

Flavonoids from the Leaves of *Loranthus kaoi* (Chao) Kiu

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ABSTRACT

In continuing a chemical examination of Loranthaceous plants, this study isolated eight flavonoids from the fresh leaves of *Loranthus kaoi*, an endemic parasitic shrub found only at median altitudes in the central part of Taiwan. These compounds were a mixture of (+)- and (-)-catechin (1), (-)-*epi*-catechin (2), 2',4',6'-trihydroxydihydrochalcone 4'-*O*- β -D-glucoside (3), pinocembrin 7-*O*- β -D-glucoside (4), kaempferol 3-*O*- α -D-rhamnoside (5), kaempferol 3,7-di-*O*- β -D-glucoside (6), quercetin 3-*O*- α -D-rhamnoside (7) and quercetin 3-*O*- β -D-glucoside (8). The structures of compounds 1-8 were established on the basis of their physical properties and spectroscopic evidence.

Key words: *Loranthus kaoi*, Loranthaceae, leaves, flavonoids.

INTRODUCTION

In previous papers⁽¹⁻⁴⁾, we reported the isolation and characterization of triterpenoids and flavonoids from *Viscum multinerve*, *Aspidixia articulata* and *A. angulata*. In continuing our chemical examination of Loranthaceous plants, we investigated the constituents of *Loranthus kaoi* (Chao) Kiu. *L. kaoi* is an endemic parasitic shrub, found only at median altitudes in the central part of Taiwan⁽⁵⁾. This paper describes the isolation and structural elucidation of eight flavonoids from the fresh leaves of this plant.

MATERIALS AND METHODS

I. Instruments and Reagents

Melting points were determined with a Fisher-Johns melting-point apparatus and were uncorrected. Optical rotation was measured with a Jasco DIP-1000 polarimeter. Mass spectra were obtained on a VG Platform Electrospray mass spectrometer. ¹H (300, 500 MHz) and ¹³C (75 MHz) NMR spectra were recorded on a Bruker AM-300WB/DMX-500 SB FT-NMR spectrometer, using the solvent peak as a reference standard. HMQC and HMBC experiments were recorded on a Varian unity 500 MHz NMR spectrometer. Column chromatography was carried out with Celite 545 (Macherey-Nagel GmbH & Co. KG, Germany), SiO₂ gel 60 (70-230 μ m, Merck), Sephadex LH-20 (25-150 μ m, Pharmacia Fine Chemical Co. Ltd.) and MCI gel CHP 20P (75-150 μ m, Mitsubishi Chemical Industries, Ltd.).

II. Plant Material

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The fresh leaves of *L. kaoi* were collected at the Ching-Ch'ing Farm, Nantou County, Taiwan, Republic of China, and verified by Mr. Muh-Tsuen Kao, Department of Botany, National Taiwan University. The voucher specimen is deposited in the National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, Republic of China.

III. Extraction and Separation

The fresh leaves of *L. kaoi* (390 g) were extracted three times with boiling methanol (3 L×3). The extract was concentrated under vacuum (ca 40°C). The residue was dissolved in methanol

and mixed with Celite 545. The solvent was removed by evaporation under reduced pressure. A brown powder thus obtained was packed in a glass column and eluted with chloroform and methanol, successively. Evaporation of the flavonoid rich eluate and the residue was subjected to column chromatography over silica gel (CHCl₃-MeOH-H₂O, 1:0:0 ← 7:3:0 ← 7:3:1) to give four fractions. Repeated column chromatography of each fraction, as shown in Scheme 1, over Sephadex LH-20, MCI gel CHP 20P and silica gel yielded compounds 1-8.

A mixture of (+)- and (-)-catechin (1)

