Pergamon

0031-9422(95)00975-2

Phytochemistry, Vol. 42, No. 3, pp. 867–869, 1996

0031-9422/96 $15.00 + 0.00

FLAVONOID GLYCOSIDES FROM *COLEBROOKEA OPPOSITIFOLIA*

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(*Received in revised form* 20 *November* 1995)

Key Word Index—*Colebrookea oppositifolia*; Labiatae; bark; flavonoid glycosides; negletein 6-glucoside; 5,7,2′-trihydroxyflavone 2′-glucoside.

Abstract—Two new flavonoid glycosides were isolated from the bark of *Colebrookea oppositifolia*, together with three known flavonoid aglycones. On the basis of spectral evidence, the structures of the two flavonoid glycosides were established as negletein 6-*O*-β-D-glucopyranoside and 5,7,2′-trihydroxyflavone 2′-*O*-β-D-glucopyranoside, respectively.

INTRODUCTION

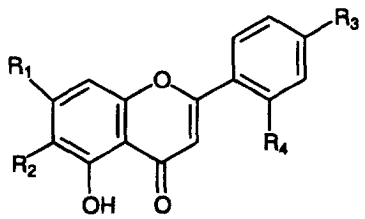
The monotypic *Colebrookea oppositifolia* Smith (Labiatae) is used in folk medicine by the Dai people in Yunnan province of China for the treatment of fractures, traumatic injuries and rheumatoid arthritis [1]. The first chemical investigation of this plant has led to the isolation and structural elucidation of two new flavonoid glycosides (**1** and **2**), together with three known flavonoid aglycones: chrysin (**3**) [2], negletein (**4**) [3] and ladanein (**4**) [4].

RESULTS AND DISCUSSION

The methanol extract of the bark of *C. oppositifolia* was repeatedly chromatographed on silica gel to yield compounds **1**–**5**. By comparing their ^{1}H and ^{13}C NMR signals with reported data, three known flavones were identified as chrysin(5,7-dihydroxyflavone) (**3**) [2], negletein (5,6-dihydroxy-7-methoxyflavone) (**4**) [3] and ladanein (5,6-dihydroxy-7,4′-dimethoxyflavone) (**4**) [4].

Compound **1** displayed several strong and broad bands in the range of 1650–1050 cm^{-1} in the IR spectrum, indicative of a flavone skeleton. Its molecular formula was determined as $C_{22}H_{22}O_{10}$ from the positive FAB-mass spectrum in conjunction with the ^{13}C NMR (DEPT) spectrum. The ^{1}H and ^{13}C NMR spectra indicated the presence of a β-glucopyranosyl unit, and the signals for the aglycone were very similar to those of **4**. Compound **1** was hydrolysed with acid to yield **4** and glucose, suggesting that **1** was a monoglycoside of **4**. Comparison of the ^{13}C NMR spectrum of **1** with that of **4** showed that the signal of C-6 was shifted upfield by 2.46 ppm, whereas the signals of C-7, C-5, C-9 were displaced downfield by 4.13, 3.06 and 5.50 ppm, respectively. However, the signal of C-4 remained unaffected. This indicated that the glucosyl unit was attached to C-6 of the aglycone. The EI-mass spectrum also supported the above deduction. Besides the base peak at *m/z* 284 $[M-Glc]^+$, fragment ion peaks at *m/z* 181 and 102 resulting from RDA cleavage were recorded (*m/z* 181 from A-ring and *m/z* 102 from B-ring). Based on the above evidence, the structure of **1** was shown to be negletein 6-O-β-D-glucopyranoside.

