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# BALANOINVOLIN, A NEW STEROID DERIVATIVE FROM *Balanophora involucrate*

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*A new compound,* β-*sitosterylglucoside-3′*-O-*linoleate, named balanoinvolin, and three known compounds coniferin, methylconiferin, and 4*-O-β-*D-glucopyranosylconiferyl aldehyde, were isolated from* Balanophora involucrate *Hook. f. and their structures were determined by MS and 1D/2D NMR spectra.*

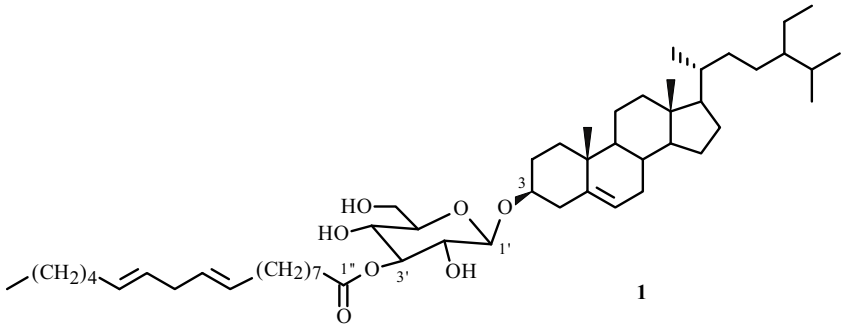
**Key words**: *Balanophora involucrate* Hook. f., balanoinvolin, *β*-sitosterylglucoside-3′-*O*-linoleate, coniferin, methylconiferin, 4-*O*-*β*-D-glucopyranosylconiferyl aldehyde.

*Balanophora involucrate* Hook. f. belongs to the genus *Balanophora* of the family Balanophoraceae. It is a dicotyledonous, perennial, and parasitic plant, and grows widely in Yunnan, Hubei, Sichuan, and Tibet of China. The whole plant is medicinally used as an antidote and hemostatic agent in China [1].

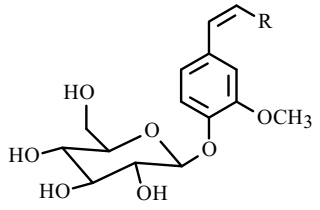
In Chinese folk medicine, the whole herb of *Balanophora involucrate* Hook. f. has been used for treatment of stomachache, asthma, irregular menstruation, injuries from falls, and traumatic bleeding symptoms [2]. In the course of our continuing search for new biologically natural compounds, we isolated a new compound, balanoinvolin. Herein we report the isolation and structural elucidation of a new steroid derivative, named balanoinvolin (**1**), together with three known compounds coniferin (**2**) [3], methylconiferin (**3**) [4], and 4-*O*-*β*-D-glucopyranosylconiferyl aldehyde (**4**) [4] from *Balanophora involucrate* from the Shennongjia Forest District of Hubei Province, a National Natural Protection District.

The ethanolic extract of dried *Balanophora involucrate* was partitioned between petroleum-ether, ethyl acetate, *n*-butanol, and water. The ethyl acetate–soluble fraction was subjected to repeated silica gel and Sephadex LH-20 column chromatography purification, affording four compounds **1**–**4**.

Balanoinvolin (**1**) was obtained as a colorless oil whose molecular formula $C_{53}H_{90}O_7$ was determined by HR-ESI-MS at *m/z* 837.2770 (calcd 837.2775) along with the analysis of NMR data. The UV and IR spectra displayed the characteristic absorption of the steroid derivative. Similarly, the $^1H$ and $^{13}C$ NMR spectra showed the presence of *β*-sitosterylglucoside and unsaturated fatty acid. After the base hydrolysis procedure, compounds **1a** and **1b** were obtained. Compound **1a** was consistent with the reference *β*-sitosterylglucoside on TLC, and the NMR data of compound **1a** conformed with the results of TLC.




**1**




**2 - 4**

**2:** R = $CH_2OH$; **3:** R = $CH_2OCH_3$; **4:** R = CHO

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TABLE 1. The NMR Data for Compound **1** ($^1$H 500 MHz and $^{13}$C 125 MHz, $CDCl_3$, J/Hz)