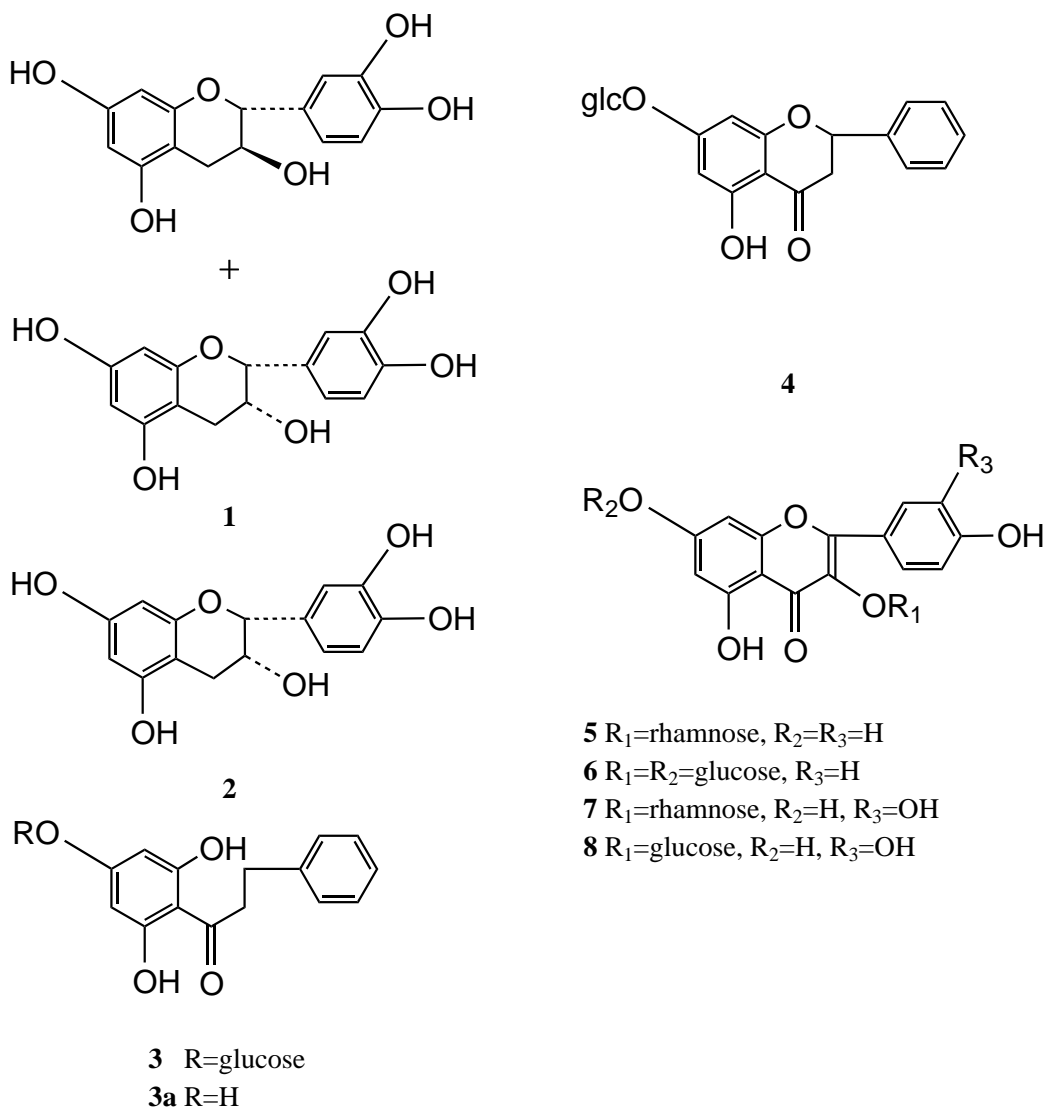


Figure 1. Structures of compounds 1-8.

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White needles (EtOH), mp 175-177°C, $[\alpha]_D^{20}$ -1.4° (c = 0.7, MeOH). $^1\text{H-NMR}$ (acetone- d_6): δ 2.48 (1H, *dd*, $J = 8.5, 16.1$ Hz, H-4), 2.86 (1H, *dd*, $J = 5.6, 16.1$ Hz, H-4), 3.96 (1H, *m*, H-3), 4.52 (1H, *d*, $J = 7.9$ Hz, H-2), 5.83 (1H, *d*, $J = 2.1$ Hz, H-6), 5.99 (1H, *d*, $J = 2.1$ Hz, H-8), 6.70 (1H, *dd*, $J = 1.7, 8.1$ Hz, H-6'), 6.77 (1H, *d*, $J = 8.1$ Hz, H-5'), 6.86 (1H, *d*, $J = 1.7$ Hz, H-2'). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 28.0 (C-4), 66.5 (C-3), 81.2 (C-2), 94.3 (C-8), 95.4 (C-6), 99.5 (C-10), 114.8 (C-2'), 115.5 (C-5'), 118.9 (C-6'), 131.0 (C-1'), 145.0 (C-3', 4'), 155.7 (C-9), 156.4 (C-5), 156.6 (C-7).

