

**GAULTHRINS C AND D, TWO NEW LIGNANS FROM THE  
ROOTS OF *GAULTHERIA YUNNANENSIS***

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**Abstract**—Two new lignans, designated as gaultherins C (**1**) and D (**2**), were isolated from roots of *Gaultheria yunnanensis*. Their structures were determined as 1,3,3 $\alpha$ ,4 $\alpha$ ,9,9 $\alpha$ -hexahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-5,7-dimethoxy-naphtho[2,3-*c*]furan-6-ol (**1**) and 1,3,3 $\alpha$ ,4 $\alpha$ ,9,9 $\alpha$ -hexahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-7-methoxynaphtho[2,3-*c*]furan-6-ol (**2**) based on the physico-chemical properties and the extensive NMR (DEPT, DQF-COSY, HETCOR, phase-sensitive NOESY, and HMBC experiments) studies.

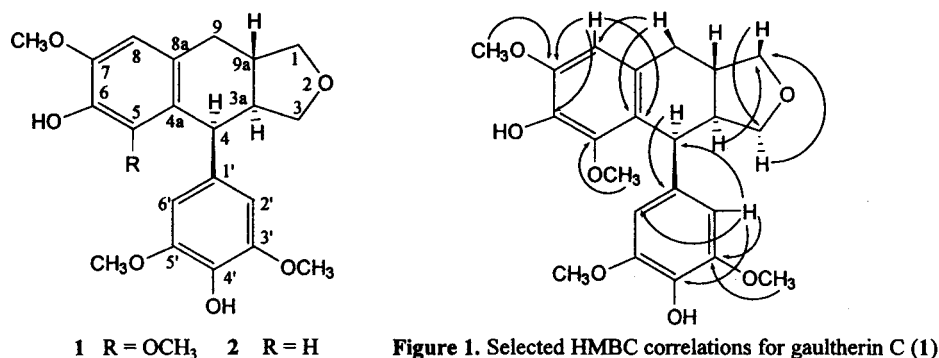
*Gaultheria yunnanensis* (Franch.) Rehd. (Ericaceae) is an herbal medicine widely used in southwest regions of the People's Republic of China for treatments of different disease. Its volatile oil, called "wintergreen oil", possessed antipyretic and analgesic effects. In previous contributions,<sup>1,2</sup> we reported two new diterpenoids, gaultheric acid and gaultheronoterpene, as well as two new lignans, gaultherins A and B. A further investigation on the CHCl<sub>3</sub> fraction furnished two additional new lignans, named

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gaultherins C (**1**) and D (**2**). In present note, we described the isolation and structural elucidation of (**1**) by means of extensive NMR studies, including DEPT, DQF-COSY, HETCOR, phase-sensitive NOESY, and HMBC experiments.



**Figure 1.** Selected HMBC correlations for gaultherin C (**1**)

The first new compound, gaultherin C (**1**), was obtained as colorless prisms, mp 185-186°C. EIMS displayed a molecular ion peak at  $m/z$  402, coupled with the  $^{13}\text{C}$  NMR information and the element analysis, suggesting its molecular formula of  $\text{C}_{22}\text{H}_{26}\text{O}_7$ . The IR spectrum exhibited characteristic absorption of hydroxyl group at  $3400\text{ cm}^{-1}$  and aromatic nucleus at  $1605$  and  $1513\text{ cm}^{-1}$ . The  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data (Table 1) of **1** were characteristic of an aryltetralin type lignan with four methoxy groups. The  $^{13}\text{C}$  NMR spectrum revealed 22 carbon signals, which were assigned by DEPT spectrum as four methoxy, one methylene, two alcoholic methylene, three methine, three olefinic methine and nine olefinic quaternary carbons. The proposed structure and unambiguous assignments for the  $^{13}\text{C}$  and  $^1\text{H}$  NMR signals were established using DQF-COSY, HETCOR and, especially, HMBC experiments. The four methoxy groups attached to C-5, C-7, C-3', and C-5' were indicated by HMBC relationships as shown in Figure 1. As observed in the HMBC spectrum, C-H relationships of  $1\beta\text{-H}$  ( $\delta$  4.14) with C-3 ( $\delta$  72.6) and  $3\alpha\text{-H}$  ( $\delta$  3.88) with C-1 ( $\delta$  72.8) confirmed the presence of a pentacycle. The stereochemistry of compound (**1**) was established by a combination of CD spectrum, 2D NOESY experiment and molecular modeling. The absolute configuration at C-4 position of compound (**1**) and the aryltetralin type lignan could be determined by examining of their CD spectra.<sup>3-5</sup> The signals of **1** in the CD spectrum indicated the absolute configuration of the aryl substituent at C-4 is *S*, since the sign of the first couplet reflects the aryl

