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8,2′-DIHYDROXYFLAVONE FROM *PRIMULA PULVERULENTA*

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Key Word Index—*Primula pulverulenta*; Primulaceae; farinose excretion; 8,2′-dihydroxyflavone; 8,2′-dimethoxyflavone.

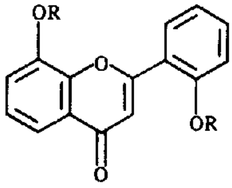
Abstract—A novel flavone, isolated from the farinose exudate of *Primula pulverulenta*, was identified as 8,2′-dihydroxyflavone. This is the first naturally occurring flavone, in which ring A is oxygenated only at C-8. The structure was deduced from spectral comparisons with the structurally related flavones: 5,2′-, 3,2′- and 2′,3′-dihydroxyflavone and confirmed by total synthesis. The spectral properties of other flavones oxygenation with a simple pattern are discussed in relation to 8,2′-dihydroxyflavone.

INTRODUCTION

The farinose excretion found on aerial parts of many *Primula* species was known previously to consist mostly of unsubstituted flavone, accompanied by 5-hydroxyflavone, 2′-hydroxyflavone, 5,8-dihydroxyflavone, and 5,2′-dihydroxyflavone, sometimes also by 5,8,2′-trihydroxyflavone [1]. Recently, 2′-methoxyflavone, 5-hydroxy-2′-methoxyflavone and 2′,4-dihydroxychalcone were found as rare minor constituents [2] and lately 2′,5′-dihydroxyflavone and its natural 5′-acetate were isolated [3] We now report the detection of a further unusual dihydroxyflavone from the exudate of *Primula pulverulenta* Duthie.

RESULTS AND DISCUSSION

Compound **1** (M^+ *m/z* 254) was isolated as a trace constituent from the powdery exudate of *Primula pulverulenta* after fractionation by column chromatography and preparative TLC. Methylation gave a dimethyl ether (**2**; M^+ 282) by usual methylation. In the MS of **1**, the fragments (*m/z* 137 $[A_1+H]^+$, 136 $[A_1^+]$, and 118 $[B_1^+]$ showed that the A and B rings of the flavone both possessed only one hydroxyl group, which was supported also by the fragments (*m/z* 150 $[A_1^+]$, 122 $[A_1-28]^+$, 132 $[B_1^+]$, and 107 $[B_2-28]^+$) in the MS of **2**. The UV spectrum gave no clear information on the oxygenation pattern because of unfamiliar shifts on addition of reagents. For example, no shifts were observed on addition of aluminium chloride or sodium acetate. This at least indicates the absence of hydroxyl groups at C-5 and C-7. A large bathochromic shift (+63 nm) with sodium methoxide showed a hydroxyl group on ring B located at C-2′ rather than at C-4′ [4]. This result explains the complex signals of the ^1H NMR spectrum of **1** [270 MHz, DMSO-d_6–$(CD_3)_2CO$ = 2:1] (see Experimental). A broad doublet at 7.51 ppm, assignable to a proton at C-5, suggested that this proton was *ortho* coupled with that of C-6 in $J = 7.69$ Hz and *meta* coupled with that of C-7 in a small J value. From the spectral data described above the most plausible position for the hydroxyl group on the A ring was concluded to be at C-8, and the possible structure for the natural flavone to be 8,2′-dihydroxyflavone. Traces of the novel 8,2′-dihydroxyflavone were also observed in the exudate of some other *Primula* species, such as *P. beesiana* Forrest, *P. bulleyana* Forrest, *P. palinuri* Petagna. Its distribution in the genus still needs to be studied in detail.



1 R = H
2 R = Me

In our present experiments the lack of available spectral data for simple oxygenated flavones became obvious. This lack of relevant data may have led to the assignment of incorrect oxygenation patterns for such compounds. In order to confirm the proposed structure of the naturally occurring flavone (**1**) and to supply additional spectral information for simple oxygenated flavones, 8,2′-dihydroxyflavone (**1**), and the related compounds: 5,2′-dihydroxy- (**3**), 3,2′-dihydroxy- (**4**) and 2′,3′-dihydroxyflavone (**5**) were prepared by synthesis. For comparison purposes, 2′-hydroxy- (**6**) and 8-methoxyflavone (**7**) were also synthesized. The flavones (**3**–**7**) are already known compounds; in the present paper we only report the synthesis of **1**. 2-Hydroxy-3-methoxyacetophenone (**8**) synthesized in four steps from *o*-vanillin (see Experimental), was condensed with 2-methoxybenzaldehyde (**9**) in the presence of potassium