(-)-*epi*-Catechin (2)

White powder (EtOH), mp 240-242°C, $[\alpha]_D^{20}$ -56° (c = 0.7, MeOH). $^1\text{H-NMR}$ (acetone- d_6): δ 2.64 (1H, *dd*, $J = 3.5, 16.7$ Hz, H-4), 2.79 (1H, *dd*, $J = 4.6, 16.7$ Hz, H-4), 4.17 (1H, *m*, H-3), 4.81 (1H, *s*, H-2), 5.88 (1H, *d*, $J = 2.3$ Hz, H-6), 5.99 (1H, *d*, $J = 2.3$ Hz, H-8), 6.73~6.79 (2H, *m*, H-5', 6'), 6.98 (1H, *d*, $J = 1.3$ Hz, H-2'). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 28.3 (C-4), 65.0 (C-3), 78.1 (C-2), 94.1 (C-8), 95.1 (C-6), 98.6 (C-10), 114.8 (C-2'), 114.9 (C-5'), 118.0 (C-6'), 130.7 (C-1'), 144.48 (C-3'), 144.54 (C-4'), 155.8 (C-9), 156.3 (C-5), 156.6 (C-7).

2',4',6'-Trihydroxydihydrochalcone 4'-*O*- β -D-glucoside (3)

White amorphous powder (H₂O), mp 140-142°C, $[\alpha]_D^{20}$ -49° (c = 1.2, MeOH). $^1\text{H-NMR}$ (acetone- d_6): δ 2.85 (2H, *t*, $J = 7.6$ Hz, H- β), 3.28 (2H, *t*, $J = 7.6$ Hz, H- α), 4.84 (1H, *d*, $J = 8.0$ Hz, H-1''), 6.03 (2H, *s*, H-3', 5'), 7.16~7.30 (5H, *m*, H-2~6), 12.2 (1H, *s*, C6'-OH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 30.1 (C- β), 45.2 (C- α), 60.5 (C-6''), 69.4 (C-4''), 73.0 (C-2''), 76.4 (C-3''), 77.1 (C-5''), 95.0 (C-3', 5'), 99.5 (C-1''), 105.2 (C-1'), 125.8 (C-4), 128.3 (C-2, 3, 5, 6), 141.5 (C-1), 163.4 (C-4'), 163.8 (C-2', 6'), 204.7 (C=O). ESI MS *m/z*: 420 (M⁺).

Enzymatic Hydrolysis of 3

A solution of **3** (15 mg) in 70% MeOH (5 mL) was incubated with β -glucosidase at 37°C for 18h. The reaction mixture was subjected to Sephadex

LH-20 chromatography with H₂O-MeOH to yield **3a** (3 mg). **3a**: White amorphous powder, $^1\text{H-NMR}$ (DMSO- d_6): δ 2.85 (2H, *t*, $J = 7.6$ Hz, H- β), 3.28 (2H, *t*, $J = 7.6$ Hz, H- α), 5.77 (2H, *s*, H-3', 5'), 7.22~7.27 (5H, *m*, H-2~6). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 30.3 (C- β), 44.9 (C- α), 94.6 (C-3', 5'), 103.8 (C-1'), 125.7 (C-4), 128.28 (C-2, 6), 128.32 (C-3, 5), 141.8 (C-1), 164.7 (C-4'), 165.0 (C-2', 6'), 203.7 (C=O).

Pinocembrin 7-*O*- β -D-glucoside (4)

White needles (MeOH), mp 134-137°C, $[\alpha]_D^{20}$ -76° (c = 0.1, MeOH). $^1\text{H-NMR}$ (DMSO- d_6): δ 2.85 (1H, *dd*, $J = 2.7, 17.0$ Hz, H-3 β), 3.30 (1H, *d*, $J = 4.7$ Hz, H-1''), 5.64 (1H, *dd*, $J = 2.7, 12.1$ Hz, H-2), 6.14 (1H, *d*, $J = 2.1$ Hz, H-6), 6.19 (1H, *d*, $J = 2.1$ Hz, H-8), 7.45 (5H, *m*, H-2'~6'), 12.01 (1H, *s*, C5-OH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 42.2 (C-3), 60.6 (C-6''), 69.5 (C-4''), 73.0 (C-2''), 76.3 (C-3''), 77.1 (C-2), 78.6 (C-5''), 95.5 (C-8), 96.7 (C-6), 99.6 (C-1''), 103.3 (C-10), 126.7 (C-2', 6'), 128.6 (C-3', 5'), 128.7 (C-4'), 138.5 (C-1'), 162.5 (C-9), 162.9 (C-5), 165.4 (C-7), 196.8 (C-4).