Compound **2** also showed characteristic IR absorptions of a flavone skeleton. It displayed a molecular ion peak at *m/z* 432 in the positive FAB-mass spectrum. Upon acid hydrolysis, **2** yielded glucose. The ^{1}H NMR spectrum of **2** exhibited a hydroxyl proton signal at δ 12.88 (5-OH), and included aromatic proton signals at δ 7.00 (1H *s*), 6.45 (1H, *d*, *J* = 2.0 Hz) and 6.20 (1H, *d*, *J* = 2.0 Hz). The former aromatic proton signal could



	R_1	R_2	R_3	R_4
1	OMe	OGlc	H	H
2	OH	H	H	OGlc
3	OH	H	H	H
4	OMe	OH	H	H
5	OMe	OH	OMe	H

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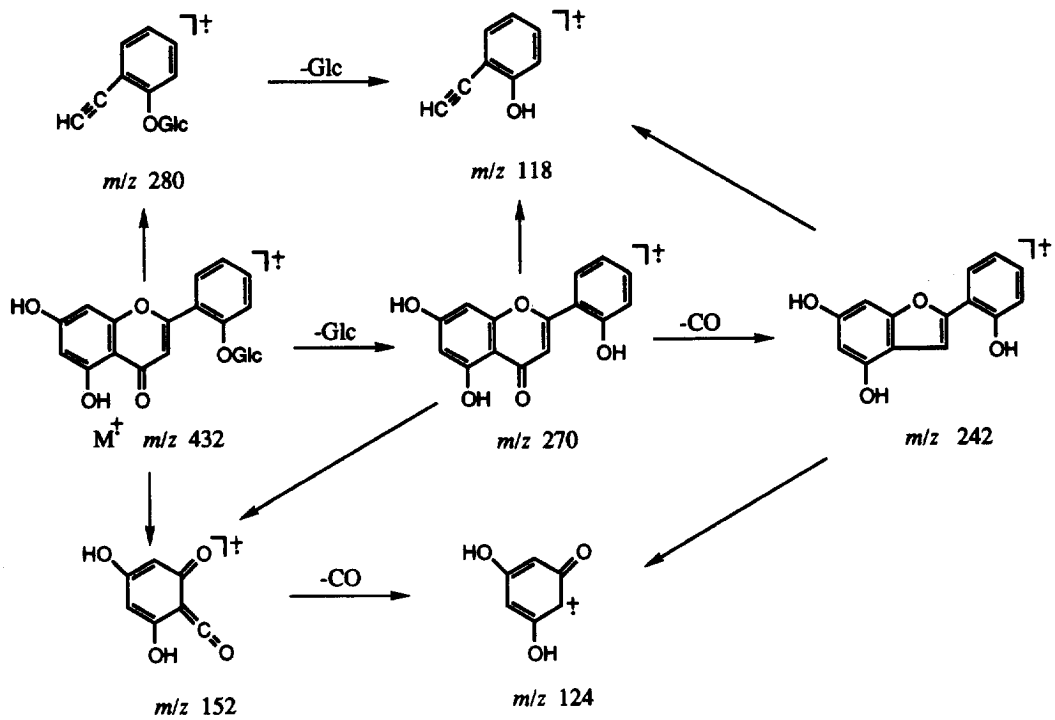

Fig. 1. The EI-mass fragmentation pattern of compound **2**.

be assigned as H-3, and the latter two upfield ones which are coupled via a ^4J coupling should be H-6 and H-8. This indicated that the A-ring had a 5,7-dihydroxy substituted pattern. In addition, two groups of doublet and double-doublet peaks at δ 7.87~7.20 (4H) suggested that the B-ring was C-2′ substituted. Comparison of the ^{13}C NMR spectrum of **2** with that of flavones lacking C-2′ oxygenation such as **1** and **3** indicated that the signal of C-2′ was markedly shifted downfield, whereas the signals of C-1′, C-3′ and C-5′ were shifted upfield. The linkage position of glucose to the aglycone was determined by the EI-mass spectrum. Fig. 1 shows the fragmentation pattern. The characteristic fragment ion peak at *m/z* 280 resulting from RDA cleavage indicated that glucose was attached to the 2′-hydroxyl. Furthermore, the carbon signals of A- and C-rings are consistent with those of C-2′ oxygenated flavonoids [5]. Thus, **2** was identified as 5,7,2′-trihydroxyflavone 2′-*O*-β-D-glucopyranoside.

