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ISOBIFLORIN, A CHROMONE *C*-GLUCOSIDE FROM CLOVES (*EUGENIA CARYOPHYLLATA*)

YONGWEN ZHANG* and YUWU CHEN

Department of Materia Medica and Pharmacology, China–Japan Friendship Institute of Clinical Medical Sciences, Hepingli, Beijing 100029, People's Republic of China

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Key Word Index—*Eugenia caryophyllata*; Myrtaceae; chromone *C*-glucoside; isobiflorin; biflorin.

Abstract—The polyoxygenated chromone *C*-glucoside, isobiflorin (5,7-dihydroxy-2-methylchromone-8-*C*-β-D-glucopyranoside), and biflorin were isolated from an ethanolic extract of cloves (*Eugenia caryophyllata*), and characterized by chemical and spectral analysis.

INTRODUCTION

The flower buds of *Eugenia caryophyllata* Thunb (cloves) are widely used in traditional Chinese medicine for the treatment of many diseases such as disorders of the digestive system, bacterial and fungal infections and toothache [1]. We have examined the non-volatile oil constituents of *E. caryophyllata* and have isolated a new chromone *C*-glucoside named isobiflorin (**1**) together with the previously described biflorin (**2**) [2].

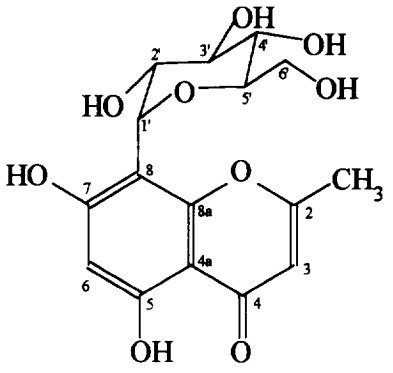
RESULTS AND DISCUSSION

The crude glycosides from the ethanolic extract of *E. caryophyllata* buds were chromatographed on a polyamide 6D column. The fractions eluted with MeOH–H_2O (7 : 3) were separated by MPLC to afford isobiflorin (**1**) and biflorin (**2**).

Compound **1** was obtained as a white powder and deduced to have the molecular formula of $C_{16}H_{18}O_9$ from its FAB-mass spectrometry data ($[M+H]^+$ ion at *m/z* 355.1029). The UV and IR data of **1** suggested the compound was a 5,7-dihydroxychromone derivative [3, 4]. Moreover, **1** gave a violet colour with ethanolic $FeCl_3$ and was resistant to acid hydrolysis with dilute trifluroacetic acid. Oxidation of **1** with $FeCl_3$ afforded both glucose and arabinose as the oxidation products, the co-occurrence of arabinose being due to oxidative cleavage of the $C_{(1)}$—$C_{(2)}$ bond of the glycosyl residue [5]. Therefore, **1** should be a chromone *C*-glucoside. The ^1H NMR spectrum of **1** showed two aromatic hydroxyl signals at δ 13.00 (*s*) and 10.70 (*br s*), respectively. The downfield chemical shift of the C-5-OH (δ 13.00) indicated that it was chelated through a strong hydrogen bond with an *ortho* carbonyl group (C-4). The spectrum also showed two aromatic proton signals at δ 6.23 (*s*, H-6) and 6.17 (*s*, H-3), and the signal of a methyl group proton at δ 2.34 (*s*, Me-2), as well as an anomeric proton signal of a glycosyl group at δ 4.63 (*d*, 9.8 Hz). The ^1H-^1H COSY spectrum of **1** confirmed the proton signals in the cyclic glycosyl residue. The ^{13}C NMR spectrum of **1** exhibited nine aromatic carbon signals and seven aliphatic carbon signals. The chemical shifts of each carbon were assigned by DEPT, C-H COSY and COLOC experiments, comparing them with those of 5,7-dihydroxy-2-methylchromone [3]. The EI-mass spectrum of **1** had its base peak at *m/z* 221 ($[M-C_5H_9O_4]^+$), whereas its $[M-C_5H_8O_5-H]^+$ peak at *m/z* 205 has a lower relative intensity, indicating the characteristic cleavage of a 5,7-dihydroxychromone-8-*C*-glycoside [6]; and the C-H COSY spectrum showed no substituent at C-6 of the chromone nucleus, indicating the *C*-glucosyl residue was attached at C-8 position. The carbon chemical shifts of the sugar moiety were congruent with those of an aromatic *C*-β-D-glucopyranosyl residue [7]. Therefore, **1** was elucidated to be 5,7-dihydroxy-2-methylchromone-8-*C*-β-D-glucopyranoside and the compound was named isobiflorin.