Kaempferol 3-*O*- α -D-rhamnoside (5)

Yellow needles (EtOH), mp 232-235°C, $^1\text{H-NMR}$ (DMSO- d_6): δ 0.78 (3H, *d*, $J = 6.0$ Hz, C6''-CH₃), 5.28 (1H, *d*, $J = 1.4$ Hz, H-1''), 6.20 (1H, *d*, $J = 2.1$ Hz, H-6), 6.40 (1H, *d*, $J = 2.1$ Hz, H-8), 6.90 (2H, *d*, $J = 8.8$ Hz, H-3', 5'), 7.74 (2H, *d*, $J = 8.8$ Hz, H-2', 6'), 12.61 (1H, *s*, C5-OH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 17.7 (C-6''), 70.3 (C-5''), 70.5 (C-2''), 71.0 (C-3''), 71.3 (C-4''), 94.3 (C-8), 99.2 (C-6), 102.1 (C-1''), 104.3 (C-10), 115.7 (C-3', 5'), 121.0 (C-1'), 131.0 (C-2', 6'), 134.6 (C-3), 157.0 (C-9), 157.6 (C-2), 160.1 (C-4'), 161.3 (C-5), 164.9 (C-7), 177.9 (C-4).

Kaempferol 3,7-di-*O*- β -D-glucoside (6)

Yellow needles (EtOH), mp 201-203°C, $^1\text{H-NMR}$ (DMSO- d_6): δ 5.07 (1H, *d*, $J = 7.5$ Hz, anomeric H), 5.47 (1H, *d*, $J = 7.4$ Hz, anomeric H), 6.43 (1H, *d*, $J = 2.1$ Hz, H-6), 6.78 (1H, *d*, $J = 2.1$ Hz, H-8), 6.89 (2H, *d*, $J = 8.9$ Hz, H-3', 5'), 8.05 (2H, *d*, $J = 8.9$ Hz, H-2', 6'), 12.61 (1H, *s*,

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C5-OH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 60.6, 60.8 (glc C-6 \times 2), 69.6, 69.9 (glc C-4 \times 2), 73.1, 74.2 (glc C-2 \times 2), 76.4 (glc C-3 \times 2), 77.1, 77.5 (glc C-5 \times 2), 94.4 (C-8), 99.3 (C-6), 99.7, 100.7 (glc C-1 \times 2), 105.6 (C-10), 115.1 (C-3', 5'), 120.8 (C-1'), 130.9 (C-2', 6'), 133.4 (C-3), 156.0 (C-2), 156.8 (C-9), 160.1 (C-4'), 160.8 (C-5), 162.8 (C-7), 177.6 (C-4). ESI MS m/z : 610 (M^+).

