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CLERODANE DITERPENES FROM *POLYALTHIA CHELIENSIS*

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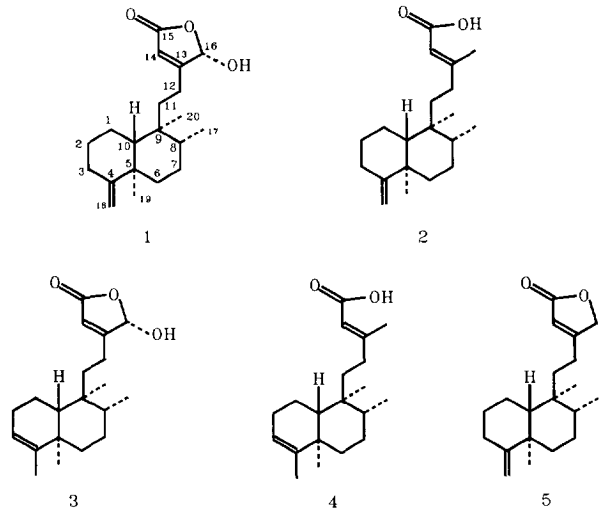
Key Word Index— *Polyalthia cheliensis*; Annonaceae; clerodane diterpenes; cytotoxic activity.

Abstract—Two new clerodane diterpenes, together with two known compounds, were isolated from the stem bark of *Polyalthia cheliensis* and identified as 16α-hydroxy-cleroda-4(18),13(14)*Z*-dien-15,16-olide and cleroda-4(18),13(14)*E*-dien-15-oic acid, respectively, on the basis of chemical and spectral properties.

INTRODUCTION

Several recent reports have described the isolation of some cytotoxic clerodane diterpenes from plants of the genus *Polyalthia* [1–6]. In the course of an investigation of the chemical constituents of the stem bark of *P. cheliensis*, two new clerodane diterpenes, 16α-hydroxy-cleroda-4(18),13(14)*Z*-dien-15,16-olide (**1**) and cleroda-4(18),13(14)*E*-dien-15-oic acid (**2**), together with two known compounds, 16α-hydroxy-cleroda-3,13(14)*Z*-dien-15,16-olide (**3**) and cleroda-3,13(14)*E*-dien-15-oic acid (**4**), were isolated and their structures identified by chemical and spectral means, respectively.



RESULTS AND DISCUSSION

Air-dried stem bark of *P. cheliensis* was powdered and extracted with EtOH. Chromatographic separation of the extract gave fractions A and B. Fraction A was a powder, which showed one spot on silica gel TLC, and showed cytotoxic activities against KB cells (IC_{50} = 1.59 μg ml^{-1}). It was easily separated into compound **1** and the known compound **3**.

Compound **1** (0.26%), $C_{20}H_{30}O_3$, contained a β-substituted butenolide [1720, 1635 cm^{-1}; δ_H 5.76 (1H, *s*); δ_C 170.9 (*s*), 116.8 (*d*), 172.1 (*s*)], two tertiary and one secondary methyl group [δ_H 1.04, 0.74 (each 3H, *s*), 0.76 (3H, *d*, *J* = 6.4 Hz); δ_C 15.9, 17.9, 20.8], an *exo*-methylene group [δ_H 4.48 (2H, *s*); δ_C 160.1 (*s*), 102.7 (*t*)] and a secondary hydroxy group [3280 cm^{-1}; δ_H 5.98 (1H, *br s*), 5.65 (1H, *br s*, OH); δ_C 99.3 (*d*)]. Reduction of **1** with $NaBH_4$ gave a new derivative, compound **5**. The ^{1}H NMR spectrum of **5** showed a doublet at δ4.68 (2H, *d*, *J* = 1.6 Hz), and its ^{13}C NMR spectrum showed a triplet at δ73.0, indicating that the hemiacetal hydroxy group at C-16 of compound **1** was reduced. The α-configuration of the hemiacetal hydroxy group of **1** was confirmed by the finding that the NMR data of C-16 and H-16 were the same for both compounds **1** and **3** [1]. Comparing with **3**, the presence of an *exo*-methylene group in compound **1** indicated the double bond in **1** could be between position 4 and 18. Thus, the structure of **1** was identified as 16α-hydroxy-cleroda-4(18),13(14)*Z*-dien-15,16-olide.

