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### A novel alkaloid from *Gaultheria nummularioides*

M. -F. Yang<sup>ab</sup>, Y. -Y. Li<sup>a</sup>; B. -G. Li<sup>a</sup>; G. -L. Zhang<sup>a</sup>

<sup>a</sup> Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu, China <sup>b</sup> Chengdu Institute of Organic Chemistry, the Chinese Academy of Sciences, Chengdu, China

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## Note

### A novel alkaloid from *Gaultheria nummularioides*

M.-F. YANG<sup>†‡</sup>, Y.-Y. LI<sup>†</sup>, B.-G. LI<sup>†</sup> and G.-L. ZHANG<sup>†\*</sup>

<sup>†</sup>Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu 610041, China

<sup>‡</sup>Chengdu Institute of Organic Chemistry, the Chinese Academy of Sciences, Chengdu 610041, China

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A novel alkaloid, gaultherialine A (**1**), along with twenty-seven known compounds were isolated from the whole plants of *Gaultheria nummularioides* D. Don. The structure of the new alkaloid was elucidated as 7,8-dimethoxy-1-(4-methoxyphenyl)-4,5-dihydro-2*H*-benzo[*e*]indole-2-one N-oxide (**1**) on the basis of spectral evidence.

**Keywords:** *Gaultheria nummularioides* D. Don; Gaultherialine A, N-oxide

## 1. Introduction

Several species of the genus *Gaultheria* (Ericaceae), such as *G. yunnanensis* (Franch.) Rehd., *G. fragrantissima* Wall. and *G. procumbens* Linn. are used in folk medicine to treat rheumatoid arthritis in China [1], India, American and Canada [2]. Phytochemical studies on some species of this genus led to the isolation of flavonoids, lignans [3], coumarines, sterols, organic acids [4], diterpenoids [5], and triterpenoids [6]. Methyl salicylate is the major component of *Gaultheria* species. *G. nummularioides* D. Don is found in China, Indonesia, Bhutan, Nepal and Sikkim. There is no report of a chemical study on this plant. In this investigation, a novel alkaloid, gaultherialine A (**1**), along with twenty-seven known compounds were isolated from an ethanolic extract of this plant. The structure of **1** was determined as 7,8-dimethoxy-1-(4-methoxyphenyl)-4,5-dihydro-2*H*-benzo[*e*]indole-2-one N-oxide (**1**) on the basis of spectral data. By spectroscopic analysis or comparison with authentic samples, the known compounds were identified as preskimmianine (**2**) [7], dictamine (**3**), confusameline (**4**) [8], squalene (**5**) [9], salicylic acid (**6**), vanillic acid (**7**) [10],  $\beta$ -sitosterol (**8**), fraxinellone (**9**), fraxinellone (**10**) [11], oleanolic acid (**11**),  $\alpha$ -amyrin (**12**), maslinsaeure (**13**), 3 $\beta$ -hydroxy-bauer-7-en-28-oic acid (**14**), (2*E*,2*R*)-24-methyl-5 $\alpha$ -cholesta-7,22-diene-3 $\beta$ ,5,6 $\beta$ -triol (**15**) [12], euscaphic acid (**16**), taraxerol (**17**),

\*Corresponding author. Email: zhanggl@cib.ac.cn

3 $\beta$ -acetoxy-urs-12-ene (**18**) [13], (+)-homoeriodictyol (**19**), hesperetin (**20**), quercetin (**21**), quercetrin (**22**) [14], hesperidin (**23**), avicularin (**24**), (+)-catechol (**25**), hirsutine (**26**), pavetannin (**27**) [15] and  $\beta$ -daucosterol (**28**).

## 2. Results and discussion

Gaultherialine A (**1**) was isolated as a red amorphous powder. Two aromatic protons resonated at  $\delta$  6.76 (1 H, s, H-6) and 7.05 (1 H, s, H-9) in its  $^1\text{H}$  NMR spectrum. The moiety  $-\text{CH}_2-\text{CH}_2-$  was deduced from  $^1\text{H}$  NMR signals at  $\delta$  3.84 (2 H, t, 6.0 Hz, H-4) and 3.07 (2 H, t, 6.0 Hz, H-5) and  $^{13}\text{C}$  NMR signals at  $\delta$  36.5 (t) and 28.8 (t). Rings B and C could be discerned from the HMBC correlations of H-4 with C-3a, 9b, H-5 with C-6, 5a, 9a, H-6 with C-7, 8, 5a, 9a, and H-9 with C-7, 8, 5a, 9a, 9b.  $^1\text{H}$  NMR signals at  $\delta$  7.28 (2 H, d, 8.4 Hz, H-2',6') and 6.96 (2 H, d, 8.4 Hz, H-3',5') suggest a 1,4-disubstituted phenyl ring. Three methoxy groups are at C-7, C-8 and C-4' on the basis of the HMBC correlations of OMe-7 ( $\delta$  3.96, 3H, s) with C-7 ( $\delta$  153.4, s), OMe-8 ( $\delta$  3.40, 3H, s) with C-8 ( $\delta$  148.0, s), and of OMe-4' ( $\delta$  3.82, 3H, s) with C-4' ( $\delta$  159.6, s).