substituents at C-4, namely negative for 4S and positive for 4R.<sup>4, 5</sup> As depicted in Figure 2, the NOEs of 9a-H ( $\delta$  2.10) with 9 $\beta$ -H ( $\delta$  2.89), 1  $\beta$ -H ( $\delta$  4.14) and 3  $\beta$ -H ( $\delta$  3.56) observed in the phase-sensitive NOESY spectrum demonstrated the 9a-H was  $\beta$ -configuration. While the spatial orientation of 3a-H was indicated by the NOEs of 3a-H ( $\delta$  2.03) with 9 $\alpha$ -H ( $\delta$  2.69), 1 $\alpha$ -H ( $\delta$  3.47), 3 $\alpha$ -H ( $\delta$  3.88), and 4 $\alpha$ -H ( $\delta$  3.84). The conformation deduced above of **1** was also confirmed by molecular mechanic and dynamic calculations (data not shown). On the basis of above evidence, compound (**1**) was determined as **1**, **3**, **3a $\alpha$ , **4a, **9**, **9a******

Table 1 <sup>13</sup>C and <sup>1</sup>H NMR Data for Compounds (**1**) and (**2**) (125 and 500MHz in CDCl<sub>3</sub>)<sup>a</sup>

| C      | <b>1</b> | DEPT            | <b>2</b> | DEPT            | H             | <b>1</b>              | <b>2</b>              |
|--------|----------|-----------------|----------|-----------------|---------------|-----------------------|-----------------------|
| 1      | 72.8     | CH <sub>2</sub> | 73.5     | CH <sub>2</sub> | 1 $\alpha$ -H | 3.47 (1H, dd,         | 3.42 (1H, dd,         |
| 3      | 72.6     | CH <sub>2</sub> | 72.6     | CH <sub>2</sub> |               | $J = 9.9, 7.7$ Hz),   | $J = 10.1, 7.7$ Hz),  |
| 3a     | 53.1     | CH              | 50.6     | CH              | 1 $\beta$ -H  | 4.14 (1H, m)          | 4.07 (1H, m)          |
| 4      | 46.8     | CH              | 51.1     | CH              | 3 $\alpha$ -H | 3.88 (1H, m)          | 3.82 (1H, m)          |
| 4a     | 125.9    | C               | 127.9    | C               | 3 $\beta$ -H  | 3.56 (1H, dd,         | 3.45 (1H, dd,         |
| 5      | 146.0    | C               | 116.5    | CH              |               | $J = 9.9, 7.7$ Hz),   | $J = 10.1, 7.6$ Hz),  |
| 6      | 137.3    | C               | 146.9    | C               | 3a-H          | 2.03 (1H, m)          | 2.01 (1H, m)          |
| 7      | 146.5    | C               | 145.5    | C               | 4 $\alpha$ -H | 3.84 (1H, d,          | 3.66 (1H, d,          |
| 8      | 106.7    | CH              | 112.8    | CH              |               | $J = 8.4$ Hz)         | $J = 7.6$ Hz)         |
| 8a     | 128.5    | C               | 133.6    | C               | 5-H           |                       | 6.26 (1H, s)          |
| 9      | 33.4     | CH <sub>2</sub> | 32.8     | CH <sub>2</sub> | 8-H           | 6.46 (1H, s)          | 6.70 (1H, s)          |
| 9a     | 41.8     | CH              | 43.3     | CH              | 9 $\alpha$ -H | 2.69 (1H, dd,         | 2.65 (1H, dd,         |