Fraction B was subjected to silica gel column chromatography to afford a mixture of compound **2** and the known compound **4**, which showed one spot on silica gel TLC. They were easily separated from each other by SiO_2–$AgNO_3$ column chromatography. Compound **2** (0.05%), $C_{20}H_{32}O_2$, contained four methyl groups [δ_H 2.13 (*d*, *J* = 1.4 Hz), 1.03 (*s*), 0.79 (*d*, *J* = 6.2 Hz), 0.72 (*s*)], an *exo*-methylene group [δ_H 4.48 (2H, *t*, *J* = 1.5 Hz); δ160.4 (*s*), 102.6 (*t*)], a β-substituted unsaturated oic acid moiety [3300–2700, 1690, 1630 cm^{-1}; δ_H 5.64 (1H, *q*,

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J = 1.0 Hz); δ_C 164.3 (*s*), 114.7 (*d*), 171.5 (*s*)]. Its ^1H–^1H COSY spectrum showed cross-peaks between δ5.64 and 2.13, indicating that the vinyl methyl group was located at C-13, as in compound **4**. The presence of a 4(18) double bond (*exo*-methylene) in **2** was assigned by comparing its NMR spectra with those of **1**. The different chemical shifts of ring A in **2** and **4** were similar with those between **1** and **3**. Compound **2**, therefore, was identified as cleroda-4(18),13(14)*E*-15-oic acid.

The structures of **3** and **4** were identified as 16α-hydroxy-cleroda-3,13(14)*Z*-dien-15,16-olide and cleroda-3,13(14)*E*-dien-15-oic acid by their MS, ^1H NMR and ^{13}C NMR spectra respectively [1–3].

EXPERIMENTAL

General. Mps: uncorr.; IR: KBr discs; ^1H NMR, ^{13}C NMR and 2D-NMR: $CDCl_3$ using TMS as int. standard; EIMS: 70 eV.

Plant material. The stem bark of *P. cheliensis* Hu was collected in Xishuangbanna, Yunnan province of China in October 1991, and identified by Mr Wang Hong, a botanist of Xishuangbanna Tropical Botanic Garden, Kunming Institute of Botany, Academia Sinica, where the voucher specimen is deposited.

Extraction and isolation. Air-dried stem bark of plant (2 kg) was extracted with 95% EtOH at room temp. After removal of solvent by evapn, the extract was partitioned between water and EtOAc. The EtOAc extract was chromatographed over silica gel (petrol–EtOAc 1:1) to afford frs A (9 g) and B (18 g). Fraction A 1.07 g was successively subjected to silica gel–$AgNO_3$ (10:1) CC (petrol–Et_2O 2:1) to give 0.47 g of **1** and 0.55 g of **3**, respectively. Fraction B 18 g was chromatographed over silica gel to give a mixture of **2** and **4** (3.87 g), which on silica gel–$AgNO_3$ short CC gave **2** (0.31 g) and **4** (0.40 g), respectively. Compounds **3** and **4** were identified by IR, ^1H and ^{13}C NMR [1–3].

Compound **1**. Needles from cyclohexane, mp 150–152°, $[\alpha]_D^{25}$ 19.5 ($CHCl_2$; *c* 0.64). Anal. calcd for $C_{20}H_{30}O_3$: C, 75.47, H, 9.43. Found: C, 75.51; H, 9.61. MS (*m/z*): 318 $[M]^+$ (60), 303 (70), 300 (85), 285 (90), 275 (38), 244 (70), 203 (90), 187 (80), IR ν_{max}^{KBr} cm^{-1}: 3280, 1720, 1635, 895, 855; ^1H NMR (400 MHz): δ5.98 (1H, *br s*, H-16β), 5.76 (1H, *s*, H-14), 5.65 (1H, *s*, OH), 4.48 (2H, *s*, 2H-18), 2.25 (2H, *m*, 2H-12), 2.08 (2H, *m*, 2H-3), 1.04 (3H, *s*, 3H-19), 0.76 (3H, *d*, J = 6.4 Hz, 3H-17), 0.74 (3H, *s*, 3H-20); ^{13}C NMR: Table 1.