Flavonoids can be useful characters for the chemotaxonomy of some plant taxa and it is known that unsubstituted B-ring flavonoids are rare in the Labiatae [6]. The discovery of such compounds (**1**, **3** and **4**) in the monotypic *C. oppositifolia* may be taxonomically significant.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured in DMSO-d_6 on a Bruker AM-400 MHz spectrometer and chemical shifts are given as δ value with TMS as an int. standard.

Plant material. The bark of *C. oppositifolia* Smith was collected in Xishuangbanna of Yunnan province and identified by Professor H. Li. A voucher specimen is preserved in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation. The air-dried bark (900 g) was extracted with hot MeOH. Removal of the solvent afforded a MeOH extract, which was suspended in H_2O and successively extracted with petrol, $CHCl_3$ and *n*-BuOH. The $CHCl_3$-soluble portion (7 g) was chromatographed on silica gel with petrol containing increasing amounts of EtOAc as eluant to give several fractions. Fractions 3, 9 and 11 were rechromatographed on silica gel with $CHCl_3$–EtOAc to yield compounds **3** (16 mg), **4** (376 mg), and **5** (15 mg). The *n*-BuOH-soluble portion (6 g) was chromatographed on MCI gel CHP 20P with MeOH–H_2O (30%–80%), and silica gel with EtOAc–MeOH as eluant to give **1** (40 mg) and **2** (25 mg).

Negletein 6-O-β-D-glucopyranoside (**1**). Light yellow powder from MeOH, $[\alpha]_D^{25}$ − 27° (DMSO; c 0.48); IR ν_{max}^{KBr} cm^{-1}: 3270 (br, OH), 1650 (C=O), 1600, 1580 and 1485 (C=C), 1441 (Ar-OMe), 1350, 1290, 1196, 1112, 1065 and 1045 (=C–O–), 840, 800, 765 and 680 (substituted aromatic ring); FAB-MS (pos.) *m/z*: 447 $[M(C_{22}H_{22}O_{10}) + H]^+$; EI-MS *m/z* (rel. int.): 284 [M-Glc]$^+$ (100), 266 $[284 - H_2O]^+$ (41), 238 $[284 - H_2O - CO]^+$ (44), 181 [A_1 (A-ring from RDA cleavage) − H]$^+$ (15), 153 $[181 - CO]^+$ (35), 139 $[153 - Me + H]^+$ (32), 102 [B_1 (B-ring from RDA cleavage)]$^+$ (48). ^1H NMR: δ 12.89 (1H, *s*, 5-OH), 8.10 (2H, *dd*, J = 7.6, 1.6 Hz, H-2′, 6′), 7.60 (3H, *m*,

Table 1. ^{13}C NMR spectral data of compounds **1–5** in DMSO-d_6 (δ, ppm)

C	1	2	3	4	5
2	163.39	161.36	163.12	163.03	163.55
3	104.93	110.19	103.04	104.57	103.37
4	182.31	181.79	181.79	182.13	182.39
5	152.75	160.76	161.43	149.69	149.89
6	128.31	98.76	98.07	130.77	130.23
7	158.77	164.30	164.38	154.51	154.64
8	91.77	93.69	94.07	91.11	91.41
9	151.60	155.35	157.01	146.10	146.46
10	105.16	103.77	105.13	105.22	105.61
1′	130.62	120.32	130.56	130.05	123.23
2′	126.35	157.64	126.33	126.14	128.41
3′	129.05	115.53	129.05	128.92	114.78
4′	132.01	132.73	131.91	131.71	162.49
5′	129.05	121.91	129.05	128.92	114.78
6′	126.35	129.00	126.33	126.14	128.41
OMe	56.56			56.18	56.61
					55.77
1″	102.05	100.26			
2″	74.13	73.28			
3″	76.54	76.73			
4″	69.95	69.62			
5″	77.21	77.12			
6″	60.91	60.63			

H-3′, 4′, 5′), 6.98 (1H, *s*, H-8), 7.03 (1H, *s*, H-3), 5.05 (1H, *d*, $J = 7.6$ Hz, Glc H-1), 3.90 (3H, *s*, OMe). ^{13}C NMR: Table 1.