| 1'     | 139.3    | C               | 136.3    | C               |               | $J = 15.0, 11.3$ Hz), | $J = 15.3, 11.6$ Hz), |
| 2'     | 105.4    | CH              | 106.7    | CH              | 9 $\beta$ -H  | 2.89 (1H, dd,         | 2.94 (1H, dd,         |
| 3'     | 147.0    | C               | 148.8    | C               |               | $J = 15.0, 4.0$ Hz)   | $J = 15.3, 4.5$ Hz)   |
| 4'     | 132.9    | C               | 135.5    | C               | 9a-H          | 2.10 (1H, m)          | 2.21 (1H, m)          |
| 5'     | 147.0    | C               | 148.8    | C               | 2'-H          | 6.30 (1H, s)          | 6.47 (1H, s)          |
| 6'     | 105.4    | CH              | 106.7    | CH              | 6'-H          | 6.30 (1H, s)          | 6.47 (1H, s)          |
| 5-OMe  | 59.1     | CH <sub>3</sub> |          |                 | 5-OMe         | 3.21 (3H, s)          |                       |
| 7-OMe  | 56.1     | CH <sub>3</sub> | 56.1     | CH <sub>3</sub> | 7-OMe         | 3.89 (3H, s)          | 3.78 (3H, s)          |
| 3'-OMe | 56.6     | CH <sub>3</sub> | 56.6     | CH <sub>3</sub> | 3'-OMe        | 3.81 (3H, s)          | 3.76 (3H, s)          |
| 5'-OMe | 56.6     | CH <sub>3</sub> | 56.6     | CH <sub>3</sub> | 5'-OMe        | 3.81 (3H, s)          | 3.76 (3H, s)          |

<sup>a</sup> The assignments are based upon DEPT, COSY, HETCOR, NOESY, and HMBC experiments.

$\beta$ -hexahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-5,7dimethoxynaphtho[2,3-*c*]furan-6-ol.

The second compound, gaultherin D (**2**), shown an EIMS molecular ion peak at  $m/z$  372, 30 mass units lower than that of **1**. This, combined with its NMR data, suggested compound (**2**) was a derivative of **1**

with one less methoxy group. A detailed NMR spectral comparison for compounds (**1**) and (**2**) indicated compound (**2**) was lack of the methoxy group at C-5 position. The absolute configuration of compound (**2**) was also established by a combination of CD spectrum and 2D NOESY experiment. The characteristic of CD spectrum of **2** was superimposable on that of **1**, indicating that compounds (**1**) and (**2**) possessed the same configuration of the aryl substituent at C-4. The orientation of 9 $\alpha$ -H and 3 $\alpha$ -H was respectively indicated by the NOEs of 9 $\alpha$ -H ( $\delta$  2.21) with 9 $\beta$ -H ( $\delta$  2.94), 1 $\beta$ -H ( $\delta$  4.07), 3 $\beta$ -H ( $\delta$  3.45), and by the NOEs of 3 $\alpha$ -H ( $\delta$  2.01) with 9 $\alpha$ -H ( $\delta$  2.65), 1 $\alpha$ -H ( $\delta$  3.42), 3 $\alpha$ -H ( $\delta$  3.82), 4 $\alpha$ -H ( $\delta$  3.66) observed in the phase-sensitive NOESY spectrum. Consequently; the structure of **2** was elucidated as 1,3,3 $\alpha$ ,4 $\alpha$ ,9,9 $\alpha$ -hexahydro-4-(4-hydroxy-3,5-dimethoxy phenyl)-7-methoxynaphtho[2,3-*c*]furan-6-ol.

