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Baeckeins L and M, two novel *C*-methylated triflavonoids from the roots of *Baeckea frutescens L.*

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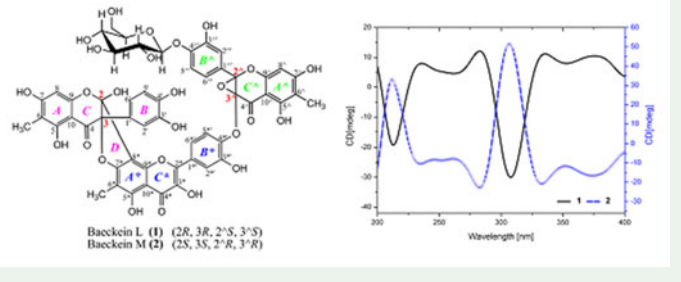
ABSTRACT

Baeckea frutescens is a medicinal plant distributing from Southeast Asia to Australia. A pair of novel diastereomeric *C*-methylated triflavonoids named baeckeins L (**1**) and M (**2**) were isolated from the roots of *B. frutescens*. The structures of these isolates were elucidated by analysis of the 1D ($^{1}H/^{13}C$) and 2D NMR (HSQC/HMBC/NOESY) and HR-ESI-MS spectroscopic data, and the absolute configurations of chiral carbons (C-2/C-3/C-2°/C-3°) were established by CD spectrometry combined with quantum chemical calculations.



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1. Introduction

Baeckea frutescens L. (Myrtaceae) distributes from Southeast Asia to Australia, and is widely used as a folk medicine in the southern part of China. Previous investigations of *B. frutescens* revealed that essential oil (Jantan et al. 1998; Tam et al. 2004), chromones (Tsui and Brown 1996a; Satake et al. 1999) and flavonoids (Tsui and Brown 1996b; Makino and Fujimoto 1999; Quang et al. 2008; Kamiya and Satake 2010) were the mainly active constituents. Recent phytochemical studies on *B. frutescens* in our laboratory have reported 11 *C*-methylated flavonoids and biflavonoids (Jia, Yang, et al. 2011; Jia, Zhou, et al. 2011; Jia et al. 2013; Jia et al. 2014; Jia et al. 2016). As the

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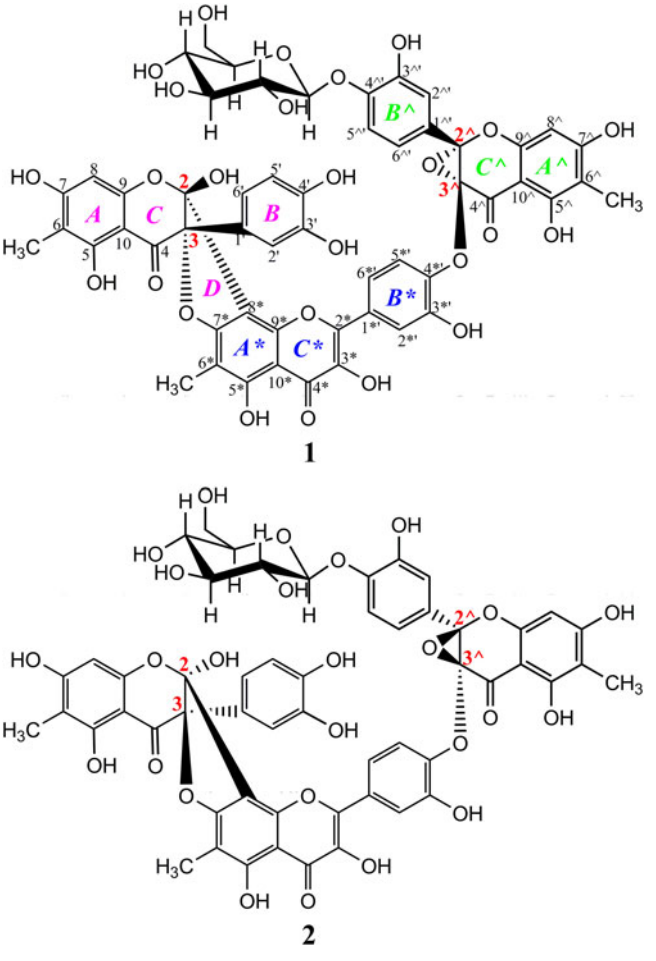

