PERGAMON

Phytochemistry 50 (1999) 303–306

PHYTOCHEMISTRY

Chromone *C*-glycosides from *Baeckea frutescens*

Toshiko Satake[a,*], Kohei Kamiya[a], Yasuhisa Saiki[a], Takao Hama[a], Yasuo Fujimoto[b], Hanani Endang[c], Mansur Umar[c]

[a]*Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan*
[b]*College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274, Japan*
[c]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok-Jawa Barat, Indonesia*

Revised 18 June 1998

Abstract

Five new chromone *C*-glycosides have been obtained from the leaves of *Baeckea frutescens*. They are: 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone, 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone, 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-methylchromone, 6-β-*C*-(2′-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone and 8-β-*C*-(2′-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone.

Keywords: *Baeckea frutescens*; Myrtaceae; Jamu; Chromone *C*-glycoside; Indonesia; Structural elucidation

1. Introduction

Baeckea frutescens L. (Myrtaceae) is widely distributed from Australia to South China. The leaves are used as a medicinal tea which is drunk for recovery from fever diseases in China. In Indonesia, *B. frutescens* is one of the raw materials of traditional folk medicine (Mardisiswojo, 1985). Chemical studies on *B. frutescens* have indicated the presence of some sesquiterpenes (Jiangsu New Medical College, 1977), a phloroglucinol derivative (Hems & Todd, 1940; Fujimoto, Usui, Makino, & Sumatra, 1995) and ellagic acid and its derivatives (Lowry, 1968). This paper describes the isolation and the structure elucidation of five new chromones (**1**–**5**).

2. Result and discussion

Compound **1** was obtained as colorless needles of m.p. 142–145°C, $[\alpha]_D$ +37.4°. Colour tests suggested that it is a glycoside possessing a phenolic hydroxyl group. The HR-FAB-mass spectrum of **1** gave a $(M + H)^+$ at *m*/*z* 383.1342 corresponding with the molecular formula of $C_{18}H_{22}O_9$. The IR spectrum showed an absorption band assignable to a conjugated carbonyl group (1658 cm^{-1}). The UV spectrum of **1** showed absorption maxima at 323, 297, 258, 251 and 231 nm, which indicated a chromone skeleton (Scott, 1964). The ^{1}H-NMR spectrum exhibited the presence of an isopropyl group from the signals at δ 2.87 (1H, hept, $J = 6.9$ Hz) and δ 1.22 (6H, d, $J = 6.9$ Hz). In the ^{13}C-NMR spectrum, carbon signals at δ 160.63, 163.33 and 156.67 suggested the presence of 1,3,5-trioxysubstituted aromatic ring. In the CH-COSY experiment, the proton signals at δ 6.37 (1H, s) and 6.11 (1H, s) were correlated with carbon signals at δ 93.40 and 105.12, respectively. Therefore, the former was an aromatic proton and the latter was an olefinic proton. The COLOC spectrum (Fig. 1) showed that the olefinic proton signal at δ 6.11 was correlated with the carbon signal at δ 103.28 (C-10), the proton signal of chelated hydroxyl group at δ 13.37 was correlated with carbon signals at δ 160.63 (C-5) and 103.28 (C-10), and the aromatic proton signal at δ 6.37 was also correlated with the carbon signals at δ 108.74 (C-6) and 103.28 (C-10). Further correlations of the methyl proton signal at δ 1.22 and methine proton signal at δ 2.87 in isopropyl group with the carbon signals at δ 174.68 (C-2) and 105.12 (C-3) were observed, respectively. These data indicated that **1** possessed a 5,7-dioxgenated chromone skeleton with a 2-isopropyl group.

* Corresponding author.