The molecular formula  $\text{C}_{21}\text{H}_{19}\text{NO}_5$  of **1** was provided by the ion peak at  $m/z$  366.1339  $[\text{M} + \text{H}]^+$  in the HR-ESIMS spectrum. Apart from the atoms assigned above, two C atoms resonated at  $\delta$  108.4 (s) and 183.7 (s), and two O atoms and one N atom remained. A carbonyl group was revealed by the IR absorption at  $1742\text{ cm}^{-1}$  and the  $^{13}\text{C}$  NMR signal at  $\delta$  183.7. The IR absorption at  $\nu_{\text{max}}$   $1268\text{ cm}^{-1}$  indicated the presence of N-oxide [16]. This was supported by the ion peak at  $m/z$  350  $[\text{M} + \text{H}]^+$  in the ESI-MS spectrum of the product yielded by the reduction of **1** [17,18]. 4-Methoxyphenyl is located at C-1 on the basis of the HMBC correlations of H-2' and H-6' with C-1 ( $\delta$  108.4) and the NOESY correlation between OMe-8 and H-3'. Thus, ring A was concluded to be as shown in figure 1.

Based on the above evidence, the structure of gaultherialine A could be determined as 7,8-dimethoxy-1-(4-methoxyphenyl)-4,5-dihydro-2H-benzo[e]indole-2-one N-oxide (**1**).

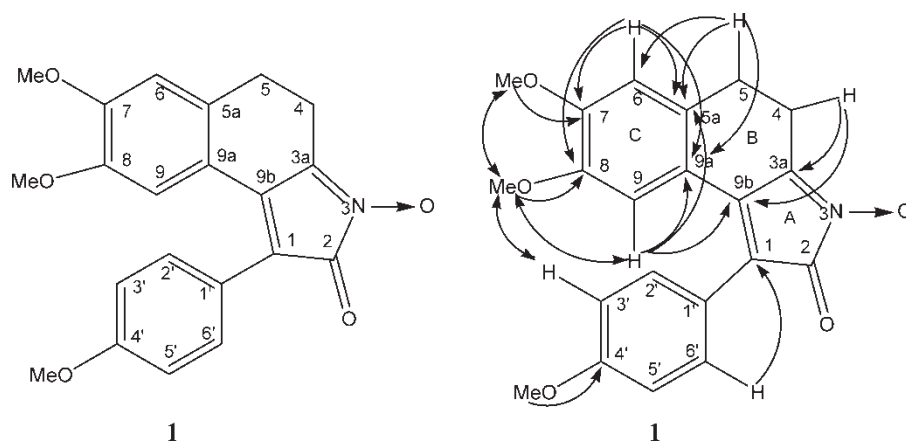


Figure 1. Structure and the major HMBC ( $\rightarrow$ ) and NOESY ( $\leftrightarrow$ ) correlations for gaultherialine A (**1**).

### 3. Experimental

#### 3.1 General experimental procedures

Melting points were determined on an XRC-1 micro-melting point apparatus and are uncorrected. IR and UV spectra were recorded on a Spectrum One FT-IR spectrometer and a Lambda 35 spectrometer, respectively. MS spectra were obtained on a Finnigan LCQ<sup>DECA</sup> ion trap mass spectrometer (ESI) and API Q-STAR PULSAR *i* Mass Spectrometer (HRESI). NMR spectra were recorded on an Advance 600 spectrometer, with TMS as internal reference. HPLC analyses were performed on a Perkin-Elmer Series 2000 with vacuo degas and UV detector using an RP-18 column (5  $\mu$ , 4 mm  $\times$  250 mm) (Merck Co. Ltd.). Silica gel H (160–200, 200–300 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China) and silica gel 60 (Merck, 230–400 mesh) were used for column chromatography. Organic solvents were distilled prior to use.

#### 3.2 Plant material

Whole plants of *Gaultheria nummularioides* D. Don were collected in August 2001 from Tianquan County, Sichuan Province of China, and identified by Professor Zuo-Cheng Zhao in Chengdu Institute of Biology, the Chinese Academy of Sciences (CAS). A voucher specimen (no. GN-1) was deposited at the Herbarium of Chengdu Institute of Biology, CAS.

