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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. ^{1}H (300, 500 MHz) and ^{13}C (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-NagelGmbH & Co. KG, Germany), SiO_2 gel 60 (70-230 μm, Merck), Sephadex LH-20 (25-150 μm, Pharmacia Fine Chemical Co. Ltd.) and MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Industries, Ltd.).

II. *Plant Material*

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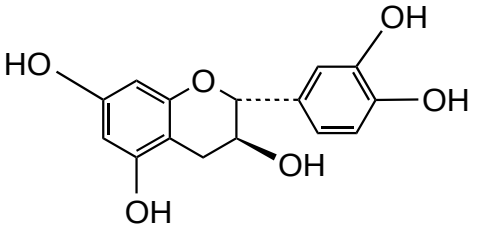
The fresh leaves of *L. kaoi* were collected at the Ching-Ch'ing Farm, Nantou County, Taiwan, Republic of China, and verified by Mr. Muh-Tsuen Kao, Department of Botany, National Taiwan University. The voucher specimen is deposited in the National Laboratories of Foods and Drugs, Department of Health,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

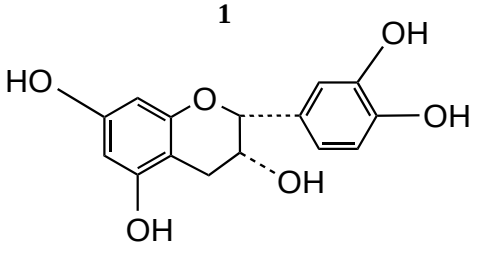
III. *Extraction and Separation*

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 ($CHCl_3$-MeOH-H_2O, 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)



1



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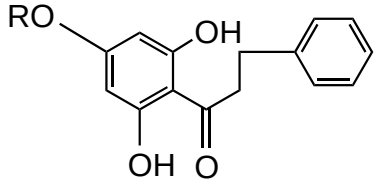
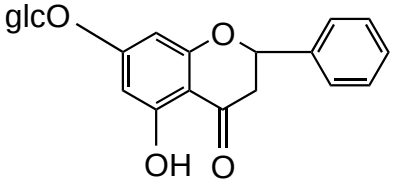


3 R=glucose
3a R=H



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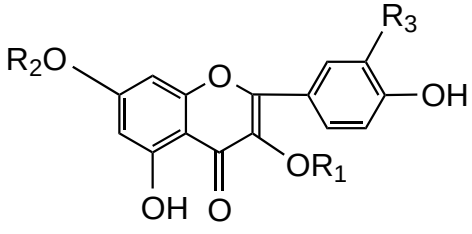


5 R_1=rhamnose, R_2=R_3=H
6 R_1=R_2=glucose, R_3=H
7 R_1=rhamnose, R_2=H, R_3=OH
8 R_1=glucose, R_2=H, R_3=OH

Figure 1. Structures of compounds **1-8**.

White needles (EtOH), mp 175-177°C, $[\alpha]_D^{20}$ -1.4° (c =0.7, MeOH). ^{1}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}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}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}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 (H_2O) , mp 140-142°C, $[\alpha]_D^{20}$ -49° (c =1.2, MeOH). ^{1}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}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 H_2O-MeOH to yield **3a** (3 mg). **3a**: White amorphous powder, ^{1}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}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}H-NMR (DMSO-d_6):δ 2.85 (1H, *dd*, *J* =2.7, 17.0 Hz, H-3β), 3.30 (1H, overlapped with other signals, H-3α), 5.33 (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}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}H-NMR (DMSO-d_6):δ 0.78 (3H, *d*, *J* = 6.0 Hz, C6"-CH_3), 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}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}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*,

