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Anti-tumour clerodane-type diterpenes from *Mitrephora thorelii*

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Bioassay-guided fractionation of the crude extract of *Mitrephora thorelii* (Annonaceae) led to the isolation of two clerodane-type diterpenes. Their structures were characterised on the basis of spectroscopic methods as 6α,16,18-trihydroxycleroda-3(4),13(14)-dien-15,16-olide (**1**) and 16-hydroxycleroda-3(4),13(14)-dien-15,16-olide (**2**). Compound **1** is a new compound. Compounds **1** and **2** exhibited inhibitory activity against the proliferation of human hepatoma BEL-7402 cells *in vitro*. Compound **2** also showed an *in vivo* anti-tumour effect against the growth of hepatoma H22 in mice.

Keywords: *Mitrephora thorelii*; Annonaceae; Diterpenes; Anti-tumour activity; Bioassay-guided isolation

1. Introduction

Natural products have been playing an important role in the discovery of anti-tumour drugs. According to a recent statistic, nearly 60% of the anti-tumour and anti-infective agents commercially available or in the later stages of clinical trials are of natural product origin [1]. In the course of our efforts to find new anti-tumour natural products from plant resources, the chloroform extract of the aerial parts of *Mitrephora thorelii* (Annonaceae) was found to exhibit significant inhibitory activity against the proliferation of human hepatoma BEL-7402 cells. Several diterpenoids, alkaloids and fatty acids have been isolated from the genus *Mitrephora* [2–8], and one *ent*-trachylobane type diterpene was recently reported to exhibit broad spectrum cytotoxicity [8]. To our knowledge, no chemical constituents have ever been reported on the species *Mitrephora thorelii*. We herein report the isolation and structure identification of two clerodane-type diterpenes (figure 1) with cytotoxic activity from *M. thorelii*, and one of them was also found to display *in vivo* anti-tumour activity.

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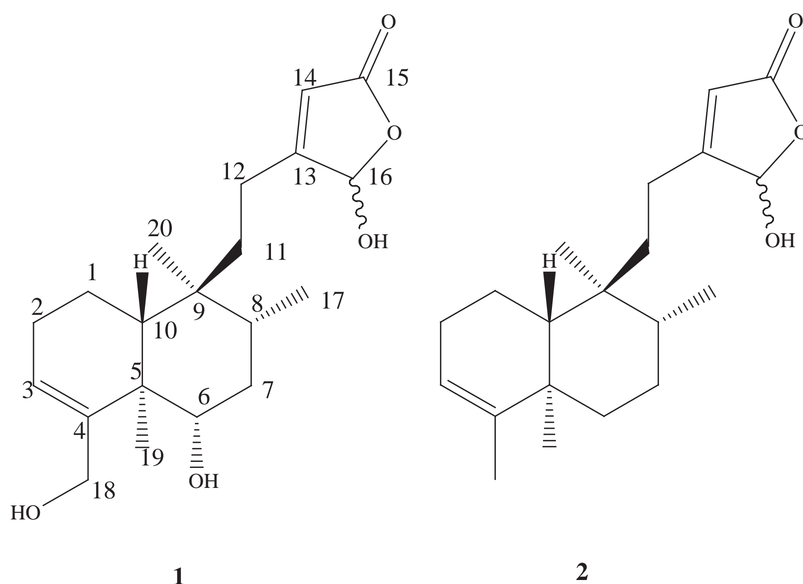

Figure 1. Structures of compounds **1** and **2**.

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2. Results and discussion

2.1 Structure elucidation

Bioassay-guided isolation of the extract of *M. thorelii* led to the purification of two cytotoxic compounds **1** and **2**. Their structures were characterised on the basis of spectroscopic methods as 6α,16,18-trihydroxycleroda-3(4),13(14)-dien-15,16-olide (**1**) and 16-hydroxycleroda-3(4),13(14)-dien-15,16-olide (**2**) [9], respectively. Compound **1** is a new clerodane-type diterpene.