#### 3.3 Extraction and isolation

Air-dried, powdered whole plants (3.6 kg) were percolated with 90% EtOH (20 L  $\times$  7 days  $\times$  3) at room temperature. The resultant solution was concentrated *in vacuo* to provide 380 g of crude residue. This residue was suspended in water and partitioned with CHCl<sub>3</sub>, EtOAc, n-BuOH (2 L each) to afford the corresponding fractions A (98 g), B (76 g) and C (59 g). Fraction A was subjected to silica-gel column (1 kg, 80–160 mesh, 10  $\times$  50 cm) using light petroleum–EtOAc (100:1, 3 L; 60:1, 3 L; 40:1, 2 L; 20:1, 2 L; 5:1, 2 L) as eluent to give subfractions 1–5. Subfraction 5 was subjected to silica-gel column (600 g, 160–200 mesh, 8  $\times$  50 cm), eluting with light petroleum–acetone (20:1). The eluates of 800–1300 and 1900–2100 ml were concentrated to give 480 mg and 320 mg mixtures, respectively. The later was separated on a silica gel 60 column (40 g, 3  $\times$  15 cm), eluted with light petroleum–acetone (12:1), to yield **1** (6 mg, 200–350 ml). The former eluate was separated on a silica gel 60 column (60 g, 4  $\times$  20 cm) to give **2** (9 mg), **3** (10 mg) and **4** (8 mg). By rechromatography on silica gel column, **5** (10 mg), **6** (30 mg) and **7** (28 mg) were obtained from subfraction 1; **8** (8.9 g), **9** (15 mg), **10** (12 mg) from subfraction 2; and **11** (130 mg), **12** (68 mg), **13** (45 mg), **14** (39 mg), **15** (90 mg), **16** (43 mg), **17** (56 mg), **18** (32 mg) from subfraction 3. Fraction B was subjected to chromatography on a silica gel column to yield **19** (38 mg), **20** (26 mg), **21** (56 mg), **22** (89 mg), **23** (32 mg), **24** (60 mg), **25** (120 mg), **26** (98 mg), **27** (75 mg), and **28** (2.6 g).

**3.3.1 Gaultherialine A (1).** A red amorphous powder, mp 170–172°C; UV (CHCl<sub>3</sub>)  $\lambda_{\max}$  (nm) (log  $\epsilon$ ): 514 (3.73), 464 (3.76), 446 (3.75), 380 (3.96), 314 (4.03), 268 (3.71); IR (KBr)  $\nu_{\max}$  (cm<sup>-1</sup>): 2924, 1742, 1697, 1581, 1498, 1428, 1288, 1261, 1248, 1172, 1103, 1033, 983,

789;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  (ppm): 3.84 (2H, t,  $J = 6.0$  Hz, H-4), 3.07 (2H, t,  $J = 6.0$  Hz, H-5), 6.76 (1H, s, H-6), 7.05 (1H, s, H-9), 7.28 (2H, d,  $J = 8.4$  Hz, H-2',6'), 6.96 (2H, d,  $J = 8.4$  Hz, H-3',5'), 3.82 (3H, s, OMe-4'), 3.96 (3H, s, OMe-7), 3.40 (3H, s, OMe-8);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 150 MHz)  $\delta$ (ppm): 108.4 (C, C-1), 183.7 (C, C-2), 158.5 (C, C-3a), 36.5 (CH<sub>2</sub>, C-4), 28.8 (CH<sub>2</sub>, C-5), 117.1 (C, C-5a), 111.4 (CH, C-6), 153.4 (C, C-7), 148.0 (C, C-8), 112.2 (CH, C-9), 133.0 (C, C-9a), 157.0 (C, C-9b), 122.6 (C, C-1'), 131.6 (CH, C-2',6'), 114.6 (CH, C-3',5'), 159.6 (C, C-4'), 55.6 (CH<sub>3</sub>, OCH<sub>3</sub>-4'), 56.4 (CH<sub>3</sub>, OCH<sub>3</sub>-7), 55.6 (CH<sub>3</sub>, OCH<sub>3</sub>-8); ESIMS  $m/z$  366.3  $[\text{M} + 1]^+$  (positive mode), 364.1  $[\text{M} - 1]^-$  (negative mode); HR-ESIMS (positive mode)  $m/z$  366.1339  $[\text{M} + \text{H}]^+$  (calcd. for  $\text{C}_{21}\text{H}_{20}\text{NO}_5$ , 366.1341).

**3.3.2 Reduction of compound 1.** Reduction was carried out according to the literature procedure [17]. Compound **1** (2 mg) was dissolved in 1 M HCl (3 ml) and Zn dust (5 mg) was added to the solution. The reaction mixture was filtered after the mixture had been continuously stirred for 12 h. Concentrated aqueous  $\text{NH}_3$  (1 ml) was added into the filtrate. The free alkaloid was then extracted with EtOAc and further purified by Si 60 column chromatography to yield a red powder (0.5 mg). ESIMS  $m/z$  350.2  $[\text{M} + 1]^+$  (positive mode);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  (ppm): 3.78 (2H, t,  $J = 6.2$  Hz, H-4), 3.12 (2H, t,  $J = 6.2$  Hz, H-5), 6.83 (1H, s, H-6), 7.01 (1H, s, H-9), 7.25 (2H, d,  $J = 8.4$  Hz, H-2',6'), 6.98 (2H, d,  $J = 8.4$  Hz, H-3',5'), 3.86 (3H, s, OMe-4'), 3.96 (3H, s, OMe-7), 3.51 (3H, s, OMe-8).

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