Figure 1. Structures of compounds **1** and **2**.

literature suggested, *C*-methylated flavonoids might be distinctive of the Myrtaceae family and provide the significance of plant chemotaxonomy (Gottlieb et al. 1972; Mayer 1990; Rao and Rao 1991; Wollenweber et al. 2000). In ongoing search for structurally interesting *C*-methylated flavonoids, two novel *C*-methylated triflavonoids named baeckeins L (**1**) and M (**2**) were obtained from the roots of *B. frutescens* (Figure 1). In this paper, the detailed isolation and structural elucidation for the isolates were described.

2. Results and discussion

Baeckein L (**1**) was obtained as a yellow amorphous solid. HR-ESI-MS in the positive ion mode gave a quasi-molecular ion at *m/z* 1105.1901 $[M - H]^-$ (calcd 1105.1891 for $C_{54}H_{42}O_{26}$), which was consistent with the molecular formula $C_{54}H_{42}O_{26}$. IR spectrum showed absorption bands for hydroxyl group (3396 cm^{-1}), carbonyl group (1636 cm^{-1}), and aromatic functionalities (1506 and 1442 cm^{-1}). UV spectrum

(λ_{max} 233, 307 and 368 nm) indicated the presence of a conjugated system. And the positive results for Mg-HCl reaction and Molish reagent suggested **1** to be a flavonoid glycoside.

^{13}C-NMR spectrum (Table S1) of compound **1** displayed a group of signals (δ_C 100.5, 76.7, 75.9, 73.3, 69.7 and 60.7) belonging to a hexosyl unit, and the remaining 48 carbon signals attributing for a triflavonoid structure, among which are three carbonyl groups (δ_C 188.2, 185.4 and 176.3), three aromatic methyl groups (δ_C 7.4, 6.9 and 6.8), 11 CH (δ_C 122.1, 119.5, 118.1, 116.4, 116.2, 115.9, 115.6, 115.3, 114.9, 95.5 and 94.3) and 31 quaternary carbons. ^{1}H-NMR spectrum (Table S1) of **1** showed signals for three sets of typical ABX coupling systems [δ_H 7.13 (1H, d, J = 8.7 Hz, H-2′), 7.82 (1H, d, J = 2.0 Hz, H-5′) and 6.18 (1H, dd, J = 8.7, 2.0 Hz, H-6′)], [δ_H 7.11 (1H, d, J = 8.7 Hz, H-2*′), 6.93 (1H, d, J = 2.0 Hz, H-5*′) and 7.05 (1H, dd, J = 8.7, 2.0 Hz, H-6*′)], and [δ_H 6.73 (1H, d, J = 8.7 Hz, H-2°′), 7.14 (1H, d, J = 2.0 Hz, H-5°′) and 7.01 (1H, dd, J = 8.7, 2.0 Hz, H-6°′)], corresponding to the 3′,4′-dihydroxy-substituted rings B, B* and B° of a triflavonoid moiety, and a series of signals in the range δ_H 5.5-3.0 related to a sugar moiety. Also two aromatic protons [δ_H 6.07 (1H, s, H-8) and 5.91 (1H, s, H-8°)] and three aromatic methyl signals [δ_H 1.91 (3H, s, Me-6), 2.10 (3H, s, Me-6*) and 1.84 (3H, s, Me-6°)] were presented. The above 1D-NMR data suggested **1** to be a triflavonoid glycoside, and the aglycone moiety was composed of three molecules of flavonoids with certain structural characteristics of 6-*C*-methylquercetin (Ibewuike et al. 1996; Quang et al. 2008).

