A New Stigmasterol Ester from *Aeschynomene indica*

CHEN Jia-yuan, TAN Xiao, LU Wen-jie, YA Qi-kang*

Guangxi Institute of Traditional Medical and Pharmaceutical Science, Nanning 530022, China

Abstract: **Objective** To study the chemical constituents of *Aeschynomene indica*. **Methods** The constituents were isolated and purified by means of silica gel column chromatography and recrytallization, and the structures were elucidated by physicochemical properties and spectral analyses. **Results** Twelve compounds were obtained and elucidated as stigmasterol tritriacontanate (**1**), monotetracontane (**2**), taraxerol (**3**), stigmasterol (**4**), stearic acid (**5**), heptatriacontanoic acid (**6**), arachidic acid (**7**), ursolic acid acetate (**8**), quercetin (**9**), myricetin (**10**), myricetin-3-*O*-rhamnoside (**11**), and rutoside (**12**). **Conclusion** All the compounds are isolated from this plant for the first time and compound **1** is a new one.

Key words: *Aeschynomene indica*; myricetin; quercetin; stigmasterol; stigmasterol tritriacontanate
DOI: 10.3969/j.issn.1674-6384.2011.04.003

Introduction

Aeschynomene indica Linn. is one of the most important crude drugs in traditional Chinese medicine (TCM), distributing in Southwest, central South, East China, North China in China. It was used to treat cold, fever, urinary tract infection, dysuria, hematuria, edema, diarrhea, cholecystitis, night blindness, cataracts, eczema, itching and other skin diseases. In the present paper, we described the isolation and structure elucidation of one new compound, named stigmasterol tritriacontanate, and eleven known compounds as well.

Materials and methods

General experimental procedures

Melting points were determined on a Chinese X—4 melting point apparatus. IR spectra were recorded on a Nicolet 4700 FT-IR Spectrometer. ^{1}H-NMR, ^{13}C-NMR, and 2D NMR spectra were taken on a Bruker Am—600 MHz Spectrometer. EI-MS and HR-MS were obtained on Agilent 1100 LC-MSD Trapsl and Waters Autospec Premier P776 Spectrometers, respectively.

Plant material

The dry leaves of *Aeschynomene indica* Linn. were collected from Lipu (Guangxi, China) in August 2009, and identified by Prof. HE Kai-jia, Guangxi Institute of Traditional Medical and Pharmaceutical Sciences. A voucher specimen (34-18) is deposited at Guangxi Institute of Traditional Medical and Pharmaceutical Sciences.

Extraction and isolation

The dried plants of *A. indica* (5 kg) were extracted with 90% ethanol for four times, 2 h each time. The combined ethanol extracts were evaporated under reduced pressure. A suspension of this crude extract in distilled water was partitioned into petroleum ether, $CHCl_3$, and EtOAc. Removal of the solvent from each phase gave the petroleum ether (90 g), $CHCl_3$ (20.5 g), EtOAc (24 g), and water-soluble extracts. The petroleum ether extract was chromatographed over a silica gel, eluting with a step gradient of petroleum ether-EtOAc (100:0→80:20) to yield 65 fractions which were recrystallizated to afford compounds **1** (1387.7 mg), **2** (3786.9 mg), **3** (901.4 mg), **4** (1021 mg), and **5** (1560 mg). The $CHCl_3$ extract combined with EtOAc extract was subjected to silica gel CC, eluting with a step gradient of $CHCl_3$-MeOH (100:0→80:20) and recrystallization repeatedly to yield compounds **6** (330.4 mg), **7** (147.7 mg), and **8** (155.7 mg). The water-soluble extract was chromatographed over polyamide, eluting with H_2O, 30% EtOH, 50% EtOH, and 70% EtOH. The 30% EtOH eluent (11.6 g) was

* Corresponding author: Ya QK Tel/Fax: +86-771-5868 986 E-mail: yaqikang@163.com
Received: October 17, 2011; Revised: October 25, 2011; Accepted: October 28, 2011
Fund: Guagnxi Administration of Traditional Chinese Medicine Science and Technology Special, China (GZKZ09-45)

separated by column chormatography on a silica gel and recrystallization repeatedly to obtain compound **12** (925 mg). The eluent of 50% EtOH (8.2 g) was subjected to silica gel CC and recrystallization repeatedly to obtain compounds **9** (83.5 mg), **10** (79.7 mg), and **11** (54.4 mg).

Results and discussion

Compound **1**: white needles (hexane-EtOAc), mp 99－101 ℃. Its molecular formula was assigned as $C_{62}H_{112}O_2$ by HR-ESI-MS at *m/z*: 911.8534 $[M + Na]^+$ (calcd 911.8560 for $C_{62}H_{112}O_2$). ^{1}H-NMR and ^{13}C-NMR data were shown in Table 1.

