Baeckeins A and B, Two Novel 6-Methylflavonoids from the Roots of *Baeckea frutescens*

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A pair of novel isomeric 6-methylflavonoids, named baeckeins A (**1**) and B (**2**), were isolated from the roots of *Baeckea frutescens*. The two compounds possess a unique C_{23} skeleton, resulting from the 6-methylation and 8-arylation of a flavonol (= 3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one) framework and the formation of an unusual lactone ring *E*. Their structures were elucidated by detailed spectroscopic analyses, including HR-ESI-MS and 1D- and 2D-NMR data (HSQC, HMBC, and ROESY). A plausible biogenetic pathway for compounds **1** and **2** is also proposed (*Scheme*).

Introduction. – *Baeckea frutescens* L. (Myrtaceae) is an aromatic low-growing shrub that ranges from Southeast Asia to Australia. It is widely used as a folk medicine in Thailand, Peninsular Malaysia, Sumatra, Borneo, Sulawesi, and New Guinea. In traditional Chinese medicine, the roots of *B. frutescens* have been used for treating rheumatism and snake bites [1]. Previous investigations of this species revealed the presence of essential oil [2], sesquiterpenes [3], phloroglucinols [4], chromones [5], flavonoids including flavanone [6], flavones, and further derivatives [7][8]. Some flavonoids exhibited strong antioxidant [9] and cytotoxic [6] properties. In our ongoing research on the flavonoids of *B. frutescens*, a pair of novel 6-methylflavonoids, baeckeins A[1]) (**1**) and B (**2**), were isolated from the roots of this plant (*Fig. 1*). In this article, we describe the isolation, structure elucidation, and possible biosynthetic pathway of compounds **1** and **2**.

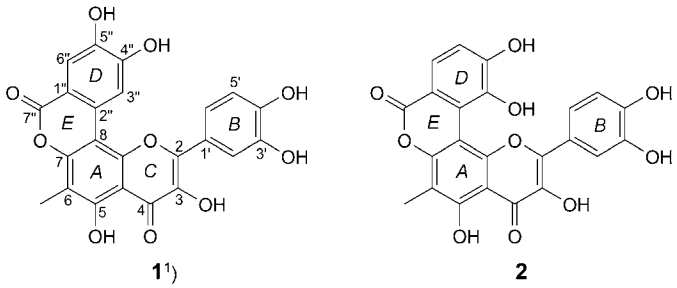

Fig. 1. *Baeckeins A* (**1**) *and B* (**2**), *isolated from* Baeckea frutescens

[1]) Trivial atom numbering; for systematic names, see *Esper. Part.*

Results and Discussion. – Baeckein A[1]) (**1**) was obtained as a yellow amorphous powder and gave a quasimolecular-ion peak at *m/z* 449.0527 ($[M-H]^-$) in the HR-ESI-MS, consistent with the elemental composition $C_{23}H_{14}O_{10}$. Its IR spectrum showed absorption bands for OH groups (3427 cm^{-1}), two C=O groups (1710 and 1643 cm^{-1}), and aromatic functionalities (1609 and 1523 cm^{-1}). The UV spectrum exhibited maxima at 254 and 345 nm, and the positive result for the Mg/HCl reaction suggested that compound **1** was a flavonoid. The ^{1}H- and ^{13}C-NMR spectra (*Table*) of **1** showed signals assignable to a flavonol (= 3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one) moiety by a typical *ABX* coupling system for H–C(2′) (δ(H) 6.95 (*d*, *J* = 8.5)), H–C(5′) (δ(H) 7.69 (*d*, *J* = 2.2)), and H–C(6′) (δ(H) 7.48 (*dd*, *J* = 8.5, 2.2)), the signal of one strongly chelated OH group, *i.e.*, of OH–C(5) (δ(H) 13.45 (*s*)), and three characteristic C-signals, *i.e.*, of C(4) (δ(C) 176.3), C(3) (δ(C) 136.5), and C(2) (δ(C) 147.9). An additional aromatic Me signal at δ(H) 2.25 (*s*) was observed, and the Me group was located at C(6) by the HMBC cross-peaks *Me*–C(6)/C(5), C(6), and C(7) (*Fig. 2*). By comparison of the NMR data with those of the known compound 6-methylquercetin [9], the above mentioned signals suggested the presence of a 6-methylquercetin unit, but the absence of a H–C(8) signal revealed that the C(8) position was substituted (6-methylquercetin = 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-6-methyl-4*H*-1-benzopyran-4-one). Except for the signals of 6-methylquercetin, the ^{13}C-NMR spectrum

