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BRICKELLIN, A NOVEL FLAVONE FROM *BRICKELLIA VERONICAEFOLIA* AND *B. CHLOROLEPIS*

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Abstract—A new highly oxygenated flavone methyl ether has been isolated from *Brickellia veronicaefolia* and *B chlorolepis* It has been identified as 5,6′-dihydroxy-6,7,2′,3′,4′-pentamethoxyflavone and given the name brickellin

INTRODUCTION

A few years ago in the course of our biochemical systematic investigation of the genus *Brickellia* [1–7], we isolated from *B veronicaefolia* (HBK) Gray (tribe Eupatorieae) [3] a few milligrams of a new highly oxygenated flavone, which we named brickellin (1) Subsequently, we also found the same compound as a trace component of *B chlorolepis* (Woot et Standl) Shinners [4] We referred to the compound as an 'unidentified flavone' in the Experimental of both papers [4, 5] Despite collecting considerable UV, mass spectral, ^{1}H NMR and ^{13}C NMR data for this new natural product over several years, including mass spectra of the permethyl (PM) and perdeuteromethyl (PDM) derivatives and NMR in different solvents, derivatized and underivatized, we were not satisfied that our unpublished structure assignment as 1 was unambiguous We have now used nuclear Overhauser enhancement (NOE) difference spectroscopy to confirm brickellin to be 5,6′-dihydroxy-6,7,2′,3′,4′-pentamethoxyflavone

In the discussion below we present the relevant data which led to the tentative structure assignment of brickellin as 1 in Table 1, where the confirming NOE results are tabulated Other data for brickellin are given in the Experimental One of the difficult aspects of the spectroscopic analysis of brickellin concerned the simplicity of the ^{1}H NMR spectrum as the trimethylsilyl (TMSi) ether, singlets were observed for three isolated skeletal protons and five methoxyl groups, in benzene-d_6 the underivated compound gave two additional singlets for two hydroxyl protons The lack of coupling between any protons frustrated the structure assignment The following discussion summarizes our structure assignment of 1 for brickellin

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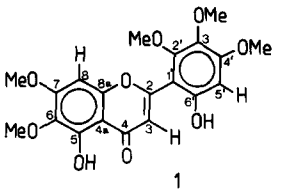
RESULTS AND DISCUSSION

Brickellin was separated from artemetin, eupatin and other pigments as described in the Experimental and on recrystallization from methanol yielded yellow crystals, mp 197° The mass spectrum of brickellin established a flavonoid with five methoxyl and two hydroxyl groups ($[M]^+$ at m/z 404 corresponding to $C_{20}H_{20}O_9$) Since the ^{1}H NMR of 1 (TMSi ether in CCl_4) exhibited three one-proton singlets at δ 6 3, 6 45 and 6 9, accounting for one isolated skeletal proton in each ring, brickellin must be a flavone with three oxygen functions in the A-ring and four in the B-ring In accord with the mass spectral data, ^{1}H NMR signals for five methoxyl groups were observed between δ 3 7 and 4 0 in CCl_4, in benzene-d_6 these signals were more clearly observable at δ 3 2, 3 35, 3 45, 3 72 and 3 95 That the A-ring contained a 5-hydroxyl was evident from the purple colour when 1 was viewed on paper in UV light, also in benzene-d_6 the signal for the hydrogen-bonded 5-hydroxyl proton appeared at δ 13 05 Since the purple colour changed to yellow-brown when 1 was viewed in UV in the presence of NH_3, the second hydroxyl must be in the B-ring, therefore, the A-ring must contain two methoxyl groups The ^{1}H NMR signal at δ 6 45 for the TMSi ether of 1 is typical for H-8, therefore, the two A-ring methoxyl groups must be at C-6 and C-7 In support of this assignment, an $[M-15]^+$ ion at 389 (85%) in the mass spectrum of 1 supported a 6-methoxyl group [9, 11] and an $[A_1-43]$ ion at m/z 153 was also consistent with an A-ring containing two methoxyls and one hydroxyl group The absence of a band III in the UV spectrum of 1 in sodium methoxide indicated the presence of a 7-methoxyl group