Compound **1** was isolated as white amorphous powder with molecular formula $C_{20}H_{30}O_5$ established by HREI-MS and NMR analysis. Its IR spectrum indicated the presences of hydroxyl ($3319\,cm^{-1}$), carbonyl ($1755\,cm^{-1}$), and olefinic ($1647\,cm^{-1}$) groups. The ^1H NMR spectrum of **1** showed signals of one hydroxymethylene at δ 3.95 (d, $J = 12.6$ Hz) and δ 4.10 (d, $J = 12.6$ Hz), one hydroxymethine at δ 3.58 (t, $J = 8.1$ Hz), and three methyls at δ 0.71 (s); 0.80 (d, $J = 6.7$ Hz); and 1.04 (s). The ^{13}C NMR spectrum of **1** exhibited 20 signals assigned by DEPT experiments into three methyls, six methylenes, six methines (two for sp^2 methines and two for oxygenated methines), and five quaternary carbons (one for ester/carboxylic carbonyl and two for sp^2 carbons). Additionally, the ^1H NMR signals at δ 5.82 and δ 6.08 together with the ^{13}C NMR signals at δ 118.0, 171.6, 101.4 and 172.1 suggested the presence of an α,β-unsaturated γ-lactone group. Analysis of the ^1H—^1H COSY and HMQC spectra revealed the following fragments —C-10—C-1—C-2—C-3; —C-17—C-8—C-7—C-6; and —C-11—C-12 in the structure. The planar structure of **1** was established on the basis of HMBC correlations (table 1 and figure 2). The relative configuration of **1** was further determined by NOE correlations observed between H-6 and H-8; H-10 and H-6, H-11, H-8; H-17 and H-20; H-19 and H-18, H-20. Therefore, the structure of **1** was elucidated to be 6α,16,

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Table 1. ^{1}H NMR and ^{13}C NMR spectral data of **1** (400 MHz for ^{1}H, 100 MHz for ^{13}C in acetone-d_6).

No.	δ_H	δ_C	*HMBC (H to C)*
1	1.53(α), m; 1.59(β), m	18.8, t	2, 3, 5, 9
2	2.10, m	27.5, t	1, 3, 4
3	5.46, t (3.2)	126.9, d	1, 2, 5, 18
4	–	148.0, s	–
5	–	45.2, s	–
6	3.58, t (8.1)	75.6, d	4, 5, 7, 8, 19
7	1.52, m	37.3, t	6, 8, 9, 17
8	1.63, m	35.7, d	6, 7, 9, 10, 11, 17, 20
9	–	39.4, s	–
10	1.28, dd (1.7, 11.9)	46.7, d	1, 2, 4, 5, 6, 8, 9, 11, 19, 20
11	1.57, m	36.0, t	8, 9, 10, 12, 13, 20
12	2.15, m; 2.27, m	22.0, t	11, 13, 14
13	–	171.6, s	–
14	5.82, s	118.0, d	12, 13, 15, 16
15	–	172.1, s	–
16	6.08, s	101.4, s	14, 15
17	0.80, d (6.6)	16.3, q	6, 7, 8, 9
18	3.95, d (12.6); 4.10, d (12.6)	66.7, t	3, 4, 5
19	1.04, s	17.2, q	4, 5, 6, 10
20	0.71, s	18.6, q	5, 8, 9, 11, 12

18-trihydroxycleroda-3(4),13(14)-dien-15,16-olide, which is a new compound named mitrephorathorol A.

Compound **2** was isolated as colourless oil. It was identified as a known diterpene isolated previously from *Polyalthia longifolia* by comparison of their physicochemical data [9,10]. It was reported for the first time from the genus *Mitrephora*.

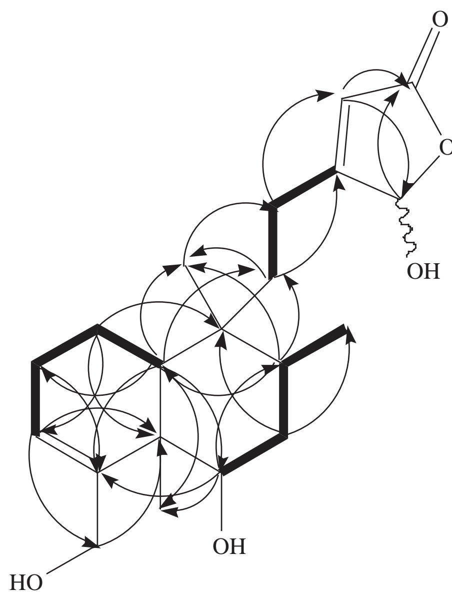

Figure 2. ^{1}H–^{1}H COSY (thick black lines) and key HMBC correlations (^{1}H to ^{13}C, curved arrows) of **1**.

