)
8	36.6 (*d*)	1.30 (*m*)	C(10), C(11)
9	49.8 (*d*)	1.39 (*m*)	C(5), C(8), C(10), C(11), C(20)
10	43.5 (*s*)	–	–
11	23.9 (*t*)	1.98 (*m*)	C(8), C(12), C(13)
12	123.0 (*d*)	5.79 (br. *d*, *J* = 5.6)	C(11), C(14), C(7)
13	137.0 (*s*)	–	–
14	50.6 (*d*)	2.48 (br. *t*, *J* = 9.6)	C(15), C(16)
15	140.4 (*d*)	5.52 (*dt*, *J* = 10.0, 16.8)	C(8), C(13), C(14), C(16)
16	117.6 (*t*)	5.18 (*dd*, *J* = 2.0, 10.0)	C(8), C(14), C(15)
		5.17 (*dd*, *J* = 1.6, 16.4)	
17	65.5 (*t*)	4.05 (*d*, *J* = 12.8)	C(12), C(13), C(14)
		3.94 (*d*, *J* = 12.8)	
18	29.2 (*q*)	0.71 (*s*)	C(2), C(3), C(4), C(5), C(19)
19	16.7 (*q*)	1.18 (*s*)	C(3), C(4), C(5), C(18)
20	14.4 (*q*)	0.83 (*s*)	C(1), C(5), C(9), C(10)

The ^{13}C-NMR (DEPT) spectrum of **1** showed 20 carbon signals: three Me groups at tertiary C-atoms, six CH_2 and seven CH groups, and four quaternary C-atoms, including a keto function at δ(C) 211.0, an oxygenated tertiary C-atom at 82.6, an oxygenated CH_2 at 65.5, and four olefinic resonances at δ(C) 117.6 (*t*), 140.4 (*d*), 123.0 (*d*), and 137.0 (*s*). Of the six degrees of unsaturation required by the molecular formula, one was accounted for by the C=O group and two by the C=C bonds, indicating that **1** was a tricyclic diterpene.

The ^{1}H,^{13}C long-range correlations between the H-atoms of the three Me groups (δ(H) 0.71, 0.83, 1.18; δ(C) 16.7, 14.4, 29.2, resp.) and the corresponding C-atoms (*Table*) established a nine-carbon residue. Moreover, HMBC experiments indicated that δ(H) 2.51 and 2.22 (CH_2(1)) had ^{1}H,^{13}C long-range correlations with δ(C) 211.0

(C(2)), 82.6 (C(3)), 53.4 (C(5)), and 43.5 (C(10)), suggesting that C(1) was linked to C(3) *via* a C=O group at C(2) (fragment **A**, *Fig. 1*).

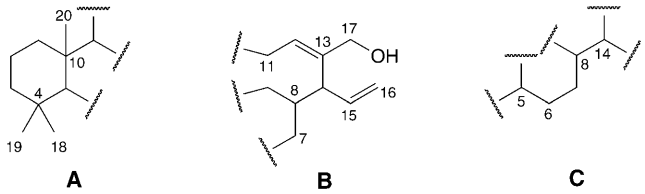

Fig. 1. *Indentified fragment structures* **A**–**C** *of compound* **1**

The $^{1}H,^{13}C$ long-range correlations of both the olefinic H-atoms (δ(H) 5.79, 5.52, 5.17, 5.18) and the OCH_2 H-atoms with the corresponding carbon resonances established the eight-carbon fragment **B** (*Fig. 1*), as supported by $^{1}H,^{1}H$-COSY cross-peaks. Moreover, H–C(9) at δ(H) 1.39 was also linked to **B**. The $^{1}H,^{1}H$-COSY data of **1** further established the five-carbon residue **C**. Based on the key H-atoms H–C(5), H–C(9), and H–C(14) in the above three fragments, they were linked together as shown in the chemical formula to form **1**.