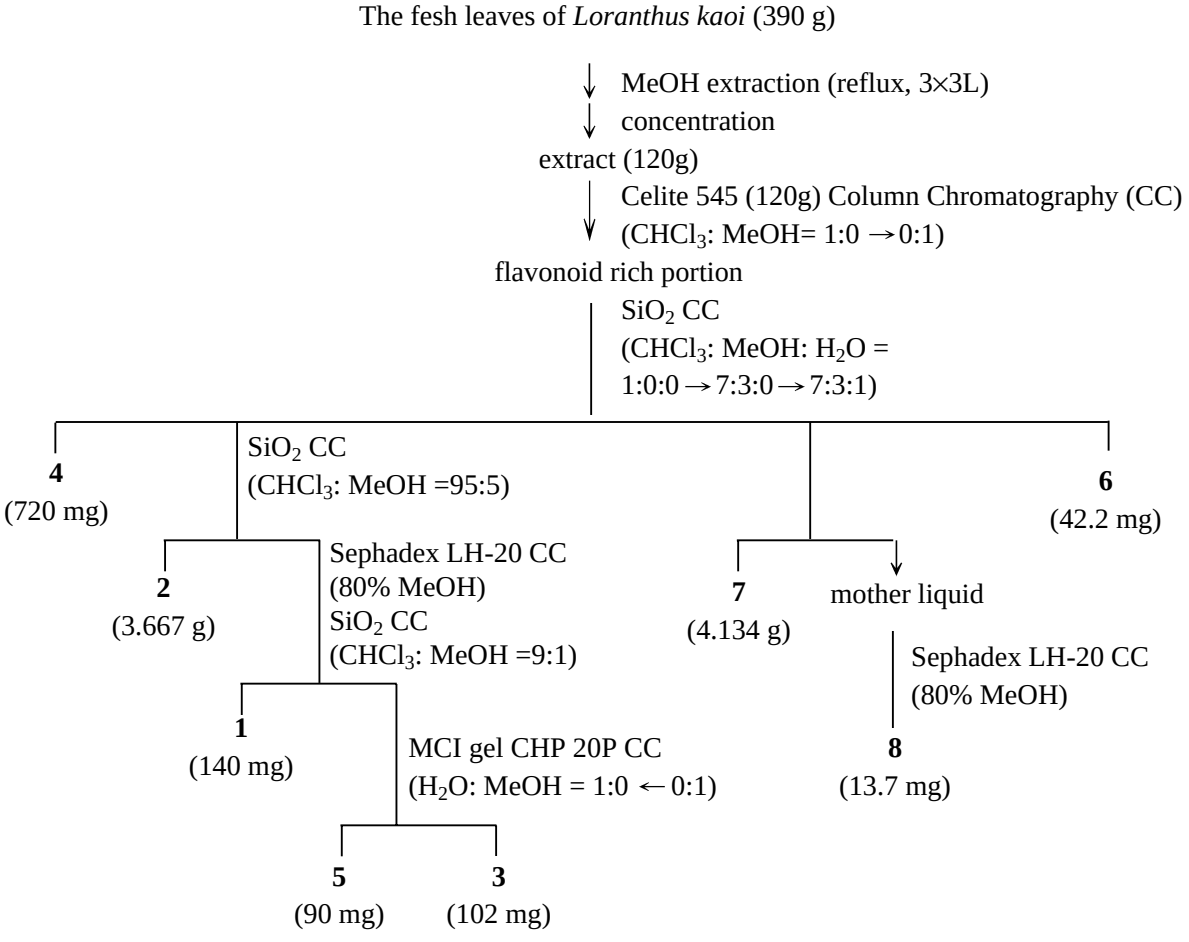
C5-OH). ^{13}C-NMR (DMSO-d_6):δ 60.6, 60.8 (glc C-6×2), 69.6, 69.9 (glc C-4×2), 73.1, 74.2 (glc C-2×2), 76.4 (glc C-3×2), 77.1, 77.5 (glc C-5×2), 94.4 (C-8), 99.3 (C-6), 99.7, 100.7 (glc C-1×2), 105.6 (C-10), 115.1 (C-3', 5'), 120.8 (C-1'), 130.9 (C-2', 6'), 133.4 (C-3), 156.0 (C-2), 156.8 (C-9), 160.1 (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}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}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}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}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**.