*5,7,2′-Trihydroxyflavone 2′-O-β-*D*-glucopyranoside* (**2**). Light yellow powder from MeOH, $[\alpha]_D^{25} - 48°$ (DMSO; *c* 0.36), IR ν_{max}^{KBr} cm^{-1}: 3430–3240 (br, OH), 1650 (C=O), 1610, 1560 and 1500 (C=C), 1442 (OMe), 1350, 1280, 1240, 1162, 1075 and 1040 (=C–O–), 850, 830, 751 and 740 (substituted aromatic ring); FAB-MS (pos.) *m/z*: 432 $[M(C_{21}H_{20}O_{10}) + H]^+$; EI-MS *m/z*: see Fig. 1. ^1H NMR: δ 12.88 (1H, *s*, 5-OH), 7.87 (1H, *d*, $J = 8.0$ Hz, H-6′), 7.53 (1H, *dd*, $J = 8.4$, 7.4 Hz, H-4′), 7.33 (1H, *d*, $J = 8.4$ Hz, H-3′), 7.20 (1H, *dd*, $J = 7.5$, 7.8 Hz, H-5′), 7.00 (1H, *s*, H-3), 6.45 (1H, *s*, H-6), 6.20 (1H, *s*, H-8), 5.10 (1H, *d*, $J = 8.0$ Hz, Glc H-1). ^{13}C NMR: Table 1.

Chrysin (**3**). Yellow pellets from MeOH, mp 297–300° (dec.). EI-MS *m/z* (rel. int.): 254 [M $(C_{15}H_{10}O_4)$] (100), 226 [M − CO] (55), 152 $[A_1]^+$ (60), 124 $[A_1 - CO]^+$ (57), 102 $[B_1]^+$ (31). ^1H NMR: δ 12.81 (1H, *s*, 5-OH), 10.92 (1H, *s*, *br*, 7-OH), 8.05 (2H, *dd*, $J = 7.6$, 1.6 Hz, H-2′, 6′), 7.57 (3H, *m*, H-3′, 4′, 5′), 6.93 (1H, *s*, H-3), 6.50 (1H, *s*, H-6), 6.20 (1H, *s*, H-8). ^{13}C NMR: Table 1.

Negletein (**4**). Orange pellets from $CHCl_3$–MeOH, mp 225–228° (dec.). EI-MS *m/z* (rel. int.): 284 [M $(C_{16}H_{12}O_5)$] (100), 266 [M − H_2O] (76), 238 [M − H_2O − CO] (87), 210 [238 − CO] (42), 152 $[A_1 - CO]^+$ (47), 139 [153 − Me + H]$^+$ (59), 102 (53) (B_1). ^1H NMR: δ 12.49 (1H, *s*, 5-OH), 8.76 (1H, *s*, 6-OH), 8.02 (2H, *dd*, $J = 8.0$, 1.6 Hz, H-2′, 6′), 7.56 (3H, *m*, H-3′, 4′, 5′), 6.92 (1H, *s*, H-3), 6.68 (1H, *s*, H-8), 3.90 (3H, *s*, OMe). ^{13}C NMR: Table 1.

Ladanein (**5**). Golden plates from $CHCl_3$–MeOH, mp 221–223° (dec.). EI-MS *m/z* (rel. int.): 314 $[M(C_{17}H_{14}O_6]$ (100), 296 [M − H_2O] (54), 268 [M − H_2O − CO] (79), 182 $[A_1]^+$ (15), 152 [182 − OMe + H]$^+$ (23), 139 [153 − Me + H]$^+$ (30), 133 $[B_1]^+$ (45). ^1H NMR: δ 12.59 (1H, *s*, 5-OH), 8.74 (1H, *s*, 6-OH), 8.03 (2H, *d*, $J = 9.2$ Hz, H-2′, 6′), 7.10 (2H, *d*, $J = 7.2$ Hz, H-3′, 5′), 6.91 (1H, *s*, H-3), 6.87 (1H, *s*, H-8), 3.90 (3H, *s*, OMe), 3.84 (3H, *s*, OMe). ^{13}C NMR: Table 1.

Acknowledgement—We are grateful to the instrument group of Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences for measuring NMR, IR and mass spectra.

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