The relative configuration of **1** was determined by NOE experiments (*Fig. 2*). Key NOEs were observed between H_β–C(1) (δ(H) 2.22) and H_β–C(7) (δ(H) 1.05); between H–C(3) and H–C(5), H_α–C(7) (δ(H) 2.18) and Me(18); between H–C(5) and both H–C(9) and Me(18); between H_α–C(6) (δ(H) 1.78) and both H–C(9) and Me (18); between H–C(9) and both H_α–C(6) and Me(18); between Me(19) and both H_β–C(6) (δ(H) 1.42) and Me(18); and between Me(20) and H_β–C(6), H–C(8), H–C(11), and H–C(14), respectively. From the above data, the structure of **1** was determined as 3β,17-dihydroxycleistantha-12,15-dien-2-one (=(3β,14α)-14-ethenyl-3-hydroxy-13-(hydroxymethyl)podocarp-12-en-2-one).

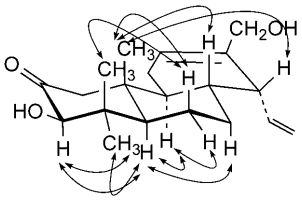

Fig. 2. *Key NOESY correlations of* **1**

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 and 80–100 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China); reverse-phase C_{18} silica gel (*Merck*) or *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated TLC plates (*Silica gel* GF_{254}; *Qingdao Marine Chemical Factory*),

detection by spraying with 5% H_2SO_4 soln. M.p.: *XRC-1* Micro-melting-point apparatus; uncorrected. Optical rotations: *JASCO DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* IR spectrometer, with KBr pellets; in cm^{-1}. NMR Spectra: *Varian Inova-400* and *Bruker AM-400* or *DRX-500* NMR spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. MS: *VG AutoSpec-3000* mass spectrometer; in *m/z* (rel. %).

Plant Material. The pericarp of *Trewia nudiflora* was collected in Xishuangbanna, Yunnan Province, P. R. China. A voucher specimen (K. M. Feng 20159) was deposited at the Herbarium of the Kunming Institute of Botany, Chinese Academy of Science, Kunming.

Extraction and Isolation. The air-dried pericarp of *T. nudiflora* (3 kg) were ground and extracted with boiling 95% EtOH (3×). The combined extracts were concentrated in vacuum on an evaporator. The residue was suspended in H_2O and then extracted successively with *a*) petroleum ether (PE), *b*) $CHCl_3$, and *c*) BuOH. The PE extract (*a*) (16 g) was subjected to CC (300 g SiO_2; PE/$CHCl_3$ 1:1 → 0:1, then $CHCl_3$/acetone 20:1 → 0:1). The fraction (70 mg) eluted with PE/$CHCl_3$ 1:2 was resubjected to CC (1. SiO_2, PE/AcOEt 100:1; 2. *Sephadex LH-20*, acetone) to yield olean-18-en-3-one (7 mg). The fraction eluted with $CHCl_3$ afforded glutin-5-en-3-ol (8 mg) after repeated fractionation by CC (1. SiO_2, PE/AcOEt 15:1; 2. *Sephadex LH-20*, acetone). The original $CHCl_3$ extract (*b*) (9.5 g) was subjected to MPLC (130 g C_{18} gel (40–63 μm); MeOH/H_2O 5:5, 7:3, 10:0), which afforded three fractions (*Fr. 1–3*). *Fr. 2* (bioactive) was further separated to yield seven subfractions: *Fr. 2.1–2.7*. *Fr. 2.3* (bioactive) was separated by CC (*Sephadex LH-20*; MeOH) to afford two further subfractions. *Fr. 2.3.2* was resubjected to CC (SiO_2; $CHCl_3$/acetone 20:1) to afford compound **1** (3 mg). *Fr. 3* was subjected to CC (80 g SiO_2; $CHCl_3$/Me_2CO 100:0 → 100:3) to afford two subfractions. *Fr. 3.a* was further purified by CC (C_{18} (40–63 μm); acetone/H_2O 3:1) to yield (22*E*,24*R*)-5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol (5 mg). *Fr. 3,b* was resubjected to repeated CC (C_{18} (40–63 μm); acetone/H_2O 4:1) to afforded (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (10 mg) and (22*E*,24*R*)-6-methoxyergosta-7,22-dien-3,5-diol (4 mg).