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Table 1 UV spectra of compounds **1**, **3–5** and bathochromic shifts on addition of reagents

	1	**3**	**4**	**5**
λ_{max}^{MeOH} nm (log ε)	264 (4 06) 330 (3 91)	269 (4 30) 340 (4 12)	240 (4 32) 290 sh (3 86) 335 (4 04) 390 (3 99)	250 (4 13) 310 (4 29)
+ NaOMe	278, 295 sh 393	250, 265 295 sh, 400	250 sh, 300 sh 332, 390	240 sh, 310 325 sh, 410 (dec)
+ $AlCl_3$	265, 331	280 sh, 290 315 sh, 355 390 sh	250, 320 sh 390	240, 315 330 sh, 386
+ $AlCl_3$–HCl	265, 290 sh 331	280 sh, 288 350, 390	250, 320 sh 390	250, 308
+ NaOAc	268, 285 sh 330	270, 340 410 sh	245 sh, 300 sh 330, 390	240 sh, 255 sh 312, 376
+ NaOAc–H_3BO_3	265, 330	270, 340	245, 295 sh 320 sh, 350	255 sh, 312 376

Table 2 MS of compounds **1–5** 2′-hydroxy- (**6**) and 8-methoxyflavone (**7**)

	1	**2**	**3**	**4**	**5**	**6**	**7**
$[M]^+$	254 (4 3)	282 (66 2)	254 (100)	254 (77 8)	254 (100)	238 (100)	252 (100)
$[M^+ - 28]^+$	226 (*t*)	254 (0)	226 (6 0)	226 (0)	226 (8 0)	210 (12 9)	224 (4 9)
A_1^+	136 (100)	150 (32 9)	136 (49 8)	120 (3 3)	120 (6 2)	120 (40 0)	150 (53 1)
$[A_1 + 1]$	137 (36 7)	151 (100)	137 (28 4)	121 (30 7)	121 (83 1)	121 (63 3)	151 (5 6)
$[A_1 - 28]^+$	108 (13 8)	122 (62 7)	108 (26 0)	92 (4 7)	92 (0)	92 (17 8)	122 (60 0)
$[A_1 - 29]^+$	107 (0)	121 (16 9)	107 (*t*)	91 (*t*)	91 (0)	91 (5 6)	121 (16 9)
B_1^+	118 (4 0)	132 (8 0)	118 (8 7)	–	134 (17 8)	118 (39 8)	102 (4 4)
B_2^+	121 (*t*)	135 (*t*)	121 (2 4)	121 (30 7)	121 (*t*)	121 (63 3)	105 (3 1)
$[B_2 - 28]^+$	93 (*t*)	107 (12 9)	93 (*t*)	93 (8 4)	109 (0)	93 (10 2)	77 (0)
Other fragments	132 (5 1)	253 (4 2)	237 (4 0)	237 (100) 197 (6 7) 181 (4 6) 134 (5 8) 127 (6 4) 100 (8 0)			112 (6 2) 76 (7 6)

t Trace

hydroxide to give 2′-hydroxy-2,3′-dimethoxychalcone (**10**) The chalcone was oxidized with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to afford 8,2′-dimethoxyflavone (**2**), which agreed well the compound obtained by methylation of the naturally occurring flavone (**1**) (TLC, MS and ^{1}H NMR) **2** was treated with boron tribromide to yield **1** It is known that the demethylation of flavones with a methoxy group at C-2′ causes a rearrangement between the A and B rings The flavone (**2**) on demethylation might also yield 2′,3′-dihydroxyflavone (**5**) instead of **1** Direct comparison of **1** and **5** showed, however, that in the present demethylation no rearrangement occurred

Spectral data (UV, MS and ^{1}H NMR) of the flavones (**1**–**5**) are shown in Tables 1–3 In the UV spectra, all flavones except **4** show the characteristic bathochromic shifts (> 60 nm) of 2′-oxygenated flavones on addition of sodium methoxide [4] Conspicuous shifts were not observed in the case of **1**, which might be peculiar to 8-oxygenated flavones. According to the general rules on mass spectral fragmentation of flavones, the mass spectrum gave important clues to evaluating the number of hydroxyl groups However, the rules showed similar fragments for **1** and **3** although the fragments for **6** and **7** provided useful information in the understanding of those of **1** and **2** In 2′-oxygenated flavones, a C-6 proton was observed at *ca* 7 9 ppm in a lower field, but in the case of the corresponding flavonols like **4**, the proton shifted up-field by *ca* 0 5 ppm and was observed at 7 5 ppm Generally in 2′-oxygenated flavones, the C-4′ and C-6′ protons were *meta* coupled in 1 7 Hz On the contrary *meta*-coupling between C-3′ and C-5′ was too small to be clarified and gave only broader signals All