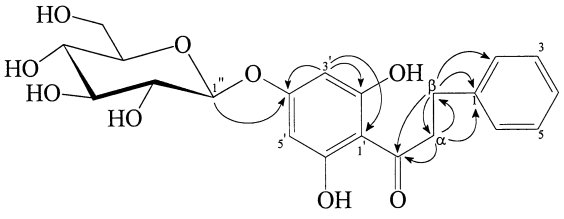


Figure 2. Selective HMBC correlations of 3.

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 SiO_2 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 ^{1}H-NMR spectra, were identical with catechin and *epi*-catechin by comparison of their ^{1}H-NMR data with literature values[6,7]. The specific rotation of catechin was 12.3° [6]. Hence the value of the specific rotation ($[\alpha]_D$ -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 ($[\alpha]_D$ -56°)[7].

Compound **3** was obtained as a white amorphous powder, mp 140-142°C. ^{1}H-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 ^{1}H- and ^{13}C-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].

ACKNOWLEDGEMENTS

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REFERENCES

1. Lin, J.-H. 1979. Studies on the constituents of the Chinese drug "Chi-Shen" (I): On the leaves of *Viscum multinerve* Hayata. J. Taiwan Pharm. Assoc. 31: 13-22.
2. Lin, J.-H. 1980. Studies on the constituents of the Chinese drug "Chi-Shen" (II): On the constituents of *Aspidixia angulata* (Heyne) Van Tieghem. J. Taiwan Pharm. Assoc. 32: 1-4.
3. Lin, J.-H. 1981. Studies on the constituents of the Chinese drug "Chi-Shen" (III): On the constituents of *Aspidixia articulata* (Burm.f.) Van Tieghem. The Annual Reports of the National Research Institute of Chinese Medicine. pp. 86-90.
4. Lin, J.-H. 1983. Studies on the constituents of the Chinese drug "Chi-Shen" (IV): On the

constituents of *Viscum multinerve* Hayata (2). The Annual Reports of the National Research Institute of Chinese Medicine. pp. 131-148.
5. Chiu, S.-T. 1996. Loranthaceae in Flora of Taiwan. 2nd ed. Vol. II. p. 272. Editorial Committee of the Flora of Taiwan, Taipei.
6. Lin, J.-H. and Huang, Y.-F. 1996. Phenolic constituents from the roots of *Rosa taiwanensis* Nakai (I). Chin. Pharm. J. 48: 231-244.
7. Kashiwada, Y., Iizuka, H., Yoshioki, K., Chen, R.-F., Nonaka, G. and Nishioka, I. 1990. Tannins and related compounds. XCIII. Occurrence of enantiomeric proanthocyanidins in the Leguminosae plants, *Cassia fistula* L. and *C. javanica* L. Chem. Pharm. Bull. 38: 888-893.
8. Tanaka, H., Ichino, K. and Ito,K. 1984. Dihydrochalcones from *Lindera umbellata*. Phytochemistry 23:1198-1199.
9. Williams, A. H. 1979. Dibenzoylmethans and flavones of Malus. Phytochemistry 18:1897-1898.
10. Liu, K. C. and Lee, S.-S. 1993. Flavonoid glucosides isolated from *Viscum liquidambaricolum*. Chin. Pharm. J. 45: 231-235.
11. Chou, C.-J. 1984. Studies on the constituents of *Diospyros kaki* leaves (I). The Annual Reports of the National Research Institute of Chinese Medicine. pp. 117-140.
12. Markham, K. R., Ternal, B., Stanley, R., Geiger, H. and Mabry, T. J. 1978. Carbon-13 NMR studies of flavonoids-III; Naturally occurring flavonoid glycosides and their acylated derivatives. Tetrahedron 34: 1389-1397.

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。