Antifungal Activity. The activity against *Penicillium avellaneum* UC-4376 of each fraction of the $CHCl_3$-soluble part of the EtOH extract was determined during compound purification by means of the disk-diffusion assay on agar plates, as described previously [13].

3β,17-Dihydroxycleistantha-12,15-dien-2-one (=*(3β,14α)-14-Ethenyl-3-hydroxy-13-(hydroxymethyl)podocarp-12-en-2-one*; **1**). Colorless gum. M.p. 106–108°. $[\alpha]_D^{25} = +27.0$ (*c* = 0.1, MeOH). IR (KBr): 3442, 3227, 2926, 1636, 1463, 1197. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 318 (100, M^+), 300 (8), 274 (27), 245 (17), 227 (38). HR-EI-MS: 318.2192 (M^+, $C_{20}H_{30}O_3^+$; calc. 318.2195).

Olean-18-en-3-one (*Germanicone*). Colorless needles. ^{1}H- and ^{13}C-NMR: see [14]. EI-MS: 424 (8, M^+), 409 (10), 218 (56), 204 (56), 203 (42), 189 (76), 177 (100).

Glutin-5-en-3-ol. Colorless needles. ^{1}H- and ^{13}C-NMR: see [15]. EI-MS: 426 (2, M^+), 408 (3), 274 (90), 259 (100).

*(22*E*,24*R*)-5α,8α-Epidioxyergosta-6,22-dien-3β-ol*. Colorless needles. ^{1}H-NMR (400 MHz, C_5D_5N): 0.75 (*s*, Me(18)); 0.84 (*m*, Me(26,27)); 0.88 (*s*, Me(19)); 0.94 (*m*, Me(28)); 1.01(*d*, *J* = 6.4, Me(21)); 4.37 (*m*, H–C(3)); 5.13 (*m*, H–C(22)); 5.27 (*m*, H–C(23)); 6.31 (*d*, *J* = 8.4, H–C(6)); 6.52 (*d*, *J* = 8.4, H–C(7)). ^{13}C-NMR (100 MHz, C_5D_5N): 13.0 (*q*, C(18)); 17.8 (*q*, C(28)); 18.4 (*q*, C(19)); 19.9 (*q*, C(27)); 20.2 (*q*, C(26)); 21.1 (*q*, C(21)); 21.2 (*t*, C(15)); 23.7 (*t*, C(11)); 29.1 (*t*, C(16)); 31.3 (*t*, C(2)); 33.0 (*d*, C(25)); 35.5 (*t*, C(1)); 37.5 (*s*, C(10)); 38.3 (*t*, C(4)); 39.6 (*t*, C(12)); 40.1 (*d*, C(20)); 43.1 (*d*, C(24)); 44.7 (*s*, C(13)); 51.9 (*d*, C(9)); 52.1 (*d*, C(14)); 56.3 (*d*, C(17)); 65.9 (*d*, C(3)); 79.3 (*s*, C(8)); 82.3 (*s*, C(5)); 130.9 (*d*, C(7)); 132.3 (*d*, C(23)); 135.9 (*d*, C(22)); 136.2 (*d*, C(6)). EI-MS: 428 (8, M^+), 410 (60), 396 (100), 376 (25), 363 (15), 337 (25), 301 (10), 251 (30).

