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CONSTITUENTS FROM THE STEMS OF ECDYSANTHERA ROSEA

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Six components were isolated from the stems of *Ecdysanthera rosea*. On the basis of spectra analysis, they were identified as 5-*O*-caffeoylquinic acid (**1**), methyl 5-*O*-caffeoyl quinate (**2**), butyl 5-*O*-caffeoyl quinate (**3**), scopoletin (**4**), β-sitisterol (**5**), and β-sitisterol glycoside (**6**). 5-*O*-caffeoylquinic acid (**1**) is the major component. Compounds **2** and **3** are methyl and butyl ester of **1**, respectively, probably artifacts formed during the isolation.

Key words: Ecdysanthera rosea, Apocynaceae, 5-O-caffeoylquinic acid, Scopoletin.

INTRODUCTION

Ecdysanthera rosea Hook. & Arn. (Apocynaceae) is a large climbing shrub scattered in forests at low altitudes of Taiwan.[1] Its roots, stems and leaves have been used as anti-inflammatory, antibacterial, antipyretic and anti-hepatitis agents in Taiwan folk medicine.[2-4] Previous work included isolation of tartaric acid, malic acid, phytosterol, triterpenoid, and saponine.[5, 6] In the present study, caffeoyl derivatives and scopoletin were isolated from the aerial parts of *E. rosea* and their structures were elucidated.

RESULTS AND DISCUSSION

The EtOH extract of *E. rosea* was partitioned successively with *n*-hexane, EtOAc, *n*-BuOH, and H_2O. Chromatography of the EtOAc and *n*-BuOH extracts yielded six components, including 5-*O*-caffeoylquinic acid (**1**), methyl 5-*O*-caffeoyl quinate (**2**), butyl 5-*O*-caffeoyl quinate (**3**), scopoletin (**4**), β-sitisterol (**5**), and β-sitisterol glycoside (**6**). The structures of these compounds elucidated from their spectra data and by comparison with the authentic samples.

Compound **1** was a caffeoyl quinic acid. It gave an $[M+H]^+$ ion peak at *m/z* 355 in the ESI mass spectrum. The

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occurrence of caffeoyl function in the molecule was easily deduced from analysis of the ^{1}H-NMR and ^{13}C NMR (see Experimental) spectra. In the ^{13}C NMR spectrum, two methylene carbons (δ 38.3, 38.9), three methine carbons bearing an oxygen function (δ 71.4, 72.0, 73.6) and a quaternary carbon atom (δ 76.3), together with a carboxyl signal (δ 177.3) were belong to the quinic acid molecular. In the ^{1}H NMR spectrum, a multiplet signal (δ 5.34) due to methine bearing a caffeoyl group was observed in the lowfield, while two methines (δ 4.17, 3.73) free from a caffeoyl group appeared in the upfield. In the ^{1}H-^{1}H COSY spectra, the cross peaks indicated that the H-4 was at δ 3.73. Therefore, **1** may be 5- or 3-*O*-caffeoylquinic acid. Compared with the literature data [7,8], **1** was in agreement with 5-*O*-caffeoylquinic acid.

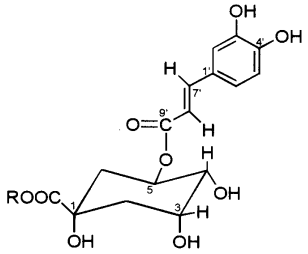
The ^{1}H- and ^{13}C-NMR spectra of **2** and **3** were similarly to those of **1**, respectively, except for the additional ester methyl group and ester butyl group. ESIMS of **2** and **3** showed a quasi-molecular ion $[M+H]^{+}$ at *m/z* 369 and 411, respectively, also confirmed the above assignments. Therefore, the structure of **2** and **3** were identified to methyl 5-*O*-caffeoyl quinate and butyl 5-*O*-caffeoyl quinate, respectively. Compounds **2** and **3** are probably artifacts formed during the isolation.