Comprehensive analysis of 1D-NMR and HSQC (Figure S4) spectra of **1** made assignments of one flavonoid molecule (the segment II), referring to rings A*, B* and C*, which were confirmed by HMBC correlations (Figure S1) from CH_3-6* (δ_H 2.10) to [δ_C 161.0 (C-5*), 102.9 (C-6*) and 163.4 (C-7*)], from H-2*′ (δ_H 7.11) to [δ_C 146.0 (C-2*), 129.3 (C-1*′), 140.0 (C-3*′) and 140.5 (C-4*′)], from H-5*′(δ_H 6.93) to [δ_C 129.3 (C-1*′), 140.0 (C-3*′), 140.5 (C-4*′) and 122.1 (C-6*′)], and from H-6*′(δ_H 7.05) to [δ_C 146.0 (C-2*) and 115.3 (C-2*′)]. Compared with literature values of 6-*C*-methylquercetin (Ibewuike et al. 1996; Quang et al. 2008), the segment II was further identified. The carbon signals of C-3*′ (δ_C 140.4) and C-4*′ (δ_C 141.5) shifting δ_C 5.0-7.5 low-frequency against those of 6-C-methylquercetin, suggested one available binding position for the segment III, and the absence of an H-8* signal indicated a substituted C-8*, revealing another binding position for the segment I.

In the segment III, except the signals assignable to rings A and B of a 6-*C*-methyl quercetin molecule, the distinctive carbon signals [δ_C 188.2 (C-4°), 100.1 (C-2°), and 90.4 (C-3°)] suggested a dihydroflavanol structure and a 2°,3°-epoxide moiety, and the carbon of C-3° provided a possible binding position for the segment II. Between segments II and III, the ether linkage (C-3°)-O-(C-4*′) was elucidated. The segment III, refering to rings A°, B°, and C°, and the 2°,3°-epoxide moiety, was confirmed by HMBC correlations (Figure S1) from CH_3-6° (δ_H 1.84) to [δ_C 160.4 (C-5°), 104.8 (C-6°), and 165.9 (C-7°)], from H-8° (δ_H 5.91) to [δ_C 104.8 (C-6°), 165.9 (C-7°), 156.5 (C-9°), and 99.3 (C-10°)], from H-2°′ (δ_H 6.73) to [δ_C 100.1 (C-2°), 146.8 (C-4°′), and 119.5 (C-6°′)], from H-5°′ (δ_H 7.14) to [δ_C 124.6 (C-1°′), 146.6 (C-3°′), and 146.8 (C-4°′)], and from H-6°′ (δ_H 7.01) to [δ_C 124.6 (C-1°′) and 115.6 (C-5°′)], and was verified by related NMR data of reported baeckeins C, D and E (Jia, Zhou, et al. 2011; Jia et al. 2013).

Inspection of the signals for segment I indicated the presence of a 5,7-dihydroxy-6-methyl-substituted ring A and a 3′,4′-dihydroxy-substituted ring B. Characteristic chemical shifts for one carbonyl carbon [δ_C 185.4 (C-4)] and two quaternary carbons [δ_C 104.7 (C-2) and 91.9 (C-3)] revealed a dihydroisoflavanol structure, and carbons of C-2 and C-3 supplied two available binding positions for the segment II. Between segments I and II, optimal connections of C-2–C-8* and C-3–O–C-7* formed a furan ring D. The segment I, including rings A, B, C and D, was confirmed by HMBC correlations (Figure S1) from CH_3-6 (δ_H 1.91) to [δ_C 161.5 (C-5), 104.8 (C-6), 167.1 (C-7), 94.3 (C-8) and 98.6 (C-10)], from H-8 (δ_H 6.07) to [δ_C 185.4 (C-4), 104.8 (C-6), 167.1 (C-7), 159.3 (C-9) and 98.6 (C-10)], from H-2′ (δ_H 7.13) to [δ_C 124.3 (C-1′), 144.5 (C-3′), 145.7 (C-4′), 115.9 (C-5′) and 118.1 (C-6′)], from H-5′ (δ_H 7.82) to [δ_C 91.9 (C-3), 124.3 (C-1′), 144.5 (C-3′), 145.7 (C-4′) and 118.1 (C-6′)], and from H-6′ (δ_H 6.18) to [δ_C 91.9 (C-3), 145.7 (C-4′) and 115.9 (C-5′)], which was further identified by literature values of baeckeins J and K (Jia et al. 2016).