2.2 Anti-tumour activity

Compounds **1** and **2** inhibited the proliferation of BEL-7402 cells *in vitro* with the IC_{50} values 44.6 and 20.1 μM, respectively. *In vivo* anti-tumour effect of compound **2** was further evaluated in murine hepatoma H22 model. It was found that compound **2** significantly inhibited the growth of hepatoma H22 with the percentage inhibition of 30.7% ($P < 0.05$ *vs* control). The alkylation agent cyclophosphamide served as a positive control (63.8%, $P < 0.01$ *vs* control). The mice were well tolerated towards compound **2**, and no significant lose of body weight was observed compared with control group ($P > 0.05$, data not shown). The above results revealed compound **2** as a potential anti-tumour natural product, and further *in vivo* anti-tumour evaluation is warranted.

3. Experimental

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3.1 General experimental procedures

Optical rotations were measured with Perkin–Elmer 241MC polarimeter. UV spectrum was recorded with a Beckman DU-7 spectrometer. IR spectrum was recorded using a Perkin–Elmer 577 spectrometer. LREI-MS was obtained on a MAT-95 spectrometer, and HREI-MS was obtained on Kratos 1H spectrometer. NMR spectra were run on a Bruker AM 400 spectrometer with TMS as internal standard. Column chromatographic separations were carried out using LiChroprep RP-18 Lobar column (40–63 μm, Merck), and using silica gel H60 (300–400 mesh) (Qingdao Haiyang Chemical Group Corp., Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC. Human hepatoma BEL-7402 cells were obtained from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China).

The cells were maintained in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated foetal bovine serum, 100 kU/L penicillin, and 200 kU/L streptomycin at 37°C in an atmosphere of 5% CO_2. Reagents for biological assays including benzidine dihydrochloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and all other reagents were purchased from Sigma Co. (St. Louis, MO, USA). Compounds **1** and **2** were stored at −20°C as 10 mM solutions in ethanol. The solutions were diluted at least 1000-fold in the growth medium so that the final concentration of ethanol was less than 0.1%, which has no effect on the cell proliferation.

3.2 Plant material

The aerial parts of *Mitrephora thorelii* were collected in the suburb of Guangzhou, Guangdong province, China, in December 2003, and identified by Professor Zexian Li of South China Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. SIMMS0312) is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

Five hundred grams of the air-dried aerial parts of *Mitrephora thorelii* was powdered and refluxed three times with methanol. The filtrate was concentrated to dryness *in vacuo*. The methanol extract (18.3 g) was suspended in water and then partitioned in turn with petroleum ether (bp 60–90°C), chloroform and *n*-butanol, yielding petroleum ether fraction (4.7 g), chloroform fraction (3.7 g), and *n*-butanol fraction (5.3 g), respectively.

The chloroform fraction (3.7 g) was subjected to chromatography over a silica gel H60 column with a chloroform/methanol gradient (50:1, 20:1, 10:1, 5:1, 2:1, 1:1) as eluent to give five fractions (I–V). Fraction IV (736.1 mg) was chromatographed over a silica gel H60 column eluting with petroleum ether/acetone (3:1) to give two fractions (IVa and IVb). Fraction IVb (156.6 mg) was successively subjected to a Sephadex LH-20 column eluting with 95% ethanol, and a LiChroprep RP-18 Lobar column eluting with a methanol/water gradient (60%) to afford compound **1** (60.6 mg). Fraction II (544.3 mg) was subjected to chromatography over a silica gel H60 column with a petroleum ether/acetone gradient (5:1, 2:1) as eluent to give three fractions (IIa–IIc). Fraction IIa (127.6 mg) was further purified by PTLC developed with petroleum ether/acetone (3:2) to afford compound **2** (62.2 mg).