| C atom | $\delta_C$ | $\delta_H$ | C atom | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| 1 | 37.3 | 1.52 (m) | 26 | 18.8 | 0.81 (d, J = 3.0) |
| 2 | 31.9 | 2.00 (m) | 27 | 19.8 | 0.83 (d, J = 7.0) |
| 3 | 70.2 | 4.35 (m) | 28 | 23.1 | 1.6 (m) |
| 4 | 42.3 | 2.23 (m) | 29 | 11.9 | 0.86 (t, J = 3.0) |
| 5 | 140.3 | – | 1′ | 101.2 | 4.40 (d, J = 7.65) |
| 6 | 122.2 | 5.36 (m) | 2′ | 76.0 | 3.55 (m) |
| 7 | 32.0 | 2.10 (m) | 3′ | 73.6 | 3.50 (m) |
| 8 | 32.0 | 1.70 (m) | 4′ | 73.9 | 3.50 (m) |
| 9 | 50.2 | 1.60 (m) | 5′ | 79.6 | 3.55 (m) |
| 10 | 36.7 | – | 6′ | 63.3 | 3.45 (m) |
| 11 | 21.1 | 1.95 (m) | 1″ | 174.6 | – |
| 12 | 39.8 | 1.08 (m) | 2″ | 34.3 | 2.38 (m) |
| 13 | 42.3 | – | 3″ | 25.0 | 2.30 (m) |
| 14 | 56.8 | 1.95 (m) | 4″–7″ | 29.7 | 1.26 (m) |
| 15 | 24.3 | 1.60 (m) | 8″ | 30.1 | 1.55 (m) |
| 16 | 28.3 | 1.70 (m) | 9″ | 129.7 | 5.38 (m) |
| 17 | 56.1 | 2.11 (m) | 10″ | 130.0 | 5.37 (m) |
| 18 | 12.0 | 0.68 (s) | 11″ | 37.1 | 1.60 (m) |
| 19 | 19.4 | 1.01 (s) | 12″ | 129.7 | 5.38 (m) |
| 20 | 36.2 | 2.09 (m) | 13″ | 130.3 | 5.37 (m) |
| 21 | 19.0 | 0.92 (d, J = 6.5) | 14″ | 30.1 | 1.60 (m) |
| 22 | 34.0 | 1.09 (m) | 15″–16″ | 29.7 | 1.26 (m) |
| 23 | 26.1 | 2.08 (m) | 17″ | 22.7 | 1.60 (m) |
| 24 | 45.8 | 2.05 (m) | 18″ | 14.1 | 0.88 (t, J = 3.0) |
| 25 | 29.7 | 1.64 (m) | | | |

Compound **1b** was determined as linoleic acid by GC-MS. Furthermore, in the HMQC and HMBC spectra of compound **1**, the key correlations, from H-3 (δ 4.35 ppm) to C-1′ (δ 101.2 ppm) and from H-1′ (δ 4.40 ppm) to C-3 (δ 70.2 ppm), indicated the linkage between the glycoside moiety and the *β*-sitosterol moiety at the 3-OH position of the steroid by an ether bond. The correlation, from H-3′ (δ 3.49 ppm) to C-1″ (δ 174.6 ppm), showed the linoleic acid unit linking at the 3′-position of the glycoside moiety. Comparing the $^{13}$C NMR spectra of compounds **1** and **1a**, the chemical shift of C-3′ was 4.3 ppm upfield, but the chemical shifts of C-2′ and C-4′ were 1.2 and 1.6 ppm downfield, respectively. The above results further confirmed the linoleic acid linkage to the 3′-position of glycoside. Therefore, compound **1** was determined as *β*-sitosterylglucoside-3′-*O*-linoleate, a new compound.

Compounds **2**, **3**, **4** were obtained as white powders. Their NMR data were similar, so they were maybe analogs. Primary NMR data analysis showed that compound **2** may be coniferin. Comparing the NMR data between coniferin and compound **2**, the results showed that compound **2** was just coniferin. Similarly, compounds **3** and **4** were determined as methylconiferin and 4-*O*-*β*-D-glucopyranosylconiferyl aldehyde, respectively, by comparing the NMR data of methylconiferin and 4-*O*-*β*-D-glucopyranosylconiferyl aldehyde to compounds **3** and **4**. Thus, compounds **2–4** were determined as coniferin, methylconiferin, and 4-*O*-*β*-D-glucopyranosylconiferyl aldehyde, respectively.

## EXPERIMENTAL

Column chromatography was carried out using silica gel (Qingdao Ocean Chemical Company, 200–300 mesh). TLC was performed with precoated silica gel GF-25-UV 254 plates, and detection was done at 254 nm or by spraying with ceric sulfate in 10% $H_2SO_4$. Mass spectra (HRESI-MS and ESI-MS) were measured in an electron spraying mode using a Finnigan-MAT LCQ DECA XP plus mass spectrometer, respectively, and ions are given in *m/z*. The $^1$H, $^{13}$C, and 2D NMR spectra were recorded on a Bruker AM-400 spectrometer in $CDCl_3$ and $CD_3OD$. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard, and scalar coupling is reported in hertz.

Analysis of the linoleic acid was performed on Finnigan GC-MS in the electronic ionization mode, and helium (99.9999%) was used as carrier gas with flow rate of 1.2 mL/min. The GC separation was performed using a Rt×25 MS capillary column (15 m × 0. 25 mm × 0. 25 μm) obtained from the USA. The following temperature program was maintained: 70° for 1 min, 4°/min from 70° to 200°, and maintained for 6 min, then 15°/min from 200° to 280°. The injections were performed in the splitless mode at 280°. The mass range of scan was 60–360 Da.