*(22*E*,24*R*)-5α,8α-Epidioxyergosta-6,9(11),22-trien-3β-ol*. Colorless, amorphous powder. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.71 (*s*, Me(18)); 0.80 (*d*, *J* = 6.4, Me(27)); 0.81 (*d*, *J* = 6.8, Me(26)); 0.89 (*d*, *J* = 6.8, Me(28)); 0.98 (*d*, *J* = 6.4, Me(21)); 1.06 (*s*, Me(19)); 4.01 (*m*, H–C(3)); 5.15 (*dd*, *J* = 15.2, 7.2, H–C(22)); 5.27 (*dd*, *J* = 15.2, 8.0, H–C(23)); 5.41 (*dd*, *J* = 6.0, 0.8, H–C(11)); 6.27 (*d*, *J* = 8.4, H–C(6)); 6.58 (*d*, *J* = 8.4, H–C(7)). ^{13}C-NMR ($CDCl_3$): 12.9 (*q*, C(18)); 17.5 (*q*, C(28)); 19.6 (*q*, C(27)); 19.9 (*q*, C(26)); 20.7 (*q*, C(21)); 20.8 (*t*, C(15)); 25.5 (*q*, C(19)); 28.6 (*t*, C(16)); 30.5 (*t*, C(2)); 32.5 (*t*, C(1)); 33.0 (*d*, C(25)); 36.0 (*t*, C(4)); 37.9 (*s*, C(10)); 39.9 (*d*, C(20)); 41.1 (*t*, C(12)); 42.7 (*d*, C(24)); 43.6 (*s*,

C(13)); 48.1 (*d*, C(14)); 55.8 (*d*, C(17)); 66.3 (*d*, C(3)); 78.3 (*s*, C(8)); 82.7 (*s*, C(5)); 119.7 (*d*, C(11)); 130.7 (*d*, C(7)); 132.4 (*d*, C(23)); 135.1 (*d*, C(22)); 135.4 (*d*, C(6)); 142.4 (*s*, C(9)). EI-MS: 426 (10, M^+), 410 (8), 394 (40), 376 (30), 299 (20), 251 (38).

*(22*E*,24*R*)-6-Methoxyergosta-7,22-dien-3,5-diol*. Colorless, amorphous powder. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.57 (*s*, Me(18)); 0.82 (*d*, *J* = 6.8, Me(26)); 0.80 (*d*, *J* = 6.4, Me(27)); 0.90 (*d*, *J* = 6.8, Me(28)); 0.97 (*s*, Me(19)); 1.00 (*d*, *J* = 6.8, Me(21)); 3.15 (*d*, *J* = 4.8, H–C(6)); 4.05 (*m*, H–C(3)); 5.16 (*dd*, *J* = 15.6, 8.0, H–C(22)); 5.18 (*dd*, *J* = 15.2, 7.2, H–C(23)); 5.38 (*m*, H–C(7)). ^{13}C-NMR ($CDCl_3$): 12.3 (*q*, C(18)); 17.6 (*q*, C(28)); 18.3 (*q*, C(19)); 19.6 (*q*, C(27)); 19.9 (*q*, C(26)); 21.1 (*q*, C(21)); 22.1 (*t*, C(11)); 22.8 (*t*, C(15)); 27.9 (*t*, C(16)); 30.8 (*t*, C(2)); 32.7 (*t*, C(1)); 33.0 (*d*, C(25)); 37.2 (*s*, C(10)); 39.3 (*t*, C(12)); 39.5 (*t*, C(4)); 40.4 (*d*, C(20)); 42.8 (*d*, C(24)); 43.8 (*s*, C(13)); 43.8 (*d*, C(9)); 54.9 (*d*, C(14)); 55.9 (*d*, C(17)); 58.3 (MeO, C(6)); 67.8 (*d*, C(3)); 76.3 (*s*, C(5)); 82.4 (*d*, C(6)); 114.9 (*d*, C(7)); 132.0 (*d*, C(23)); 135.4 (*d*, C(22)); 143.6 (*s*, C(8)). EI-MS: 444 (6, M^+), 426 (80), 411 (26), 393 (40), 377 (100), 301 (11), 269 (32), 251 (76).

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