The sugar moiety was identified as D-glucose by TLC and GC analysis (Tang et al. 2005; Gao et al. 2008) . The large $^3J_{H\text{-}1,H\text{-}2}$ coupling constant of the anomeric proton [δ_H 4.93 (1H, d, $J = 7.0$ Hz, H-1′′)] suggested a β-configuration for the glucose unit, and the HMBC cross-peak (Figure S1) from H-1′′ to C-4°′ and NOE correlations (Figure S6) between H-1′′ and aromatic protons of ring B (H-2°′, H-5°′, and H-6°′) indicated the location of the glucose residue should be at C-4°′. And the β-D-glucose moiety was further confirmed by related data in the literature (Quang et al. 2008).

In the aglycone structure of compound **1**, referring to segments I, II and III, the quaternary carbons C-2, C-3, C-2° and C-3° provided four chiral centers. However, our NOESY experiment (not giving useful NOE increments) and CD spectrum (with positive Cotton effects (CEs) at 236, 283, 336 and 375 nm, and negative CEs at 213 and 308 nm) (Figure S2A) were not sufficient enough to determine their absolute configurations. In this case, quantum chemical CD calculations were employed (Jia, Zhou, et al. 2011; Jia et al. 2013; Jia et al. 2014; Jia et al. 2016). All 16 geometries were optimized by M06-2X functional (Zhao and Truhlar 2008) with 6-311G* basis-set (Krishnan et al. 1980), and ωB97XD functional (Chai and Head-Gordon 2008) with 6-31G* basis-set (Hariharan and Pople 1973) was employed to conduct the TD-DFT calculations. Based on the resulting electronic excitation energies and rotatory strengths, Multiwfn 3.3.8 (Lu and Chen 2012) in combination with Origin software were used to obtain ECD spectra. The calculated CD spectrum (Figure S2B) for the configuration (2*R*, 3*R*, 2°*S*, 3°*S*) displayed diagnostic negative and positive CEs, which exhibited good agreements with the experimental CD, and allowed the assignments of absolute configurations of **1** as (2*R*, 3*R*, 2°*S*, 3°*S*). Based on the above results, the structure of baeckein L (**1**) was unambiguously established.

Baeckein M (**2**) was also obtained as a yellow amorphous powder and possessed the molecular formula $C_{54}H_{42}O_{26}$, as established by HR-ESI-MS (*m/z* 1107.2028 $[M+H]^+$, calcd 1107.2037 for $C_{54}H_{43}O_{26}$). The ^1H, ^{13}C and 2D-NMR (Table S1, Figure S1) for compound **2** were very similar to those of **1**, and careful analysis of these spectral data suggested that both the carbon skeleton and functional groups presented in **2** were much the same as those of **1**. And the CD spectrum of **2** displayed positive CEs at 212 and 307 nm and negative CEs at 235, 283, 333 and 374 nm, nearly a mirror

image of that of **1** (Figure S2A), which disclosed a pair of diastereomers. Also the absolute stereochemistry of chiral centers C-2, C-3, C-2° and C-3° in compound **2** was assigned as (2*S*, 3*S*, 2°*R*, 3°*R*) by the similar TD-DFT calculations that **1** used (Figure S2C).

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References

Chai J-D, Head-Gordon M. 2008. Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections. Phys Chem Chem Phys. 10(44):6615–6620.

Gao H, Wang Z, Yang L, Yu Y, Yao Z-H, Wang N-L, Zhou G-X, Ye W-C, Yao X-S. 2008. Five new bidesmoside triterpenoid saponins from *Stauntonia chinensis*. Magn Reson Chem. 46(7): 630–637.

Gottlieb OR, Da Silva ML, Maia JGS. 1972. Eucalyptin from *Eugenia* and *Myrcia* species. Phytochemistry. 11(3):1185–1189.

Hariharan PC, Pople JA. 1973. The influence of polarization functions on molecular orbital hydrogenation energies. Theoretica Chimica Acta. 28(3):213–222.

