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SYNTHESIS AND REVISED STRUCTURE OF THE FLAVONE BRICKELLIN

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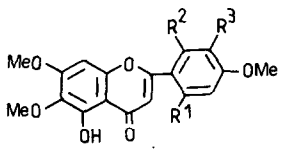
Key Word Index—*Brickellia veronicaefolia*; *B. chlorolepis*; Asteraceae; brickellin; synthesis; high field ^{1}H NMR; HPLC; methoxylated flavone aglycones.

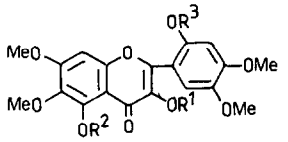
Abstract—The structure of the flavone brickellin has been revised and confirmed by synthesis. The correct structure is 5,2-dihydroxy-3,6,7,4′,5′-pentamethoxyflavone.

INTRODUCTION

In previous work [1] brickellin isolated from *Brickellia veronicaefolia* and *B. chlorolepis* was assigned structure **1** on consideration of its UV, ^{1}H and ^{13}C NMR, MS and NOE data. However the lack of suitable models at the time made the positional assignment of the B-ring methoxyl groups difficult, particularly since brickellin and one other compound [2] were the most heavily oxygenated B-ring flavonoids isolated. As a result of the recent synthesis of a number of flavonoid aglycones with a tetra-oxygenated B-ring the ^{13}C NMR assignments for brickellin were questioned [3, 4]. In order to attempt to elucidate this problem the flavone **1**, the proposed structure for brickellin and the isomers **2** and **3** were synthesized [5]. The UV, ^{1}H NMR, ^{13}C NMR and MS data of these compounds clearly were all different from those of brickellin. An alternative structure **4** was therefore proposed which was still consistent with the spectroscopic data. The movement of a methoxyl group from C-2′ in **1** to C-3 in **4** does not affect the major MS fragmentation pathways. In structure **4**, the A-ring assignments in the ^{1}H NMR spectrum are the same as for structure **1** since these were not in doubt. The three proton singlets are now accounted for by H-8 and by a pair of *para* protons H-3′ and H-6′ in **4** which must have a zero coupling constant. NOEs between the H and OMe groups which have exchanged position would not be affected. Compound **4** was therefore synthesized for comparison with natural brickellin. 2′-Hydroxy-2-isopropyloxy-4,5,4′,5′,6′ pentamethoxychalcone [5] prepared by condensation of 2-hydroxy-4,5,6-trimethoxyacetophenone with 2-isopropyloxy-4,5-dimethoxybenzaldehyde in the presence of potassium hydroxide, was converted into the corresponding flavonol **5** by the AFO oxidation [6]. After methylation of **5** under the usual conditions, the resulting flavonol **6** was partially demethylated at C-5 and deisopropylated with boron trichloride [7] to give 2′,5-dihydroxy-3,6,7,4′5′-pentamethoxyflavone (**4**) as yellow needles, mp 188–189°. Compound **4** had very similar UV, ^{13}C NMR and MS data to those previously reported for natural brickellin [1]. Samples of the two compounds were compared directly by highfield ^{1}H NMR (300 MHz) and were found to be superimposable when run under the same conditions.



	R^1	R^2	R^3
1	OH	OMe	OMe
2	OMe	OH	OMe
3	OMe	OMe	OH



	R^1	R^2	R^3
4	Me	H	H
5	H	Me	iPr
6	Me	Me	iPr

As a further check HPLC examination of synthetic compounds **1–4** and natural brickellin was carried out. The four isomers were readily resolved under reverse-phase conditions (Table 1) and brickellin was found to have an identical retention time to **4**. Thus the correct structure for brickellin is that shown and not **1** as reported previously [1].

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Table 1. HPLC of brickellin and isomeric flavonoids

Compound	R_t^* (min)	k'
1	24.1	17.85
2	28.0	20.74
3	21.9	16.22
4	19.75	14.63
Brickellin	19.75	14.63

*Conditions: 25 cm × 0.4 cm i.d. column of 5 μm ODS-Hypersil eluted with 30% MeCN, 70% (v/v) potassium hydrogen phthalate buffer pH 3.00 (0.1 M monopotassium phthalate + 0.1 M HCl) at 2.0 ml min. Detector, 345 nm.

EXPERIMENTAL

Melting points are uncorr. MS were obtained at 70 eV. ^{1}H NMR spectra were taken on a Hitachi R-20B at 60 MHz (Japan) and a Varian XL300 at 300 MHz (U.K. comparative highfield ^{1}H NMR). Chemical shifts are given in δ values (ppm) with tetramethylsilane as an internal standard. ^{13}C NMR spectra were obtained on a JEOL FX-60FT spectrometer operating at 25.15 MHz, spectral width 4000 Hz and 4096 data points. HPLC was carried out using a Waters 6000 M pump, Rheodyne 7125 injector fitted with a 20 μl loop and a Cecil 2012 variable wavelength detector.