0031-9422/98/$ - see front matter
PII: S0031-9422(98)00513-5

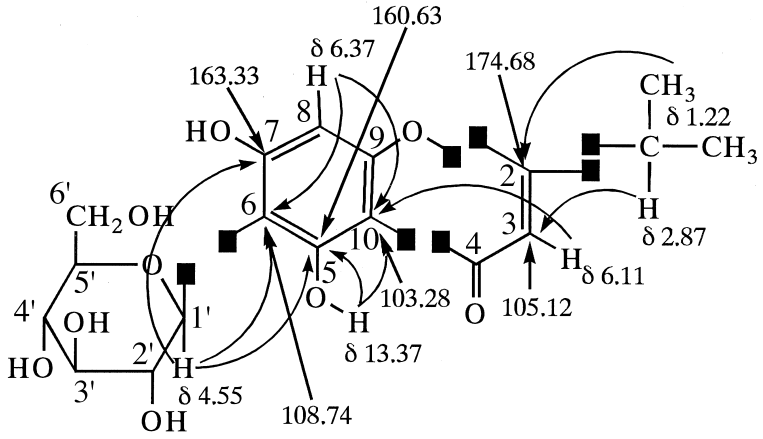

Fig. 1. Long range correlations observed in COLOC spectrum of **1**.

Moreover, as the anomeric proton at δ 4.55 (1H, d, $J = 9.8$ Hz) was correlated with a carbon signal at δ 73.02, its carbon signal was assignable to the anomeric carbon. The carbon signals at δ 73.02, 70.18, 78.88, 70.59, 81.55 and 61.46 agreed very well with those of glucose moiety of isoorientin (Okuyama, Okamoto, Yamazaki, & Satake, 1996), suggesting the *C*-glucosyl structure. In the COLOC spectrum, the cross peaks were observed between the anomeric proton at δ 4.55 and the aromatic carbon signals at δ 108.74 (C-6), 160.63 (C-5) and 163.33 (C-7), respectively. On the basis of the above mentioned evidence and the large coupling constant of the anomeric proton, the glucose was determined to be attached to C-6 in the β-configuration. Consequently, **1** is 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropylchromone.

Compound **2** was obtained as colorless needles of m.p. 145–153°C. The molecular formula of **2** was determined as $C_{18}H_{22}O_9$ from HR-FAB-mass spectrum ($(M + H)^+$, *m/z* 383.1369). The IR, UV and ^{1}H-NMR spectra of **2** showed a similar pattern to those of **1**, indicating it to be chromone glycoside. The ^{13}C-NMR spectrum of **2** was also similar to that of **1**, except for the signals at δ 98.14 (C-6) and 104.65 (C-8). Furthermore, the COLOC spectrum showed the cross peaks between the anomeric proton signal at δ 4.63 (1H, d, $J = 9.8$ Hz) and the aromatic carbon signals at δ 104.65 (C-8), 156.57 (C-9) and 162.67 (C-7), respectively. Therefore, the structure of **2** was determined to be 8-β-*C*-glucopyranosyl-5,7-dihydroxy-2-isopropyl chromone.

Compound **3**, $C_{16}H_{18}O_9$, was obtained as colorless needles of m.p. 158–164°C. The IR and UV spectra of **3** resembled those of **1**, also indicating the chromone skeleton. The ^{1}H- and ^{13}C-NMR spectra of **3** were analogous to those of **1**, except for that signals corresponding to methyl group were observed instead of signals assignable to isopropyl group in **1**. From these spectral data, the structure of **3** must be 6-β-*C*-glucopyranosyl-5,7-dihydroxy-2-methylchromone.