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_6)DMSO) *of* **1** *and* **2**[1]). δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)		147.9		147.9
C(3)		136.5		136.4
C(4)		176.3		176.0
C(5)		156.7		157.6
C(6)		106.7		105.6
C(7)		153.2		153.4
C(8)		99.4		98.3
C(9)		150.2		149.4
C(10)		106.3		106.1
C(1′)		121.4		121.8
H–C(2′)	6.95 (*d*, *J* = 8.5)	115.6	6.83 (*d*, *J* = 8.5)	115.3
C(3′)		145.2		144.7
C(4′)		149.4		148.3
H–C(5′)	7.69 (*d*, *J* = 2.2)	116.2	7.84 (*d*, *J* = 2.2)	116.1
H–C(6′)	7.48 (*dd*, *J* = 8.5, 2.2)	120.2	7.64 (*dd*, *J* = 8.5, 2.2)	120.7
C(1″)		110.8		112.5
C(2″)		126.5		119.3
H–C(3″) or C(3″)	8.30 (*s*)	112.2		140.9
C(4″)		146.1		152.9
C(5″) or H–C(5″)		153.4	7.12 (*d*, *J* = 8.4)	115.4
H–C(6″)	7.62 (*s*)	114.4	7.70 (*d*, *J* = 8.4)	122.4
C(7″)		159.1		160.0
Me–C(6)	2.25 (*s*)	7.5	2.25 (*s*)	7.4
OH–C(5)	13.45 (*s*)		13.50 (*s*)	

displayed seven additional signals, among which two arose from CH moieties and five from quaternary C-atoms (two of them O-bearing), and the ^{1}H-NMR spectra exhibited two for two aromatic H-atoms. Comprehensive analysis of the ^{1}H-NMR and HSQC spectra established the presence of a 4″,5″-dioxy-substituted ring *D*, characterized by δ(C) 110.8 (C(1″)), 126.5 (C(2″)), 112.2 (C(3″)), 146.1 (C(4″)), 153.4 (C(5″)), and 114.4 (C(6″)) and δ(H) 8.30 (*s*, H–C(3″)) and 7.62 (*s*, H–C(6″)). The unusual ester C=O signal at δ(C) 159.1 (C(7″)) was consistent with the IR absorption band at 1710 cm^{-1} and suggested the presence of an aromatic lactone ring *E*, in accord with the remaining one degree of unsaturation. The HMBCs H–C(3″)/C(1″), C(4″), and C(5″), and H–C(6″)/C(2″), C(5″), and C(7″) (*Fig. 2*) confirmed the presence of the C_7 unit, which was connected to the 6-methylquercetin moiety *via* the C(8)–C(2″) bond as suggested by the cross-peaks H–C(3″)/C(8). The presence of the lactone ring *E* (C(7), C(8), C(2″), C(1″), and C(7″)) was confirmed by comparison of the ^{13}C-NMR data with those reported for 3,4-benzo-fused furocoumarins [10]. The NOE correlations H–C(3″)/H–C(6′) and H–C(5′) (*Fig. 2*) definitively established the structure of compound **1**.