**Plant Material**. *Balanophora involucrate* Hook. f. was collected from Muyu town of the Shennongjia Forest District in July 2005 and identified by Dr. Fa-Ju Chen. A voucher specimen was deposited at the Hubei Key Laboratory of Natural Products Research and Development, China Three Gorges University, Yichang, P. R. China.

**Extraction and Isolation**. The air-dried whole plant (1.8 kg) was exhaustively extracted with 95% ethanol (5 L × 5) on refluxing at 60°C. The extract was evaporated in vacuo to yield a residue and freeze-dried, affording a powder, which was divided into petroleum-ether (64.3 g), ethyl acetate (289.1 g), *n*-butanol (197.6 g), and water-soluble fractions. The ethyl acetate–soluble extract (100 g out of 289.1 g) was chromatographed with gradient elution (100% petroleum-ether→100% ethyl acetate→100% methanol). Repeated silica gel column and Sephadex LH-20 chromatography afforded compounds **1**–**4**, **1** (20 mg), **2** (500 mg), **3** (30 mg), **4** (15 mg).

**Base Hydrolysis**. Ten mg of **1** was dissolved in a mixture of methanol and tetrahydrofuran at 1:1 volume ratio, and a 2 N NaOH solution was added to the mixture, which was stirred at 50° for 2 hours. After the base hydrolysis was finished, 10 mL of water was added, the precipitation was filtrated and recrystallizated with $CHCl_3$ and methanol (2:1), and compound **1a** was obtained. The filtrate was acidified by adding 2 N HCl unless the value of pH was less than 7, extracted with $CHCl_3$ three times, evaporated in vacuo, and dissolved in 1 mL $CHCl_3$ to test for GC-MS.

**Compound 1**, coloreess oil. HR-ESI-MS at *m/z* 837.2770 (calcd 837.2775). The NMR data, see Table 1.

**Compound 2**, white powder, mp 149–151°C. $^1$H NMR ($CD_3OD$, 400 MHz, δ, ppm, J/Hz): 3.42–3.90 (6H, m), 3.89 (3H, s), 4.22 (2H, d, J = 5.5), 4.90 (1H, d, J = 7.2), 6.28 (1H, dt, J = 15.9, 5.5), 6.56 (1H, d, J = 15.9), 6.96 (1H, dd, J = 8.4, 1.4), 7.08 (1H, d, J = 1.4), 7.12 (1H, d, J = 8.4). $^{13}$C NMR (MeOD, 100 MHz, δ, ppm): 133.8 (C-1), 111.5 (C-2), 150.9 (C-3), 147.7 (C-4), 118.0 (C-5), 120.8 (C-6), 131.3 (C-7), 128.9 (C-8), 63.8 (C-9), 56.8 ($OCH_3$), 102.8 (C-1′), 74.9 (C-2′), 77.9 (C-3′), 71.4 (C-4′), 78.2 (C-5′), 62.6 (C-6′).

**Compound 3**, white powder, mp 193–195°C. $^1$H NMR ($CD_3OD$, 400 MHz, δ, ppm, J/Hz): 3.20–3.90 (6H, m), 3.30 (3H, s), 3.83 (3H, s), 4.17 (2H, d, J = 5.2), 4.8 (1H, d, J = 7.3), 6.24 (1H, dt, J = 14.6, 5.2), 6.49 (1H, d, J = 14.6), 6.92 (1H, dd, J = 8.4, 1.2), 7.02 (1H, d, J = 1.2), 7.05 (1H, d, J = 8.4). $^{13}$C NMR ($CD_3OD$, 100 MHz, δ, ppm): 134.0 (C-1), 111.7 (C-2), 150.9 (C-3), 147.7 (C-4), 117.9 (C-5), 121.3 (C-6), 131.7 (C-7), 129.3 (C-8), 64.1 (C-9), 50.3 (C-10), 57.2 ($OCH_3$), 102.9 (C-1′), 75.1 (C-2′), 77.9 (C-3′), 71.6 (C-4′), 78.4 (C-5′), 62.7 (C-6′).

**Compound 4**, white powder, mp 212–213°C. $^1$H NMR (DMSO-$d_6$, 400 MHz, δ, ppm, J/Hz): 3.20–3.90 (6H, m), 3.78 (3H, s), 4.98 (1H, d, J = 6.8), 6.69 (1H, dd, J = 16.0, 8.0), 7.11 (1H, d, J = 8.4), 7.25 (1H, d, J = 8.4), 7.30 (1H, s), 7.63 (1H, d, J = 16.0), 9.50 (1H, d, J = 8.0). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, δ, ppm): 129.4 (C-1), 112.6 (C-2), 155.5 (C-3), 149.9 (C-4), 115.0 (C-5), 116.2 (C-6), 127.8 (C-7), 124.5 (C-8), 197.1 (C-9), 56.9 ($OCH_3$), 100.5 (C-1′), 73.9 (C-2′), 77.0 (C-3′), 70.5 (C-4′), 77.5 (C-5′), 61.6 (C-6′).

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