The IR spectrum indicated the presence of ester group (1742 and 1196 cm^{-1}). The ^{1}H-NMR data of compound **1** showed characteristic signals for two double bond prodons at δ 5.02 (1H, dd, J = 8.5, 16.0 Hz), 5.15 (1H, dd, J = 8.5, 16.0 Hz), 5.37 (1H, dd, J = 1.8, 2.4 Hz), multiple aliphatic protons at δ 1.25 (mH, m) (Table 1), suggesting the presence of long-chain aliphatic groups. The ^{13}C-NMR data of compound **1** analyzed with the aid of DEPT spectra revealed there were seven methyl carbons, multiple methylene carbons, ten methine carbons, and four quaternary carbons. There were two double bond carbons at δ 122.7, 129.5, 138.5, and 139.9. The analyses of NMR data showed that there were stigmasterol units in compound **1**. The fragment ion at *m/z* 394 and other ions at *m/z* 379, 351, 255, 214, 145, attributable to fragment ion in EI-MS, showed the presence of stigmasterol units. Also the ion at *m/z* 478 and a series of fragment ions with the difference of *m/z* 28 or 14 indicated as tritriacontanate. The IR, NMR, and MS data of compound **1** were similar with those of stigmasterol arachidate (Li, Jiang, and Wang, 2001). Therefore, the structure of compound **1** was assigned as stigmasterol tritriacontanate (Fig. 1).

The ^{1}H-NMR and ^{13}C-NMR data of monotetracontane (**2**, Zhang *et al*, 2002), araxerol (**3**, Li, Li, and Chen, 2010), stigmasterol (**4**), stearic acid (**5**), heptatriacontanoic acid (**6**), arachidic acid (**7**), ursolic acid acetate (**8**, Jin *et al*, 2008), quercetin (**9**), myricetin (**10**), myricetin-3-*O*-rhamnoside (**11**, Lu *et al*, 2008), and rutoside (**12**) were consistent with those of physicochemical constants and references and so identified, respectively.

Table 1 ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound 1 (in $CDCl_3$)

No.	δ_H	δ_C
1		37.2
2		32.1
3	3.51 (1H, m)	73.9
4		39.6
5		139.9
6	5.37 (1H, d, J = 4.2 Hz)	122.6
7		34. 9
8		32.1
9		51.4
10		36.8
11		21.2
12		38.4
13		42.4
14		57.0
15		22.9
16		29.1
17		56.2
18	0.70 (3H, s)	12.0
19	0.90 (3H, s)	19.5
20		40.6
21	1.02 (3H, s)	21.2
22	5.02 (1H, dd, J = 8.5, 16.0 Hz)	129.5
23	5.15 (1H, dd, J = 8.5, 16.0 Hz)	138.5
24		50.3
25		21.4
26	0.85 (3H, d, J = 6.6 Hz)	32.1
27	0.85 (3H, d, J = 6.6 Hz)	12.4
28		28.0
29	0.82 (3H, m)	14.3
1′		173.5
2′	2.26 (2H, d, J = 7.8 Hz)	39.8
3′	1.62 (2H, m)	25.2
4′	1.25 (m)	31.4
5′~27′	1.25 (m)	29.9
28′	1.25 (m)	29.6
29′	1.25 (m)	29.5
30′	1.25 (m)	29.4
31′	1.25 (m)	29.3
32′	1.29 (2H, m)	22.9
33′	0.87 (3H, t, J = 6.5 Hz)	12.2

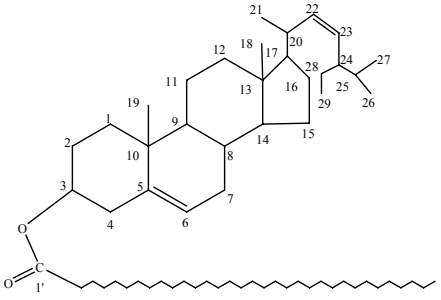

Fig. 1 Chemical structure of compound 1

References

Li GY, Jiang HX, Wang H, 2001. Studies on chemical constituents of *Frullania muscicola*. *Chin Tradit Herb Drugs* 32(8): 678-681.

Zhang HL, Wang S, Chen RY, Yu DQ, 2002. Studies on chemical constituents of *Uvaria macrophylla*. *Acta Pharm Sin* 37(2): 124-127.

Li P, Li X, Chen JW, 2010. The study of chemical substances of alcohol extract part of Taohong Siwu Decoction. *J Nanjing Univ Tradit Chin Med* 26(4): 286-288.

Jin YP, Zhang GG, Zheng HT, Shang Q, Ou YJ, Du SS, 2008. Chemical constituents of *Boschniakia himalaica*. *Cent South Pharm* 6(1): 43-45.

Lu WJ, Ya QK, Chen JY, Liu BM, 2008. A new flavonol glycoside from *Baeckea Frutescens* L. *Acta Pharm Sin* 43(10): 1032-1035.