The remaining problem concerned the assigning of three methoxyls and one hydroxyl to five available positions in the B-ring In the mass spectrum of 1, a B_1-fragment at m/z 208 was consistent with a B-ring with three methoxyls and one hydroxyl moiety and supported the data presented above Of significance was the appearance of ions at $[M-17]^+$ (m/z 387, 30%) and $[M-31]^+$

Table 1 NOE results for brickellin



1

Proton	δ	NOEs observed on irradiation
5-OH	13 07	6'-OH (−ve)
6'-OH	8 24	5-OH (−ve), 3-H
5'-H	7 20	4'-OMe
3-H	6 65	2'-OMe
8H	6 05	7-OME
3'-OME	3 89	—
4'-OME	3 59	5'-H
6-OMe	3 52	5-OH, 6'-OH
2'-OMe	3 24	H-3
7-OMe	3 17	H-8

The solvent used in these experiments was C_6D_6

(*m/z* 372, 60%), which are in accord with C-6' hydroxyl and C-2' methoxyl functions, respectively, in the mass spectrum of the perdeuteromethyl (PDM) derivative of **1**, an ion appeared at $[M-34]^+$ in accord with 6'-hydroxyl in **1** having been converted to an $-OCD_3$ group [8–11]

In addition to giving signals for five methoxyl carbons between δ 55 and 60 6, the ^{13}C NMR of **1** gave signals for the carbons bearing protons at δ 91 35, 102 27 and 114 2 There were also ten carbon singlets ranging from δ 132 0 to 158 5 and a characteristic flavonoid carbonyl carbon at δ 176 9 All the above data suggested structure **1** for brickellin Confirmatory evidence was sought through the observation of nuclear Overhauser enhancements (NOEs) In preliminary experiments using the conventional integration method for measuring NOEs, irradiation of 4'-methoxyl gave a 25–35% enhancement of the 5'-H, irradiation of 7-methoxyl gave a similar enhancement of the 8-H and irradiation of the 2'-methoxyl gave *ca* 10% enhancement of the 3-H

Because of the limited accuracy of the conventional integration method, the observation of NOEs below 5–10% is extremely difficult by this technique Hall and Sanders [12, 13] have recently developed a refined method of observing NOEs by means of difference spectroscopy, which permits NOEs to be detected reliably at levels well below 1% This technique is rapidly proving its value in structural assignments [14, 15] When the NOE difference spectra of brickellin were recorded at 400 MHz in perdeuterobenzene, the results shown in Table 1 were obtained With the exception of the 3'-methoxyl (presumably because of its unique placement between two adjacent methoxyl groups), irradiation of each signal in the spectrum gave rise to at least one observable NOE It will be noted that irradiation of each of the two hydroxyl groups gave rise to a negative NOE on the other hydroxyl group This results from saturation transfer confirming the exchanging nature of these hydrogens Similarly, irradiation of 6-methoxyl resulted in NOEs on both the 5-hydroxyl and the 6'-hydroxyl

Several aspects of the spectral analysis of brickellin require further comment Brickellin exhibited an unusual UV spectrum in methanol exhibiting band I at 342 nm (in contrast, we found 5,7,2',4'-tetrahydroxy-6,5'-dimethoxyflavone gave band I at 366 nm in methanol [16]), moreover for **1** the band I was only about one-third as intense as band II (at 262 nm) We suggest that these peculiar spectral features result from the two groups at the 2'- and 6'-positions interfering with the coplanarity of the B-ring with the remainder of the molecule Another unusual UV characteristic of **1** was the band I shift of *ca* 32 nm with $AlCl_3$/HCl (6-methoxyflavones typically give a shift of 18 nm with $AlCl_3$/HCl), this may be a peculiarity of 6-methoxy-6'-hydroxyflavones since another 6-methoxy-2'-hydroxyflavone, 5,7,2',4'-tetrahydroxy-6,5'-dimethoxyflacone, gave a similar shift of 30 nm [16]