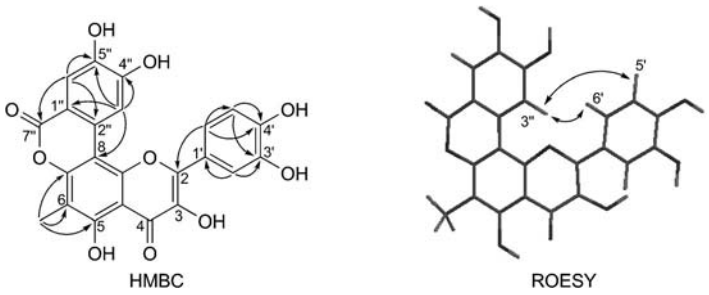

Fig. 2. *Selected 2D-NMR correlations of compound* **1**

Baeckein B[1]) (**2**) was also obtained as a yellow amorphous powder, and possessed the molecular formula $C_{23}H_{14}O_{10}$, as established by the HR-ESI-MS (*m*/*z* 449.0498 ($[M-H]^-$), indicating an isomer of **1**. Its IR and UV spectra showed similar patterns to those of **1**. Careful inspection of the ^{1}H- and ^{13}C-NMR data of compound **2** (*Table*) revealed some structural characteristics of compound **1** and also suggested the presence of a 6-methylquercetin unit, a C_7 unit, and the lactone ring *E*. The differences between compounds **1** and **2** concerned only ring *D*. The easily visible changes were a pair of *ortho*-coupled aromatic H-atoms, *i.e.*, the signals of H–C(5″) (δ(H) 7.12 (*d*, $J=8.4$)) and H–C(6″) (δ(H) 7.70 (*d*, $J=8.4$)), instead of the two *s* at δ(H) 8.30 and 7.62 (each 1 H), for **1** suggesting a 3″,4″-dioxy-substituted ring *D* in **2**, characterized by δ(C) 112.5 (C(1″)), 119.3 (C(2″)), 140.9 (C(3″)), 152.9 (C(4″)), 115.4 (C(5″)), and 122.4 (C(6″)). The above conclusion was confirmed by the HMBC H–C(5″)/C(1″), C(3″), and C(4″), and H–C(6″)/C(2″), C(4″), and C(7″) and the ROESY data of compound **2** (no NOE between ring *D* and ring *B*) (*Fig. 3*). On the basis of these evidences, the structure of **2** was determined.

From a chemical point of view, compounds **1** and **2** possess a unique C_{23} skeleton, resulting from the 6-methylation and 8-arylation of a flavonol framework and the

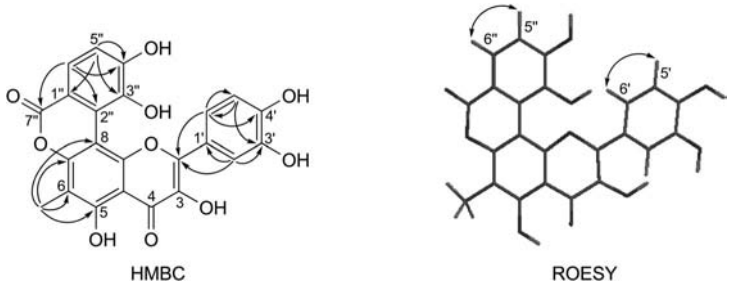

Fig. 3. *Selected 2D-NMR correlations of compound* **2**

formation of an unusual lactone ring *E*. *C*-Methylation of ring *A* is common in flavonoids from *B. frutescens* and more generally in the family Myrtaceae [9], but 8-arylflavones (except biflavonoids with a C–C connection to C(8)) are rare in nature [11][12]. A plausible biogenetic pathway for baeckeins A (**1**) and B (**2**) is proposed in the *Scheme*. The 6-Methylquercetin (**3**), which has been already isolated from *B. frutescens* [9], and protocatechuic acid (**4**), a natural phenol-derived acid which provides the C_7 unit, are suggested as precursors. The mechanism involves a phenolic coupling *via* a radical mechanism. Such a coupling takes place typically between *ortho–para*, *ortho–ortho*, or *para–para* positions [13][14]. Compounds **3** and **4** can generate free radicals **3b** and **4b** and **4c**, respectively. For compound **1**, the coupling is between *ortho*- and *para*-C-atoms (**3b** and **4c**), while two *ortho*-C-atoms (**3b** and **4b**) are coupled in the case of **2**. The lactone ring *E* can be easily formed by subsequent intramolecular esterification. Compounds **1** and **2** are, to the best of our knowledge, the first conjugates of protocatechuic acid and a flavonol leading to a unique C_{23} skeleton.