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3.4 Identification

3.4.1 6α,16,18-Trihydroxycleroda-3(4),13(14)-dien-15,16-olide (1). White amorphous powder; $[\alpha]_D^{20}$ -16.7 (*c* 0.51, MeOH); UV_{max} (MeOH): 204 (log ε 4.00) nm; IR bands (KBr): 3319, 2958, 1755, 1647, 1456, 1387, 1136, 1001, 949, 756, 667 cm^{-1}; LREI-MS *m/z* (% base peak): 350 $[M^+]$ (7), 332 (15), 314 (35), 203 (100); HREI-MS *m/z*: 350.2093 $[M]^+$ (calcd for $C_{20}H_{30}O_5$, 350.2099). ^1H NMR and ^{13}C NMR data: see table 1.

3.4.2 16-Hydroxycleroda-3(4),13(14)-dien-15,16-olide (2). Colourless oil; $[\alpha]_D^{20}$ -30.5 (*c* 0.56, MeOH) {lit. $[\alpha]_D^{26}$: -70.6 (*c* 0.01, MeOH) [10]}; LREIMS *m/z* (% base peak): 318 $[M^+]$ (30), 285 (40), 189 (100). ^1H NMR data and ^{13}C NMR data were consistent with those reported in literature [9].

3.5 In vitro cytotoxic activity assay

Cells (2×10^4 cells/well) were plated into 96-well plates. Following 24 h of incubation, cells were incubated with different concentrations of compounds **1** and **2** for another 72 h. Cell proliferation was determined by MTT assay as described previously [11]. The assays were carried out in triplicate in at least three independent experiments.

3.6 In vivo evaluation of anti-tumour activity of compound 2

Female mice (5–6 weeks old) of Kunming strain were purchased from Shanghai SLAC Laboratory Animal Co. (Shanghai, China). All mice were housed in sterile room and fed with irradiated nutrients and filtered water *ad libitum*. All animal studies were conducted under the guidelines approved by Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). Murine hepatoma H22 is maintained by serial intraperitoneal passage in Kunming mice. H22 cells were subcutaneously implanted into Kunming mice at

1×10^6 cells/mouse. Each experimental group was composed of 6 mice. Compound **2** was first dissolved in ethanol and further diluted with normal saline. After 24 h of tumour implantation, mice were intraperitoneally administered with compound **2** (40 mg/kg) for 7 consecutive days and the anti-tumour activity was determined by weighing the tumour masses removed from the mice at the end of experiment. The alkylation agent cyclophosphamide was included as a positive control in this study and was given intraperitoneally for 7 days at the dose of 24 mg/kg.

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References

[1] Y.Z. Shu. *J. Nat. Prod.*, **61**, 1053 (1998).
[2] J.R. Zgoda-Pols, A.J. Freyer, L.B. Killmer, J.R. Porter. *Fitoterapia*, **73**, 434 (2002).
[3] J.R. Zgoda, A.J. Freyer, L.B. Killmer, J.R. Porter. *J. Nat. Prod.*, **64**, 1348 (2001).
[4] N.H. Lee, Y.J. Xu, S.H. Goh. *J. Nat. Prod.*, **62**, 1158 (1999).
[5] R. Yu, B.G. Li, Q. Ye, G.L. Zhang. *Nat. Prod. Res.*, **19**, 359 (2005).
[6] B. Supudompol, T. Chaowasku, K. Kingfang, K. Burud, S. Wongseripipatana, K. Likhitwitayawuid. *Nat. Prod. Res.*, **18**, 387 (2004).
[7] J. Brophy, R. Goldsack, P. Forster. *J. Essent. Oil Res.*, **16**, 95 (2004).
[8] C. Li, D. Lee, T.N. Graf, S.S. Phifer, Y. Nakanishi, J.P. Burgess, S. Riswan, F.M. Setyowati, A.M. Saribi, D.D. Soejarto, N.R. Farnsworth, J.O. Falkinham III, D.J. Kroll, A.D. Kinghorn, M.C. Wani, N.H. Oberlies. *Org. Lett.*, **7**, 5709 (2005).
[9] N. Hara, H. Asaki, Y. Fujimoto, Y.K. Gupta, A.K. Singh, M. Sahai. *Phytochemistry*, **38**, 189 (1995).
[10] A.P. Phadnis, S.A. Patwardhan, N.N. Dhaneshwar, S.S. Tavale, T.N. Guru Row. *Phytochemistry*, **27**, 2899 (1988).
[11] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Woff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd. *J. Natl. Cancer Inst.*, **83**, 757 (1991).