Compound **2** was identified as the known biflorin, i.e. 5,7-dihydroxy-2-methylchromone-6-*C*-β-D-glucopyranoside [2], based on the same analytical methods as above; and it is the 6-*C*-isomer of isobiflorin. This type of structure is rarely distributed and is different from those recently found 8-*C*-glycosyl-5-methylchromones [8]. Both **1** and **2** were found to exist in *E. caryophyllata* for the first time.

*Author to whom correspondence should be addressed.



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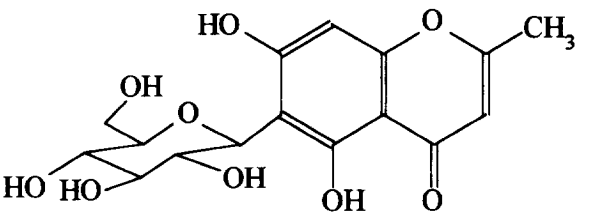

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EXPERIMENTAL

General. Mps: uncorr.; $[\alpha]_D$: MeOH; UV: MeOH; IR: KBr; ^1H and ^{13}C NMR: 500 MHz and 125 MHz, respectively, DMSO-d_6, TMS as int. st. and correlation experiments (DEPT, COSY, COLOC); MS: JMS-AX505 HA mass spectrometer; MPLC: Merck LiChroprep RP-8 column (40–63 μm).

Plant material. The cloves were purchased from Beijing Medicine Materials Co. in October, 1991 and identified as *Eugenia caryophyllata* Thunb. by Prof. Yuheng Chen of the Institute of Materia Medica, Academia Medica Sinica, Beijing. A voucher sample is deposited at the title address.

Extraction and isolation. The powdered cloves (1 kg) were defatted with Et_2O and extracted with 95% EtOH, yielding 101 g dry residue after concentration. The residue was partitioned between H_2O and EtOAc. The aqueous layer was passed through a macro-reticular resin (H-103) (Nankai University Chemicals, Tianjin, China) column and the column was washed with 95% EtOH giving 26.2 g dry residue as the crude glycosides. The crude extract was then chromatographed on a polyamide 6D column (5.5 × 100 cm) using MeOH–H_2O (7:3), (8:2) and (9:1) as eluents. The MeOH–H_2O (7:3) frs were then separated on MPLC using MeOH–H_2O (4:6) as eluent. Workup of the eluates followed by crystallization in MeOH permitted the isolation of compounds **1** (510 mg) and **2** (1022 mg).

Isobiflorin (**1**). Amorphous powder, mp 158–161°; $[\alpha]_D^{20}$, 43.2° (MeOH, *c* 0.37); UV λ_{max} (log ε): 208 (4.37), 226 sh (4.23), 249 sh (4.31), 256 (4.33), 295 (3.81); λ_{max} (MeOH–$AlCl_3$): 208, 264, 308, 360; λ_{max} (MeOH–MeONa): 208, 267, 330; IR ν_{max} cm^{-1}: 3400 (*br*, OH) 1661 (benz-pyrone CO), 1618, 1594, 1090, 1058, 1034; EI-MS (70eV) *m/z* (rel. int.): 354 (M^+, 28.6), 336 (M^+-H_2O), 221 (M^+—$C_5H_9O_4$, 100), 205 (M^+—$C_5H_8O_5$—H, 70.3); FAB-MS *m/z*: 355 ($[M+H]^+$); HRFAB-MS *m/z*: 355.1037 ($[M+H]^+$) for $C_{16}H_{18}O_9$, required: 355.1029; ^1H NMR (DMSO-d_6): 13.00 (*s*, 5-OH), 10.70 (*br s*, 7-OH), 6.23 (*s*, 6-H), 6.17 (*s*, 3-H), 4.63 (*d*, J = 9.8 Hz, 1′-H), 3.86 (*t*, J = 9.8 Hz, 2′-H), 3.67 (*dd*, ^1J = 11.5, ^2J = 5.0 Hz, 6′-H), 3.41 (*br d*, ^1J = 11.5 Hz, 6′-H), 3.20 (*t*, J = 8.7 Hz, 3′-H), 3.16 (*br s*, 4′-H, 5′-H), 2.34 (*s*, 2-Me) and ^{13}C NMR (DMSO-d_6): 181.94 (C-4), 167.02 (C-2), 162.51 (C-7), 160.35 (C-5), 156.14 (C-8a), 107.43 (C-3), 104.27 (C-8), 103.51 (C-4a), 98.44 (C-6), 81.08 (C′-5), 78.46 (C′-3), 73.12 (C′-1), 70.95 (C′-2), 70.34 (C′-4), 61.25 (C′-6), 19.63 (Me-2).