The only other flavone which, like brickellin, has four substituents in the B-ring is 6,7,2',3'-tetramethoxy-5,4',6'-trihydroxyflavone, recently isolated as one of the main constituents of the farinose exudate on fronds of *Notholaena aschenborniana* [17] The B-ring substitution pattern of brickellin differs from this compound only in that the hydroxyl group at C-4' is further methoxylated

EXPERIMENTAL

Plant material Collection data for *Brickellia veronicaefolia* (from south of Monterrey, Mexico) and *B chlorolepis* (from Alpine, Texas) were previously reported (refs [3] and [4], respectively)

General techniques Chromatography and spectral analyses were made using the standard procedures described in detail in earlier reports on *Brickellia* species [1–7] Brickellin was isolated as described in ref [3] and was finally separated from artemetin using PC on Whatman No 1 with 15% HOAc, the material was further purified by Sephadex L-20 chromatography and recrystallization from MeOH as yellow plates or needles, mp 197°

Brickellin (**1**) gave the following data Colour UV purple; NH_3 yellow/brown, NA* yellow/brown PC R_f 0 87 TBA, 0 35 15% HOAc UV λ_{max}^{MeOH} nm 345, 262, + NaOMe 402, 254, + $AlCl_3$ 394, 322sh, 272, + $AlCl_3$ + HCl 386, 318sh, 272, NaOAc 344, 260, + NaOAc + H_3BO_3 344, 260 Accurate mass $C_{20}H_{20}O_9$ calc 404 1107, obs 404 1102 MS underivatized *m/z* (rel int) 404 $[M]^+$ (100), 403 $[M-H]^+$ (14), 389 $[M-Me]^+$ 85, 372 $[M-OMe]^+$ (60), 361 $[M-CH_2-CO]^+$ (1), 296 A_1 (1), 181 $[A_1-Me]$ (33), 153 $[A_1-Me-CO]$ (29), 208 B_2 (31) MS derivatized (PDM), 438 $[M]^+$ (50), 423 $[M-Me]^+$ (100), 404 $[M-OCD_3]^+$ (34) ^1H NMR (100 MHz, CCl_4, TMS) δ 3 7–4 0 (15H, *s*, for 5-OMe's), 6 35 (1H, *s*, for 3-H), 6 45 (1H, *s*, for 3-H) and 6 88 (1H, *s*, for 5'-H) ^1H NMR (100 MHz, C_6D_6, TMS) δ 3 2 (3H, *s*, for 2'-OMe), 3 35 (3H, *s*, for 7-OMe), 3 48 (3H, *s*, for 6-OMe), 3 72 (3H, *s*, for 4'-OMe), 3 92 (3H, *s*, for 3'-OMe), 6 35 (1H, *s*, for 3-H), 6 45 (1H, *s*, for 8-H) and 6 88 (1H, *s*, for 5'-H) ^{13}C NMR (90–25 MHz, $CDCl_3$, ppm) δ 176 9 (*s*, C=O), 158 5 (*s*, C-2), 155 5 (*s*, C-8a), 153 4 (*s*, C-7), 152 3 (*s*), 150 8 (*s*), 143 1 (*s*), 136 3 (*s*), 132 0 (*s*, C-6), 110 9 (*d*, C-3), 108 2 (*s*, C-1'), 105 9 (*s*, C-4a), 102 6 (*d*, C-8), 90 4 (*d*, C-3'), 61 7 (*q*, OMe), 60 5 (*q*, OMe), 56 5 (*q*, OMe), 56 1 (*q*, OMe) and 55 7 (*q*, OMe) [18] A Bruker WH 360 spectrometer was used at 90 25 MHz for ^{13}C NMR and at 360 MHz for ^1H NMR A Bruker WH 400 was used at 400 MHz for ^1H NMR and for the NOE difference spectra

*NA refers to Naturstoggreagenz A in MeOH

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