Reduction of compound **1**. $NaBH_4$ (300 mg) was dissolved in 30 ml MeOH. To this soln was added 260 mg of **1** (in 15 ml of MeOH) and the reaction soln was stirred for 6 hr at room temp. The reaction soln was then neutralized with HCO_2H, and the MeOH removed by evapn. The residue was partitioned between H_2O and CH_2Cl_2. Removal of the CH_2Cl_2 gave the crude product, which was chromatographed over silica gel (short column) (petrol–Et_2O 2:1) to give 226 mg of **5** (90%) as a gum: $C_{20}H_{30}O_2$, IR ν_{max}^{KBr} cm^{-1}: 1730, 1640; ^1H NMR (400 MHz): δ5.77 (1H, *t*, J = 1.6 Hz, H-14), 4.68 (2H, *d*, J = 1.6 Hz, 2H-16), 4.47 (2H, *d*, J = 1.5 Hz, 2H-18), 2.23 (2H, *m*, 2H-12), 2.09 (2H, *m*, 2H-3), 1.02 (3H, *s*, 3H-19), 0.77 (3H, *d*, J = 6.5 Hz, 3H-17), 0.75 (3H, *s*, 3H-20); ^{13}C NMR: Table 1.

Table 1. ^{13}C NMR shifts of compounds **1**, **2** and **5** ($CDCl_3$, 100.6 MHz)*

C	1	2	5
1	21.3	21.8	21.8
2	21.7	27.5	22.2
3	32.9	33.1	32.9
4	160.1	160.4	160.4
5	39.2	39.5	39.3
6	27.4	28.7	27.4
7	37.2	36.3	37.3
8	36.7	36.8	36.8
9	40.0	40.1	40.0
10	48.7	48.8	48.8
11	28.5	34.9	28.6
12	34.7	37.5	35.3
13	170.9	164.3	170.9
14	116.8	114.7	115.0
15	172.1	171.5	174.0
16	99.3	19.4	73.0
17	15.9	16.0	15.9
18	102.7	102.6	102.8
19	17.9	18.1	18.3
20	20.8	20.8	20.8

*Multiplicities were established by the DEPT pulse sequence.

Compound **2**. Needles from benzene, mp 111–113°, $[\alpha]_D^{23}$ 8.7° ($CHCl_3$; *c* 1.74). HR-FABMS (*m/z*): 305.2508 $[M + H]^+$. Calcd for $C_{20}H_{32}O_2$: 305.2481. MS (*m/z*): 304 $[M]^+$ (55), 289 (65), 280 (20), 271 (40), 261 (25), 236 (60), 222 (55); IR ν_{max}^{KBr} cm^{-1}: 3300–2700, 1690, 1630, 900, 870; ^1H NMR (400 MHz): δ5.64 (1H, *q*, J = 1.0 Hz, H-14), 4.48 (2H, *t*, J = 1.5 Hz, 2H-18), 2.13 (3H, *d*, J = 1.4 Hz, 3H-16), 2.28 (1H, *dt*, J = 5.0, 12.0 Hz, H-3), 2.10 (1H, *m*, H-12), 1.97 (1H, *dt*, J = 4.6, 12.6 Hz, H-3), 1.86 (1H, *m*, H-12), 1.03 (3H, *s*, 3H-19), 0.79 (3H, *d*, J = 6.2 Hz, 3H-17), 0.72 (3H, *s*, 3H-20); ^{13}C NMR: Table 1.

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