Compound **4**, $C_{25}H_{26}O_{13}$, was obtained as a colorless amorphous powder. The ^{13}C and ^{1}H-NMR spectra of **4** were homologous to those of **1**. The carbon signals for chromone skeleton of **4** were very similar to those of the corresponding skeleton of **1** (Table 1). However, in the comparison between the ^{13}C-NMR spectrum of **1** and those of **4**, the spectrum of **4** showed additional signals at δ 164.71 and δ 119.85, 108.67, 145.39 and 138.13 belonging to an aromatic ring. Furthermore, the ^{1}H-NMR data of **4** showed an additional signal at δ 6.79 (2H, s) attributable to an aromatic proton. The IR spectrum showed an absorption band at 1706 cm^{-1} due to the conjugated ester. The alkaline hydrolysis of **4** yielded **1** and gallic acid. From above mentioned evidence, **4** was suggested that gallic acid was ester linked to the hydroxyl group of **1**. In the ^{13}C-NMR spectrum of **4**, the esterification shifts (Tanaka, 1985) were observed for the signals corresponding to C-1′ (−2.33 ppm), C-2′ (+1.78 ppm) and C-3′ (−2.24 ppm) as compared with those of **1**. From this finding, the position of gallic acid was determined to be at C-2′ in **1**. Thus, the structure of **4** is 6-β-*C*-(2′-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone.

Compound **5**, $C_{25}H_{26}O_{13}$, was obtained as a colorless amorphous powder. The IR, UV and ^{1}H-NMR spectra of **5** showed a similar pattern to those of **4**. The ^{13}C-NMR spectrum of **5** was analogous to that of **4**, except for the signals at δ 97.76 (C-6) and 102.79 (C-8). In the COLOC spectrum, the cross peaks were observed between anomeric proton signal at δ 4.89 (1H, d, $J = 10.2$ Hz) and aromatic carbon signals at δ 102.79 (C-8), 157.15 (C-9) and 162.25 (C-7). From this finding, the structure of **5** was elucidated as 8-β-*C*-(2′-galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone.

Table 1
^{13}C-NMR spectral data for compounds **1**–**5** in DMSO-d_6

C	1	2	3	4	5
2	174.68	174.39	167.29	174.39	174.82
3	105.12	105.08	107.80	105.31	105.42
4	182.22	182.28	181.83	182.20	182.43
5	160.63	160.37	160.57	160.38	160.74
6	108.74	98.14	108.69	106.97	97.76
7	163.33	162.67	163.20	163.40	162.25
8	93.40	104.65	93.34	93.69	102.79
9	156.67	156.57	156.59	156.88	157.15
10	103.28	104.13	103.01	103.09	103.98
1′	73.02	73.14	72.96	70.69	70.70
2′	70.18	70.81	70.15	71.96	72.51
3′	78.88	78.60	78.87	76.64	75.99
4′	70.59	71.18	70.55	70.69	71.15
5′	81.55	81.64	81.44	81.87	81.98
6′	61.46	61.69	61.40	61.45	61.76
CH	32.33	32.83		32.43	33.10
CH_3	19.76	19.56	19.79	19.77	19.81
		20.16			20.34
1″				119.85	119.58
2″,6″				108.67	108.71
3″,5″				145.39	145.40
4″				138.13	138.28
7″				164.71	165.03

3. Experimental

3.1. General procedures and plant materials

Melting points were determined on a Yanagimoto micromelting point apparatus. The leaves of *B. frutescens* L. were purchased in Jakarta, Indonesia and the plant was identified by Dr. Suhardjono, the Botanical Garden, Bogor and a herbarium specimen has been deposited at the Botanical Garden, Bogor.

3.2. Extraction and isolation

The leaves of *B. frutescens* (3.8 kg) were extracted with MeOH under reflux. The solvent were evaporated off under reduced pressure to yield MeOH extract (1448 g). The MeOH extract was suspended in MeOH/H_2O (400/1200 ml) mixture and was extracted successively with $CHCl_3$, AcOEt and *n*-BuOH. The *n*-BuOH extract (270 g) was chromatographed on silica gel using a gradient of $CHCl_3$ and MeOH (100:0–0:100) to give crude chromone-containing fractions. These fractions were subjected repeatedly to silica gel column chromatography using AcOEt–MeOH–H_2O (92:5:3) and to Rp-8 column chromatography using MeOH–H_2O (3:7) and finally were purified by CC on Sephadex LH-20 using MeOH to give compounds **1**–**5** (**1**: 300 mg, **2**: 200 mg, **3**: 30 mg, **4**: 560 mg, **5**: 480 mg).