Ibewuike JC, Ogundaini AO, Ogungbamila FO, Martin MT, Gallard JF, Bohlin L, Païs M. 1996. Piliostigmin, a 2-phenoxychromone, and C-methylflavonols from *Piliostigma thonningii*. Phytochemistry. 43(3):687–690.

Jantan I, Ahmad AS, Bakar SAA, Ahmad AR, Trockenbrodt M, Chak CV. 1998. Constituents of the essential oil of *Baeckea frutescens* L. from Malaysia. Flavour Fragr J. 13(4):245–247.

Jia B-X, Ren F-X, Jia L, Chen X-Q, Yang J, Wang Q. 2013. Baeckein E, a new bioactive *C*-methylated biflavonoid from the roots of *Baeckea frutescens*. Nat Prod Res. 27(22):2069–2075.

Jia B-X, Yang J, Chen X-Q, Cao Y, Lai M-X, Wang Q. 2011. Baeckeins A and B, two novel 6-Methylflavonoids from the roots of *Baeckea frutescens*. Helv Chim Acta. 94(12):2283–2288.

Jia B-X, Zeng X-L, Ren F-X, Jia L, Chen X-Q, Yang J, Liu H-M, Wang Q. 2014. Baeckeins F–I, four novel *C*-methylated biflavonoids from the roots of *Baeckea frutescens* and their anti-inflammatory activities. Food Chem. 155:31–37.

Jia BX, Zhou YX, Chen XQ, Wang XB, Yang J, Lai MX, Wang Q. 2011. Structure determination of baeckeins C and D from the roots of *Baeckea frutescens*. Magn Reson Chem. 49(11):757–761.

Kamiya K, Satake T. 2010. Chemical constituents of *Baeckea frutescens* leaves inhibit copper-induced low-density lipoprotein oxidation. Fitoterapia. 81(3):185–189.

Krishnan R, Binkley JS, Seeger R, Pople JA. 1980. Self-consistent molecular orbital methods. XX. A basis set for correlated wave functions. J Chem Phys. 72(1):650–654.

Lu T, Chen F. 2012. Multiwfn: A multifunctional wavefunction analyzer. J Comput Chem. 33(5): 580–592.

Makino M, Fujimoto Y. 1999. Flavanones from *Baeckea frutescens*. Phytochemistry. 50(2):273–277.

Mayer R. 1990. Flavonoids from *Leptospermum scoparium*. Phytochemistry. 29(4):1340–1342.

Quang TH, Cuong NX, Minh CV, Kiem PV. 2008. New flavonoids from *Baeckea frutescens* and their antioxidant activity. Nat Prod Commun. 3(5):755–758.

Rao JR, Rao RS. 1991. Syzalterin, a new 6,8-di-C-methyflavone from *Syzygium alternifolium* leaves. Indian J Chem B. 30(1):66–67.

Satake T, Kamiya K, Saiki Y, Hama T, Yasuo F, Endang H, Umar M. 1999. Chromone C-glycosides from *Baeckea frutescens*. Phytochemistry. 50(2):303–306.

Tam NGT, Thuam DT, Bighelli A, Castola V, Muselli A, Richomme P, Casanova J. 2004. *Baeckea frutescens* leaf oil from Vietnam: composition and chemical variability. Flavour Fragr J. 19(3): 217–220.

Tang L, Jiang Y, Chang HT, Zhao MB, Tu PF, Cui JR, Wang RQ. 2005. Triterpene saponins from the leaves of *Ilex kudingcha*. J Nat Prod. 68(8):1169–1174.

Tsui W-Y, Brown GD. 1996a. Chromones and chromanones from *Baeckea frutescens*. Phytochemistry. 43(4):871–876.

Tsui W-Y, Brown GD. 1996b. Unusual metabolites of *Baeckea frutescens*. Tetrahedron. 52(29): 9735–9742.

Wollenweber E, Wehde R, Dörr M, Lang G, Stevens JF. 2000. *C*-Methyl-flavonoids from the leaf waxes of some Myrtaceae. Phytochemistry. 55(8):965–970.

Zhao Y, Truhlar D. 2008. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. Theor Chem Account. 120(1–3):215–241.