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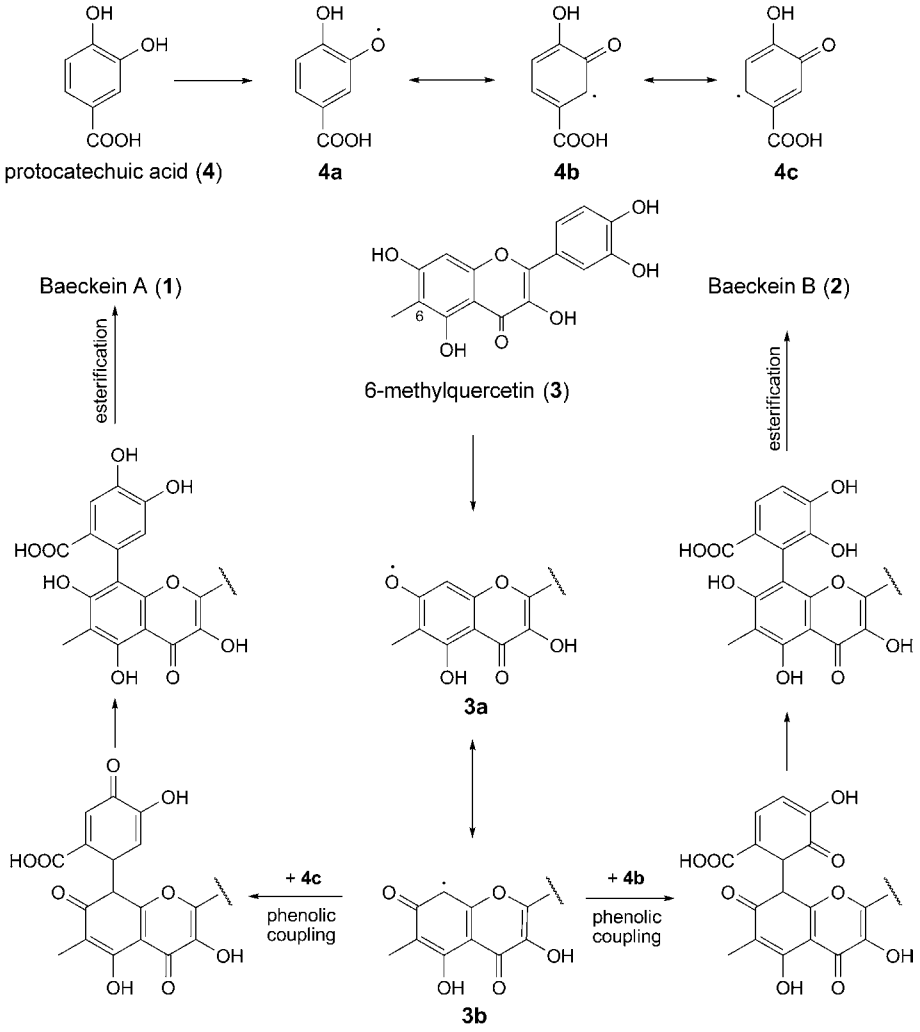
Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*) and *Sephadex LH-20* (20–100 μm; *Pharmacia*). UV Spectra: *Shimadzu-UV-2450* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Impact-410* spectrometer; KBr discs; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AV-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI and HR-ESI-MS: *Agilent LC/MSD TOF*; in *m*/*z*.

Plant Material. The roots of *B. frutescens* were collected from Nanning, Guangxi Province, P. R. China, in Nov. 2009, and identified by *Q. W.*, China Pharmaceutical University. A voucher sample (No. GS001) is kept with the Department of Chinese Material Medica Analysis, China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation. The dried roots of *B. frutescens* (10 kg) were extracted with 90% EtOH (3×30 l) at 80° for 3 h and concentrated to give 600 g of extract, which was suspended in H_2O (5 l) and partitioned successively with petroleum ether (3×5 l), AcOEt (10×5 l), and BuOH (10×5 l). The AcOEt-soluble part (203.6 g) was subjected to CC ($CHCl_3$/MeOH 1:0 → 1:1): *Fractions 1–8*. *Fr. 5* (15.8 g) was submitted to repeated CC ($CHCl_3$/MeOH 25:1 → 5:1), and the obtained **1** was purified by

Scheme. *Plausible Biogenetic Pathway for Compounds* **1** *and* **2**



CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) **1** (15 mg). *Fr. 4* (21.6 g) was subjected to repeated CC ($CHCl_3$/MeOH 50:1 → 5:1), the obtained **2** was purified by CC (*Sephadex LH-20*, MeOH): **2** (25 mg).

*2-(3,4-Dihydroxyphenyl)-3,5,10,11-tetrahydroxy-6-methyl-4*H,*8*H-*[2]benzopyrano[3,4-*h*]-1-benzopyran-4,8-dione* (**1**): Yellow amorphous powder. UV (MeOH): 254 (3.52), 345 (3.10). IR (KBr): 3427, 1710, 1643, 1609, 1523, 1361, 1288, 1185, 1160, 794. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 449 ($[M-H]^-$). HR-ESI-MS: 449.0527 ($[M-H]^-$, $C_{23}H_{13}O_{10}^-$; calc. 449.0514).

*2-(3,4-Dihydroxyphenyl)-3,5,11,12-tetrahydroxy-6-methyl-4*H,*8*H-*[2]benzopyrano[3,4-*h*]-1-benzopyran-4,8-dione* (**2**): Yellow amorphous powder. UV (MeOH): 252 (3.50), 343 (3.11). IR (KBr): 3397, 1714, 1637, 1602, 1527, 1346, 1289, 1190, 1148, 795. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 449 ($[M-H]^-$). HR-ESI-MS: 449.0498 ($[M-H]^-$, $C_{23}H_{13}O_{10}^-$; calc. 449.0514).

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