3.3. 6-β-C-Glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (*1*)

Colorless needles, m.p. 142–145°C. $[\alpha]_D^{21}$ +37.4° (c = 0.51, MeOH). HR-FAB-MS m/z: $[M+H]^+$ 383.1332 (calcd for 383.1342). IR ν_{max}^{KBr} cm^{-1}: 3368, 1658, 1632, 1540, 1496 1470. UV λ_{max}^{MeOH} nm (log ε): 297 (3.81), 258 (4.24), 251 (4.22), 231 (4.25), 210 (4.31). ^{1}H-NMR (DMSO-d_6) δ: 13.37 (1H, s), 6.37 (1H, s), 6.11 (1H, s), 4.55 (1H, d, J = 9.8 Hz), 3.99 (1H, br t, J = 8.9 Hz), 3.67 (1H, br d, J = 11.2 Hz), 3.41 (1H, m), 3.14 (3H, m), 2.87 (1H, hept, J = 6.9 Hz), 1.22 (6H, d, J = 6.9 Hz). ^{13}C-NMR (DMSO-d_6): Table 1.

3.4. 8-β-C-Glucopyranosyl-5,7-dihydroxy-2-isopropylchromone (*2*)

Colorless needles, m.p. 145–153°C. $[\alpha]_D^{21}$ +6.0° (c = 1.05, MeOH). HR-FAB-MS m/z: $[M+H]^+$ 383.1369 (calcd for 383.1342). IR ν_{max}^{KBr} cm^{-1}: 3408, 1658, 1624, 1592, 1514, 1460. UV λ_{max}^{MeOH} nm (log ε): 293 (3.19), 257 (4.45), 251 (4.39), 229 (4.41). ^{1}H-NMR (DMSO-d_6) δ: 13.02 (1H, s), 6.24 (1H, s), 6.15 (1H, s), 4.63 (1H, d, J = 9.8 Hz), 3.88 (1H, br t, J = 9.0 Hz), 3.71 (1H, br d, J = 11.2 Hz), 3.23 (1H, br t, J = 8.4 Hz), 3.15 (3H, m), 2.88 (1H, hept, J = 6.8 Hz), 1.26, 1.24 (each 3H, each d, J = 6.8 Hz). ^{13}C-NMR (DMSO-d_6): Table 1.

3.5. 6-β-C-Glucopyranosyl-5,7-dihydroxy-2-methylchromone (*3*)

Colorless needles, m.p. 158–164°C. $[\alpha]_D^{21}$ +40.9° (c = 0.33, MeOH). HR-FAB-MS m/z: $[M+H]^+$ 355.1058 (calcd for 355.1029). IR ν_{max}^{KBr} cm^{-1}: 3404, 1660, 1622, 1584, 1552, 1520, 1462. UV λ_{max}^{MeOH} nm (log ε): 296 (3.76), 257 (4.21), 251 (4.19), 230 (4.20), 209 (4.29). ^{1}H-NMR (DMSO-d_6) δ: 13.39 (1H, s), 6.36 (1H, s), 6.16 (1H, s), 4.56 (1H, d, J = 9.9 Hz), 4.01 (1H, br t, J = 9.0 Hz), 3.68 (1H, br d, J = 11.0 Hz), 3.40 (1H, m), 3.14 (3H, m), 2.35 (3H, s). ^{13}C-NMR (DMSO-d_6): Table 1.