Synthesis of 2',5-dihydroxy-3,6,7,4',5'-pentamethoxyflavone (4). To a methanol solution (60 ml) containing 2'-hydroxy-2-isopropyloxy-4,5,4',5',6'-pentamethoxychalcone [5] (1.5 g, 3.4 mmol), were added H_2O_2 (35%, 9 ml) and 10% NaOH (20 ml), successively [6]. The solution was stirred at room temperature for 15 min. After acidification with 1 N HCl, the reaction mixture was extracted with EtOAc. The EtOAc extract was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (eluent: C_6H_6–Me_2CO, 3:1) to give 0.8 g of 3-hydroxy-2'-isopropyloxy-4,5,6,7,5'-pentamethoxyflavone (5) as yellow prisms. Mp 155–156° (MeOH). ^{1}H NMR ($CDCl_3$): δ1.37 (6H, *d*, J = 6.0 Hz, Me_2CH), 3.89, 3.90 (3H, each *s*, OMe), 3.92 (6H, *s*, 2 × OMe), 4.05 (3H, *s*, OMe), 4.27 (1H, hept., CH<), 6.67 (1H, *s* H-3'), 6.76 (1H, *s*, H-8), 7.14 (1H, *s*, H-6'), OH proton not observed. MS *m/z* (rel. int.): 446 $[M]^+$ (31), 431 (6), 404 (13), 389 (47), 375 (14), 371 (21), 369 (10), 331 (7), 234 (100), 211 (42), 207 (23). Calc. for $C_{23}H_{26}O_9$: C, 61.87; H, 5.87. Found: C, 61.60; H, 5.87. Flavone 5 (200 mg, 0.4 mmol) was methylated with Me_2SO_4 (126 mg, 1 mmol) and KOH (112 mg, 2 mmol) to give 200 mg 2'-isopropyloxy-3,5,6,7,4',5'-hexamethoxyflavone 6 as a pale yellow oil. ^{1}H NMR (CCl_4): δ1.25 (6H, *d*, J = 6.0 Hz, ($\underline{Me}_2CH$), 3.71 (3H, *s*, OMe), 3.78 (6H, *s*, 2 × OMe), 3.86, 3.87, 3.90 (3H, each *s*, OMe), 4.37 (1H, hept., CH<), 6.51 (1H, *s*, H-3'), 6.53 (1H, *s*, H-8), 6.88 (1H, *s*, H-6'). MS *m/z* (rel. int.): 460 $[M]^+$, (51), 445 (48), 429 (10), 417 (14), 403 (78), 401 (56), 387 (35), 375 (10), 371 (9), 369 (10), 357 (9), 345 (10), 343 (10), 259 (19), 241 (20), 224 (21), 212 (20), 195 (23), 182 (71), 180 (68), 167 (100). Treatment of 6 (200 mg) with BCl_3 (1 mmol) in CH_2Cl_2 at −60° gave crude 4 which was purified by silica gel chromatography (eluent: C_6H_6–Me_2CO, 2:1). Mp 188–189° (C_6H_6–C_6H_{14}) yellow needles (natural product mp 197°). ^{1}H NMR ($CDCl_3$): δ3.87, 3.89 (3H, each *s*, OMe), 3.91 (6H, *s*, 2 × OMe), 3.93 (3H, *s*, OMe), 6.49 (1H, *s*, H-3'), 6.61 (1H, *s*, H-8), 7.10 (1H, *s*, H-6'), 7.88, 12.30 (1H, each *s*, OH). MS *m/z* (rel. int.): 404 $[M]^+$ (100), 389 (66), 387 (25), 373 (42), 371 (16), 361 (9), 355 (10), 343 (8), 208 (19), 202 (10), 193 (29), 181 (14), 179 (10), 153 (10). Calc. for $C_{20}H_{20}O_9$: C, 59.40; H, 4.99: Found: C, 59.66; H, 5.03. UV λ_{max}^{MeOH} (log ε): 262 (4.50), 307 sh (4.08), 349 (4.24). λ + MeONa: 266, 300 sh, 393. λ + $AlCl_3$: 275, 323, 398. λ + $AlCl_3$ + HCl: 274, 321, 386. Unchanged with NaOAc and NaOAc + H_3BO_3. ^{13}C NMR ($CDCl_3$)*: δ177.4 (*s*, C-4), 159.0 (*s*, C-7), 155.9 (*s*, C-9), 153.9 (*s*, C-4'), 152.7 (*s*, C-2'), 151.2 (*s*, C-5), 143.0 (*s*, C-2 and 5'), 136.6 (*s*, C-3), 132.6 (*s*, C-6), 111.3 (*d*, C-6'), 108.6 (*s*, C-1'), 106.3 (*s*, C-1), 103.0 (*d*, C-3'), 90.7 (*d*, C-8), 62.1 (*q*, OMe at C-3), 60.9 (*q*, OMe at C-6), 56.8, 56.4, 56.0 (each *q*, OMe at C-2', 5' and 7).

Comparative high field ^{1}H NMR (300 MHz), synthesized 4 and natural brickellin: ^{1}H NMR ($CDCl_3$) δ3.8 (3H, *s*, OMe); 3.90 (3H, *s*, OMe); 3.93 (6H, *s*, 2 × OMe); 3.96 (3H, *s*, OMe); 6.59 (1H, *s*, H-3'); 6.62 (1H, *s*, H-8); 7.1 (3H, *s*, H-6'); 7.87 and 12.36 (1H, each *s*, OH).

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*Each chemical shift of skeletal carbons agreed well with the following data: The A and C ring moieties are in agreement with those of casticin (5,3'-dihydroxy-3,6,7,4'-tetramethoxyflavone) isolated from *Vitex rotundifolia* unpublished data. The B-ring moiety is in agreement with that of arcarpillin (5,2',4'-trihydroxy-6,7,5'-trimethoxyflavone) isolated from *Artemisia capillaris* [8].