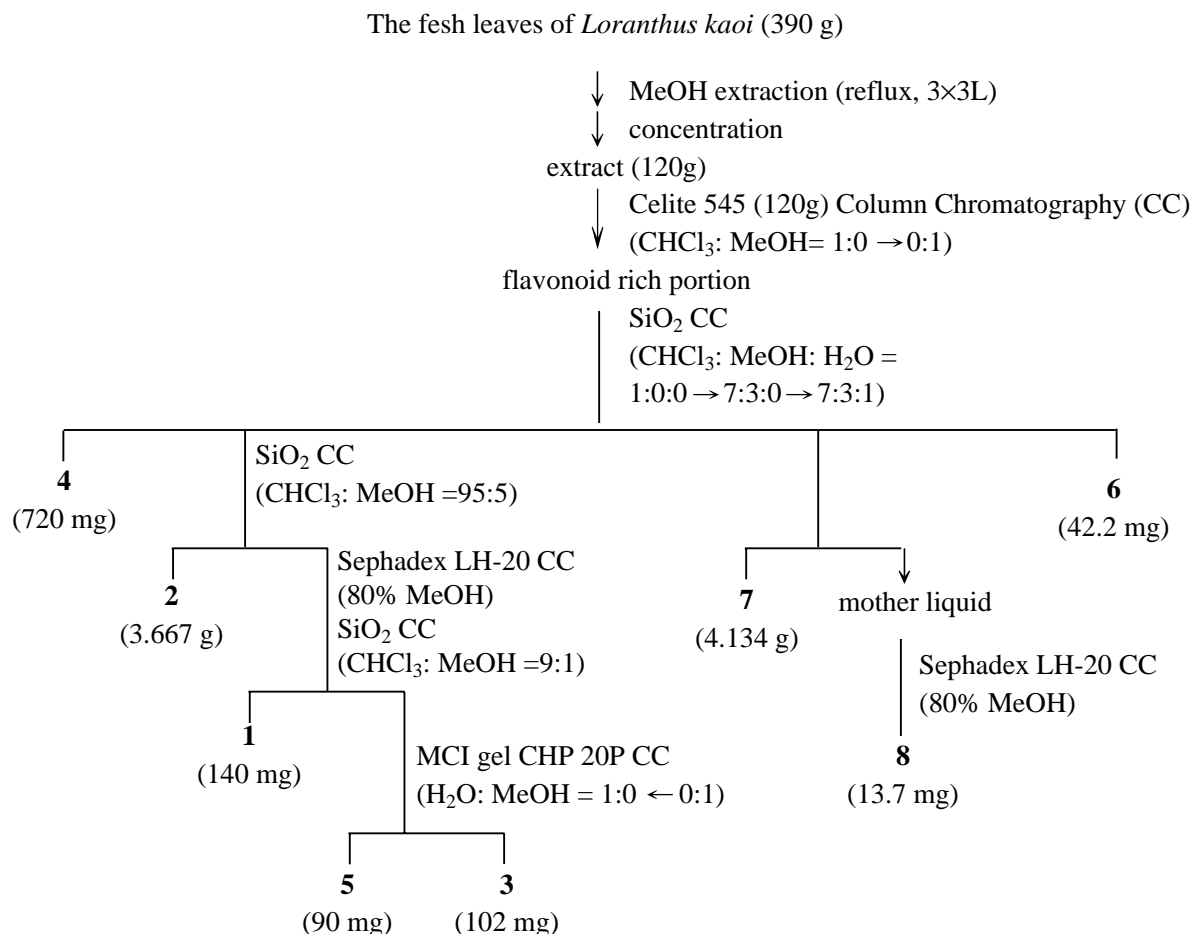
Quercetin 3-O- α -D-rhamnoside (7)

Yellow needles (EtOH), mp 179°C, $^1\text{H-NMR}$ (DMSO- d_6): δ 0.80 (3H, d , $J = 5.8$ Hz, C6''-CH $_3$), 5.24 (1H, d , $J = 1.2$ Hz, H-1''), 6.19 (1H, d , $J = 1.8$ Hz, H-6), 6.38 (1H, d , $J = 1.8$ Hz, H-8), 6.85 (1H, d , $J = 8.1$ Hz, H-5'), 7.24 (1H, dd , $J = 2.2$, 8.1 Hz, H-6'), 7.29 (1H, d , $J = 2.2$ Hz, H-2'),

12.64 (1H, s , C5-OH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 17.5 (C-6''), 70.1 (C-5''), 70.4 (C-2''), 70.6 (C-3''), 71.2 (C-4''), 93.7 (C-8), 98.7 (C-6), 101.9 (C-1''), 104.1 (C-10), 115.5 (C-2'), 115.7 (C-5'), 120.8 (C-1'), 121.2 (C-6'), 134.3 (C-3), 145.2 (C-3'), 148.5 (C-4'), 156.5 (C-2), 157.3 (C-9), 161.3 (C-5), 164.2 (C-7), 177.8 (C-4).

Quercetin 3-O- β -D-glucoside (8)

Yellow needles (EtOH), mp 225-227°C, $^1\text{H-NMR}$ (DMSO- d_6): δ 5.45 (1H, d , $J = 7.2$ Hz, H-1'), 6.19 (1H, d , $J = 1.9$ Hz, H-6), 6.39 (1H, d , $J = 1.9$ Hz, H-8), 6.83 (1H, d , $J = 9.1$ Hz, H-5'), 7.57 (2H, m , H-2', 6'), 12.63 (1H, s , C5-OH). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 61.1 (C-6''), 70.0 (C-4''), 74.3 (C-2''), 76.6 (C-3''), 77.6 (C-5''), 93.9 (C-8),



Scheme 1. Isolation of compounds 1-8.