3.6. 6-β-C-(2′-Galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (*4*)

Colorless amorphous powder. $[\alpha]_D^{21}$ −93.1° (c = 2.50, MeOH). HR-FAB-MS m/z: $[M+H]^+$ 535.1471 (calcd for 535.1451). IR ν_{max}^{KBr} cm^{-1}: 3396, 1706, 1652, 1634, 1584, 1534, 1490, 1464. UV λ_{max}^{MeOH} nm (log ε): 278 (5.12), 258 (5.37), 251 (5.33), 218 (5.61). ^{1}H-NMR (DMSO-d_6) δ: 13.40 (1H, s), 6.79 (2H, s), 6.29 (1H, s), 6.05 (1H, s), 5.68 (1H, br s), 4.87 (1H, d, J = 9.9 Hz), 3.75 (1H, br d, J = 11.4 Hz), 3.47–3.27 (4H, m), 2.79

(1H, hept, $J = 6.9$ Hz), 1.18 (6H, d, $J = 6.9$ Hz). ^{13}C-NMR (DMSO-d_6): Table 1.

3.7. Alkaline hydrolysis of **4**

A mixture of **4** (70 mg) and 0.17 N NaOMe in MeOH (3 ml) was treated at 1°C for 30 min. The mixture was diluted with H_2O (17 ml), then neutralized with Dowex 50W × 8 and the resin was filtered off. The filtrate was evaporated under the reduced pressure to yield a product (50 mg), which was purified by CC on SiO_2 to afford **1** (25 mg) and gallic acid.

3.8. 8-β;-C-(2′-Galloylglucopyranosyl)-5,7-dihydroxy-2-isopropylchromone (**5**)

Colorless amorphous powder. $[\alpha]_D^{21}$ −136.9° ($c = 2.53$, MeOH). HR-FAB-MS m/z: $[M + H]^+$ 535.1428 (calcd for 535.1451) IR ν_{max}^{KBr} cm^{-1}: 3392, 1704, 1662, 1622, 1584, 1532, 1446. UV λ_{max}^{MeOH} nm (log ε): 279 (5.22), 257 (5.45), 250 (5.40), 217 (5.46). ^{1}H-NMR (DMSO-d_6) α: 12.98 (1H, s), 6.75 (2H, s), 6.18 (1H, s), 6.10 (1H, s), 5.60 (1H, t, $J = 9.7$ Hz), 4.89 (1H, d, $J = 10.2$ Hz), 3.78 (1H, br d, $J = 11.0$ Hz), 3.54 (2H, m) 3.30 (2H, m), 2.96 (1H, hept, $J = 6.9$ Hz), 1.36, 1.32 (each 3H, each d, $J = 6.9$ Hz). ^{13}C-NMR (DMSO-d_6): Table 1.

Acknowledgements

The authors are grateful to Dr. K. Hori, Akita Research Institute of Food and Brewing, for measurements of HR-FAB-MS and to Mr. T. Kageno, Mr. T. Nakano, Mr. Y. Okamura, Miss C. Watanabe, Miss K. Yonehara and Miss T. Yorimitsu, Kobe Gakuin University, for their cooperation in experiments. This work was financially supported by the Ministry of Education, Science, Sports and Culture of Japan for University-to-University Cooperative Research (No. 05045053).

References

Fujimoto, Y., Usui, S., Makino, M., & Sumatra, M. (1995). *Phytochemistry, 41,* 923.

Hems, B. A., & Todd, A. R. (1940). *J. Chem. Soc.*, 1208.

Jiangsu New Medical College (1977). *Chinese drug dictionary*. Shanghai: Shanghai Science and Technology Co. p. 1121.

Lowry, J. B. (1968). *Phytochemistry, 7,* 1803.

Mardisiswojo, S. (1985). *Cabe Puyang Warisan Nenek Moyang*. PN Jakarta: Balai Pustaka, p. 95.

Okuyama, E., Okamoto, Y., Yamazaki, M., & Satake, M. (1996). *Chem. Pharm. Bull., 44,* 333.

Scott, A.I. (1964). *Ultraviolet spectra of natural products*. Oxford: Pergamon Press, pp. 149–150.

Tanaka, O. (1985). *Yakugaku Zasshi, 105,* 323.