Biflorin (**2**). ^1H NMR (DMSO-d_6): 13.37 (*s*, 5-OH), 10.47 (*br s*, 7-OH), 6.35 (*s*, 8-H), 6.13 (*s*, 3-H), 4.56 (*d*, J = 9.8Hz, 1′-H), 3.99 (*t*, J = 9.8 Hz, 2′-H), 3.67 (*dd*, ^1J = 11.5, ^2J = 5.0 Hz, 6′-H), 3.41 (*br d*, ^1J = 11.5 Hz, 6′-H), 3.17 (*t*, J = 8.7 Hz, 3′-H), 3.12 (*br s*, 4′-H, 5′-H), 2.34 (*s*, 2-Me) and ^{13}C NMR (DMSO-d_6): 181.76 (C-4), 167.01 (C-2), 162.96 (C-7), 160.48 (C-5), 156.57 (C-8a), 108.56 (C-6), 107.76 (C-3), 103.06 (C-4a), 93.36 (C-8), 81.18 (C′-5), 78.75 (C′-3), 72.95 (C′-1), 70.41 (C′-2), 70.25 (C′-4), 61.28 (C′-6), 19.66 (Me-2).

Oxidation of **1** *and* **2** *with* $FeCl_3$. A sample (1 mg) of each glycoside was mixed with $FeCl_3 \cdot 6\ H_2O$ (8 mg) in H_2O (0.5 ml) and was heated at 100° for 10 hr. The mixture was desalted with an electrodialyzer and the soln was reduced with $NaBH_4$ (10 mg). The reduced sugars with an electrodialyzer and the soln was reduced with $NaBH_4$ (10 mg). The reduced sugars were acetylated with Ac_2O at 121° for 3 hr to form the peracetylated alditol derivatives; these were identified with authentic peracetylated glucitol and arabitol by GLC comparison. GLC conditions: SP-2380 capillary column (0.2 μm film, 0.25 mm i.d. × 30 m, Supelco, USA); FID; He at 78 ml min^{-1}; temp program, 60°, 1 min, 60 → 215° (30° min^{-1}), 215° (18.8 min), 215 → 250° (8° min^{-1}) and 250° (5.7 min); **1**, t_R, 1,2,3,4,5-Ac_5-arabitol, 15.08 min; 1,2,3,4,5,6-Ac_6-glucitol, 28.20 min; **2**, t_R, 15.02 min and 28.13 min, respectively.

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REFERENCES

1. *Dictionary of Chinese Crude Drugs*. Jiangsu New Medical College, Shanghai Scientific Technological Publishers, Shanghai, 1977, p. 13.
2. Ghosal, S., Kumar, Y., Singh, S. and Ahad, K., *Phytochemistry*, 1983, **22**, 2591.
3. Ali, A. A., Makboul, M. A., Attia, A. A. and Ali, D. T., *Phytochemistry*, 1990, **29**, 625.
4. Robeson, D. J., Ingham, J. L. and Harborne, J. B., *Phytochemistry*, 1980, **19**, 2171.
5. Koeppen, B. H. and Roux, D. G., *Biochemistry Journal*, 1965, **97**, 444.
6. Prox, A., *Tetrahedron*, 1968, **24**, 3697.
7. Ikeya, Y., Sugama, K. and Maruno, M., *Chemistry and Pharmacology Bulletin*, 1994, **42**, 2305.
8. Jay, M. in *The Flavonoids*: *Advances in Research Since* 1986, ed. J. B., Harborne. Chapman and Hall, London, 1994, p. 57.