Table 3 ^{1}H NMR spectra in DMSO-d$_6$ of compounds **1**, **3**–**5**

	1	**3**	**4**	**5**
3	7.14 *s*	7 20 *s*	—	7 11 *s*
5	7 239 *d**	—	8.15 *dd*	8 03 *dd*
	(5 86)		(1 46, 8 06)	(1 46, 7 51)
6	7.43 *dd*	7.14 *dd*	7 43–7 49 *m*	7 46 *dt*
	(4 01, 5 86)	(8.43, 0 74)		(1 46, 7 51)
7	7 236 *d**	7 56 *t*	7 76 *ddd*	7 79 *ddd*
	(4 01)	(8.43)	(1 46, 6 96, 8 06)	(1 46, 7 51, 8.41)
8	—	6 78 *dd*	7 60 *d*	7 68 *dd*
		(0.74, 8 43)	(8 06)	(1 46, 8 41)
2′			—	—
3′	7.05 *br d*	7 07 *dd*	6 99 *d*	—
	(7 33)	(1.09, 8.24)	(7 33)	
4′	7 37 *dt*	7.41 *dd*	7 34 *dt*	6 98 *dd*
	(1 46, 7 33)	(1 47, 7 51, 8 24)	(1 46, 7 33)	(1 46, 7.70)
5′	7 01 *br t*	7 01 *dt*	6 93 *t*	6.81 *dd*
	(7 33)	(1 09, 7 51)	(7.33)	(7 70, 8 06)
6′	7 99 *dd*	7 92 *dd*	7 43–7 49 *m*	7 35 *dd*
	(1 46, 7 33)	(1 47, 7.51)		(1 46, 8 06)
OH	10.35 *br s*	10 83 *br s*	9.01 *br s*	9 72 *br s*
	10 68 *br s*	12.72 *s*	9 76 *br s*	

*Interchangeable

the present assignments supply important informations for the structural elucidation of flavones with a simple oxygenation pattern.

EXPERIMENTAL

Mps uncorr ^{1}H NMR spectra were obtained at 270 MHz, MS were determined at 70 eV by direct inlet

Plant material and isolation of compound **1**. Aerial parts of *Primula pulverulenta* (leaves and inflorescences) were rinsed with Me$_2$CO to dissolve the farinose exudate, which was concd *in vacuo* and fractionated by CC on silica gel and polyamide Compd **1** was isolated in minute amt after prep TLC on silica gel of a minor fraction On polyamide TLC with toluene–dioxane–MeOH (8 · 1 · 1) it occurs as a pale greeish spot at R_f 0.20 that does not change its colour on spraying with Naturstoffreagenz A

Compound **1** Yellow rectangles, mp >250° (dec). ^{1}H NMR (DMSO-d_6–acetone-d_6) δ 7 04 (1H, *br t*, J = 7 69 Hz, H-5′). 7 15 (1H, *br d*, J = 8.42 Hz, H-3′), 7 23 (1H, *d*, J = 7.69 Hz, H-7), 7 28 (1H, *s*, H-3), 7 29–7 36 (2H, *m*, H-6 and 4′), 7.51 (1H, *br d*, J = 7 69 Hz, H-5), 8.10 (1H, *br d*, J = 7 69 Hz, H-6′) The UV spectrum is identical with that of a synthetic sample shown in Table 1 Methylation of **1** was achieved in the usual manner with MeI in Me$_2$CO in the presence of K_2CO_3