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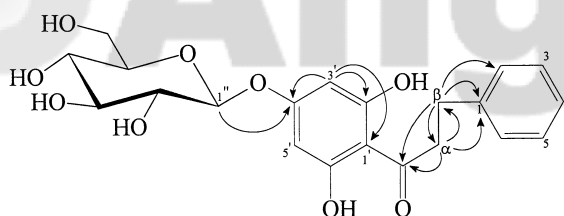


Figure 2. Selective HMBC correlations of **3**.

98.9 (C-6), 101.2 (C-1''), 104.3 (C-10), 115.5 (C-2'), 116.4 (C-5'), 121.5 (C-1'), 122.0 (C-6'), 133.7 (C-3), 144.9 (C-3'), 148.5 (C-4'), 156.6 (C-2), 156.7 (C-9), 161.2 (C-5), 164.3 (C-7), 177.7 (C-4).

RESULTS AND DISCUSSION

The fresh leaves of *L. kaoi* were extracted with methanol and the extract was coated on Celite 545 and eluted with chloroform and methanol, successively. The flavonoid-rich portion was repeatedly chromatographed over SiO₂ and reversed phase gels as shown in Scheme 1 to yield eight compounds (**1-8**).

Compounds **1** and **2** showed the characteristic signals of flavan-3-ol in the ¹H-NMR spectra, were identical with catechin and *epi*-catechin by comparison of their ¹H-NMR data with literature values^(6,7). The specific rotation of catechin was 12.3°⁽⁶⁾. Hence the value of the specific rotation ([α]_D -1.4°) of **1** indicated that it was a mixture of (+)- and (-)-catechin; and containing more (-)- than (+)-catechin. The structure of **2** was established as (-)-*epi*-catechin by its specific rotation ([α]_D -56°)⁽⁷⁾.

Compound **3** was obtained as a white amorphous powder, mp 140-142°C. ¹H-NMR spectra of **3** showed signals indicating the presence of a monosubstituted benzene ring [δ 7.16~7.30 (5H, *m*, H-2~6)], an 1,2,4,6-tetra-substituted benzene ring [δ 6.03 (2H, *s*, H-3', 5')], an ethylene [δ 2.85 (2H, *t*, *J* = 7.6 Hz, H-β), 3.28 (2H, *t*, *J* = 7.6 Hz, H-α)] and a sugar moiety [δ 4.84 (1H, *d*, *J* = 8.0 Hz, H-1'')]. Enzymatic hydrolysis of **3** with β-glucosidase yielded **3a**. **3a** was identified as 2', 4', 6'-trihydroxydihydrochalcone by comparison of

the ¹H- and ¹³C-NMR spectral data with the literature values⁽⁸⁾. The *meta*-coupled protons of **3** represented by the singlet at δ 6.03 (2H, *s*, H-3', 5') indicated the symmetrical nature of the benzene ring. Hence, the glucosyl moiety has been placed at the 4'-OH position. The conjugated position was further confirmed by the HMQC and HMBC experiments (Fig. 2). Thus, the structure of **3** was established as 2',4', 6'-trihydroxydihydrochalcone 4'-*O*-β-D-glucoside which has been cited previously⁽⁹⁾ without any spectral data.

The structures of compounds **4-8** were identified as pinocembrin 7-*O*-β-D-glucoside (**4**), kaempferol 3-*O*-α-D-rhamnoside (**5**), kaempferol 3, 7-di-*O*-β-D-glucoside (**6**), quercetin 3-*O*-α-D-rhamnoside (**7**) and quercetin 3-*O*-β-D-glucoside (**8**), by the analysis of their spectral data and comparisons with the values in literature⁽¹⁰⁻¹²⁾.

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高氏桑寄生葉部之類黃酮成分研究

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摘 要

高氏桑寄生為桑寄生科植物，由其葉部之甲醇萃取物中分離到八種類黃酮類成分，從各成分之物理性質及光譜數據確認其結構分別為：(+)-和(-)-catechin之混合物(1)，(-)-epi-catechin(2)，2',4',6'-trihydroxydihydrochalcone 4'-O-β-D-glucoside(3)，pinocembrin 7-O-β-D-glucoside(4)，kaempferol 3-O-α-D-rhamnoside(5)，kaempferol 3,7-di-O-β-D-glucoside(6)，quercetin 3-O-α-D-rhamnoside(7)及quercetin 3-O-β-D-glucoside(8)。化合物3之光譜數據為首次提出。

關鍵詞：高氏桑寄生，桑寄生科，葉部，類黃酮化合物。