Compound **4**, a yellow needle, had mp 208-210°C from EtOAc. The ^{1}H NMR spectrum of **4** displayed a pair of one-proton doublets at δ 6.18 (J = 9.5 Hz, H-3) and 7.83 (H-4), two aromatic protons at δ 6.75 (s, H-8) and 7.09 (s, H-5), and a methoxyl signal at δ 3.90 (s, H-3). These data together with the IR spectrum with peaks at 1700 cm^{-1} (δ-lactone) and 1620, 1610, 1560 cm^{-1} (aromatic C = C stretch) support that **4** is a coumarin. ESIMS of **4** showed a quasi-molecular ion $[M+H]^{+}$ at *m/z* 193. From an nOe experiment, the MeO (δ 3.90) and H-4 (δ 7.83) signals were enhanced when irradiated at δ 7.09 (H-5). This phenomenon indicated that the OMe group was attached at C-6. Based on the above-mentioned and compared with the authentic sample,[9] **4** was determined as scopoletin.

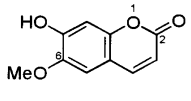
EXPERIMENTAL

General Method

Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were obtained as KBr pellets on a Perkin-Elmer 781 IR spectrometer. UV spectra were obtained on a Hitachi U-3200 spectrophotometer in MeOH.^{1} H, ^{13}C and 2D NMR spectra were measured with a Varian Inova-500 spectrometer with deuterated solvent as internal standard. APCIMS and ESIMS were recorded on a Finnigan LCQ spectrometer.



1: R= H
2: R= Methyl
3: R= Butyl



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Plant Material

The stems of *Ecdysanthera rosea* were collected at Shihting, Taipei, Taiwan, in October 1999. A voucher specimen (No. 222478) has been deposited in the herbarium of the Department of Botany of the National Taiwan University.

Extraction and Isolation

The air-dried stems of *E. rosea* (6.9 Kg) were extracted with 95% EtOH (40 L × 3). A solvent was evaporated *in vacuo* at ca. 50 °C. The concentrated extract was partitioned in succession between H_2O and EtOAc, followed by *n*-BuOH, respectively. The *n*-BuOH extract (170 g) was subjected to Sephadex LH-20 CC with a gradient of MeOH in H_2O and 5 fractions (1-5) were collected. Fraction 4 was repeatedly rechromatographed over Sephadex LH-20, eluting with MeOH to give compound **1** (728.7 mg), **2** (196.9 mg) and **3** (484.0 mg). The EtOAc extract (300 g) was subjected to Si gel CC with a gradient of EtOAc in *n*-hexane and 10 fractions (I-X) were collected. Fraction II was further purified by silica gel column (EtOAc/*n*-hexane) to give β-sitosterol (**5**, 813.7 mg). Fraction VII was further purified by Sephadex LH-20 column (MeOH) to give **4** (113.5 mg) and β-sitosterol glycoside (**6**, 804.2 mg).

5-*O*-Caffeoylquinic acid (1)

Brown granules from MeOH-H_2O, mp 206-208 °C; IR (KBr) ν max 3335, 1709, 1635, 1598, 1514, 1283 cm^{-1}; ^1H NMR (CD_3OD): δ 2.04 - 2.21 (4H, *m*, H_2-2 and H_2-6), 3.73 (1H, *dd*, *J* = 2.5, 8.0 Hz, H-4), 4.17 (1H, *br s*, H-3), 5.34 (1H, *m*, H-5), 6.27 (1H, *d*, *J* = 16.0 Hz, H-8'), 6.79 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.87 (1H, *dd*, *J* = 2.0, 8.5 Hz, H-6'), 7.05 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.57 (1H, *d*, *J* = 16.0 Hz, H-7'); ^{13}C NMR (CD_3OD): δ 38.3, 38.9 (C-2/6), 71.4 (C-3), 72.0 (C-5), 73.6 (C-4), 76.3 (C-1), 115.2 (C-8'), 115.3 (C-2'), 116.5 (C-5'), 123.0 (C-6'), 127.8 (C-1'), 146.8 (C-3'), 147.1 (C-7'), 149.6 (C-4'), 168.7 (C-9'), 177.3 (C-7); ESIMS *m/z* 377 $[M+Na]^+$, 355 $[M+H]^+$, 163 [caffeoyl] $^+$.