Synthesis of 2-hydroxy-3-methoxyacetophenone (**8**). 2-Hydroxy-3-methoxybenzaldehyde (*o*-vanillin) (7 5 g, 49 mmol), hydroxylanime hydrochloride (3.4 g, 49 mmol), HCOONa (6 3 g), and HCOOH (70 ml) were refluxed for 1 hr The reaction mixture was poured into H_2O and extracted with EtOAc *o*-Vanillonitrile was obtained as a colourless oil (6.2 g), IR ν^{film} cm^{-1} 2260 (C≡N) The nitrile (5 g, 33 mmol) was methylated with dimethyl sulphate (5 g, 40 mmol), K_2CO_3 (25 g, excess) in Me$_2$CO (50 ml) to give *o*-veratronitrile (4 8 g) as colourless needles, mp 43–45° An Et$_2$O soln containing *o*-veratronitrile (4.5 g, 28 mmol) was added to an Et$_2$O soln of Grignard reagent [MeI (7 9 g, 56 mmol), and Mg (1 3 g, 56 mmol)]. The soln was stirred under reflux for 15 hr, and then heated in the usual way to give **8** (2 8 g) as pale yellow needles, mp 48–50°, ^{1}H NMR (60 MHz, CDCl$_3$) δ 2 57 (3H, *s*, COMe), 3.82 (3H, *s*, OMe), 6 52 (1H, *t*, J = 7 5 Hz, H-5), 6 93 (1H, *dd*, J = 7 5 Hz, H-4), 7 10 (1H, *dd*, J = 2 0, 7.5 Hz, H-6), 12 16 (1H, *br s*, OH)

Synthesis of 8,2′-dihydroxyflavone (**1**) To a soln of methylcellosolve (50 ml) containing **8** (1 7 g, 10 mmol) and **9** (1 4 g, 10 mmol) a 5 N KOH (10 ml) was added and the soln stirred overnight After acidification with 1 N HCl and extraction with EtOAc **10** was obtained as orange needles, 2 4 g, mp 109–110° (MeOH) MS *m/z* (rel int) 284 [M$^+$] (91 6), 269 (8.7), 253 (8 0), 177 (11 1), 161 (11 6), 150 (95 6), 142 (8 0), 134 (25 1), 122 (100) ^{1}H NMR (DMSO-d_6) δ 3 83, 3 92 (3H, *br t*, J = 7.69 Hz, H-5), 7 11 (1H, *br d*, J = 7 69 Hz, H-3), 7 23 (1H, *dd*, J = 8,06, 1.47 Hz, H-6′), 7 46 (1H, *dt*, J = 1 47, 7.69 Hz, H-4), 7 74 (1H, *dd*, J = 1 46, 8 06 Hz, *H*-4′), 7 93 (1H, *d*, J = 15 75 Hz, *H*-α), 7 95 (1H, *dd*, J = 1 46, 7 69 Hz, H-6), 8 15 (1H, *d*, J = 15 75 Hz, H-β), 12 69 (1H, *s*, OH) A dry dioxane soln containing **10** (2 3 g, 8.1 mmol) and DDQ (4 5 g, 16 mmol) was boiled under reflux for 7 hr By silica gel CC of the reaction mixture (eluent EtOAc–C_6H_{14}), **2** was obtained as colourless needles (1 8 g), mp 136° (EtOH) ^{1}H NMR [270 MHz, (CD$_3$)$_2$CO] δ 4 01, 4 04 (3H, each *s*, OMe), 7 02 (1H, *s*, H-3), 7.16 (1H, *dt*, J = 1 1, 7 51 Hz, H-5′), 7 24 (1H, *br d*, J = 8 06 Hz, H-3′), 7 35 (1H, *d*, J = 6 59 Hz, H-5), 7 37 (1H, *d*, J = 3 30 Hz, H-7), 7 56 (1H, *ddd*, J = 1 46, 7 51, 8 06 Hz, H-4′) 7 64 (1H, *dd*, J = 3 30, 6 59 Hz, H-6), 8 02 (1H, *dd*, J = 1 46, 7.51 Hz, H-6′) To a dichloromethane soln containing **2** (1 2 g, 4 mmol) added BBr$_3$ (1 ml) at −70° The reaction mixture was left at room temp overnight, poured into H_2O and extracted with EtOAc to give **1** as a yellow powder, mp >250° (EtOH) The spectral data are shown in Tables 1–3

Syntheses of **3**–**7**. According to our previous paper [5], **3** (mp 271–272°) (lit [6] mp 273–274°), **4** (mp 197–198°) (lit [7] mp 209°), **5** (mp 232–233° (dec) (lit [8] mp 246° [dec]), **6** (mp 218–220°) (lit [6] mp 250–251°), and **7** (mp 200–203°) (lit [8] mp 200–202°) were synthesized and these samples used for the spectral measurements

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