## EXPERIMENTAL

Melting points were uncorrected. Optical rotations were performed with a JASCO DIP-370 digital polarimeter. CD and UV spectra were measured by JASCO-720W and UV-260 spectrophotometer in MeOH respectively. IR spectra were run at a JASCO D-300 FTIR spectrophotometer. EIMS was conducted using AEI-MS-50 mass spectrometer. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR measurements were carried out at 500 MHz using an standard JEOL sequences for 1D and 2D NMR measurement in  $\text{CDCl}_3$  solution and chemical shifts were expressed in  $\delta$  (ppm) with reference to TMS. HMBC experiment was run with the delay time set at 100 ms. Phase-sensitive NOESY spectra were recorded with the mixing time set at 600 ms. Molecular mechanics and dynamics calculations were performed using Discover/INSIGHT II 97 (force field, CVFF) on a Silica graphics 02 R 1000 workstation (MSI). The roots of *G. yunnanensis* were collected from Guiyang, Guizhou Province, the People's Republic of China, and were identified by Professor Junhua Zheng. A voucher specimen was deposited in the Division for Pharmacognostical Biotechnology; School of Pharmaceutical Sciences, Beijing Medical University.

## Extraction and isolation

The powdered roots (5 kg) were refluxed with 95% EtOH (25 L) three times for 3 h at 60 °C. The combined solutions were evaporated to yield an extract (500 g), which was suspended in water, and then

partitioned successively with petroleum ether,  $\text{CHCl}_3$ , EtOAc, and BuOH. The  $\text{CHCl}_3$  fraction (30.5 g) was applied to a column of silica gel eluting with  $\text{CHCl}_3$ -EtOAc mixtures of increasing polarity to furnish parts A (8.2 g), B (1.2 g), C (5.6 g), D (10.8 g), and E (2.6 g). The part B was repeatedly chromatographed over polyamide column eluting with acetone-water (1 :3) to afford **1** (15 mg) and **2** (7 mg).

### Gaultherin C (**1**)

A colorless prism from MeOH, mp 185 -186 °C,  $[\alpha]_{\text{D}}^{21} +56^\circ$  ( $c$  0.025, MeOH); *Anal.* Calcd for  $\text{C}_{22}\text{H}_{26}\text{O}_7$ : C, 65.66; H, 6.51. Found: C, 65.68; H, 6.62. UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 219 (2.21), 276 (0.26); IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3400, 2930, 2834, 1605, 1513, 1424, 1308, 1264, 1047; CD ( $c$  0.0015 MeOH)  $[\theta]^{21}$  (nm): +8835 (245), +4134 (273), -1422 (286); EIMS  $m/z$  402 ( $\text{M}^+$ , 100%), 371, 301, 248, 203, 167; for  $^{13}\text{C}$  and  $^1\text{H}$  NMR data are shown in Table 1.

### Gaultherin D (**2**)

A colorless prisms from MeOH, mp 196-197 °C,  $[\alpha]_{\text{D}}^{21} +55^\circ$  ( $c$  0.04, MeOH); *Anal.* Calcd for  $\text{C}_{21}\text{H}_{24}\text{O}_6$ : C, 67.73; H, 6.50. Found: C, 67.63; H, 6.60. UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 218 (2.18), 278 (0.30); IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3400, 2931, 2835, 1606, 1512, 1423, 1308, 1265, 1047; CD ( $c$  0.0017 MeOH)  $[\theta]^{21}$ (nm): +9690 (245), +4655 (273), -1329 (286); EIMS:  $m/z$  372 ( $\text{M}^+$ , 100%), 341, 271, 218, 203, 167; for  $^{13}\text{C}$  and  $^1\text{H}$  NMR data are given in Table 1.

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