Methyl 5-*O*-caffeoyl quinate (2)

Amorphous brown solid; ^1H NMR (CD_3OD): δ 2.00-2.21 (4H, *m*, H_2-2 and H_2-6), 3.69 (3H, *s*, -OCH_3), 3.74 (1H, m, H-4), 4.14 (1H, *m*, H-3), 5.28 (1H, *m*, H-5), 6.21 (1H, *d*, *J* = 15.9 Hz, H-8'), 6.78 (1H, *d*, *J* = 8.2 Hz, H-5'), 6.94 (1H *dd*, *J* = 1.7, 8.2 Hz, H-6'), 7.04 (1H, *d*, *J* = 1.7 Hz, H-2'), 7.52 (1H, *d*, *J* = 15.9 Hz, H-7'); ^{13}C NMR (CD_3OD): δ 38.0 (C-2/6), 53.1 (C-8), 70.4 (C-3), 72.0 (C-5), 72.6 (C-4), 75.9 (C-1), 115.0 (C-8'), 115.2 (C-2'), 116.6 (C-5'), 123.0 (C-6'), 127.6 (C-1'), 146.8 (C-3'), 147.2 (C-7'), 149.6 (C-4'), 168.4 (C-9'), 175.5 (C-7); ESIMS *m/z* 369 $[M+H]^+$.

Butyl 5-*O*-caffeoyl quinate (3)

Amorphous brown solid; ^1H NMR (CD_3OD): δ 0.86 (3H, *t*, *J* = 7.0 Hz, -CH_3), 1.30 (2H, m, -CH_2-), 1.56 (2H, *m*, -CH_2-), 1.99-2.21 (4H, *m*, H_2-2 and H_2-6), 3.75 (1H, *m*, H-4), 4.06 (2H, *m*, -OCH_2-), 4.15 (1H, *m*, H-3), 5.29 (1H, *m*, H-5), 6.21 (1H, *d*, *J* = 16.0 Hz, H-8'), 6.77 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.92 (1H *dd*, *J* = 1.7, 8.5 Hz, H-6'), 7.03 (1H, *d*, *J*

= 1.7 Hz, H-2'), 7.52 (1H, *d*, *J* = 16.0 Hz, H-7'); ^{13}C NMR (CD_3OD): δ 14.0 (-CH_3), 19.9 (-CH_2-), 31.5 (-CH_2-), 37.9 (C-2/6), 66.3 (-OCH_2-), 70.3 (C-3), 72.0 (C-5), 72.6 (C-4), 75.8 (C-1), 115.0 (C-8'), 115.1 (C-2'), 116.5 (C-5'), 122.9 (C-6'), 127.5 (C-1'), 146.7 (C-3'), 147.1 (C-7'), 149.5 (C-4'), 168.3 (C-9'), 175.0 (C-7); ESIMS *m/z* 411 $[M+H]^+$.

Scopoletin (4)

Yellow needle from EtOAc, mp 208-210°C, IR (KBr) νmax 3450 (OH), 1700 (C = O), 1620, 1610 (aromatic) cm^{-1}; UV λ_{max} nm (MeOH): 345, 297, 253, 228; ^{1}H NMR (CD_3OD): δ 3.90 (3H, s, 6-OCH_3), 6.18 (1H, *d*, *J* = 9.5 Hz, H-3), 6.75 (1H, *s*, H-8), 7.09 (1H, *s*, H-5), 7.83 (1H, *d*, *J* = 9.5 Hz, H-4); ^{13}C NMR (CD_3OD): δ 56.8 (6-OCH_3), 104.0 (C-8), 110.0 (C-5), 112.6 (C-3, 4a), 146.1 (C-4), 147.1 (C-6), 151.4 (C-8a), 152.9 (C-7), 164.0 (C-2); ESIMS *m/z* 193 $[M+H]^+$.

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酸藤莖部之成分研究

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由酸藤的莖部分離得到六個化合物，經光譜分析，確定其結構分別為 5-*O*-caffeoyl quinic acid (**1**), methyl 5-*O*-caffeoyl quinate (**2**), butyl 5-*O*-caffeoyl quinate (**3**), scopoletin (**4**), β-sitisterol (**5**), and β-sitisterol glycoside (**6**)。其中化合物 (**1**) 是含量較多的主成分，而化合物 (**2**) 及 (**3**) 分別是化合物 (**1**) 的甲酯及丁酯衍生物。化合物 (**2**) 及 (**3**) 的產生可能是來自於分離過程中使用了甲醇及正丁醇。

關鍵詞：酸藤，夾竹桃科，咖啡醯基奎寧酸，東莨菪素。

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