2′,5′-Dihydroxyflavone and its 5′-Acetate – Novel Compounds from the Farinose Exudate of *Primula*

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2′,5′-Dihydroxyflavone and its 5′-acetate were isolated from the farinose exudate of *Primula japonica* and *P. pulverulenta*. Their structures were elucidated by spectroscopic methods and confirmed by synthesis. Both flavones are novel natural products.

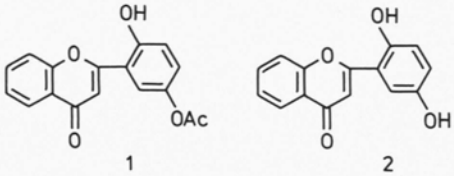
Introduction

In botany textbooks the farinose coating on leaves and inflorescences of many *Primula* species is still often said to "consist of flavone". We have shown earlier [1, 2] that it is in fact formed by varying amounts of unsubstituted flavone (at least 50%), 5-hydroxy flavone and 2′-hydroxy flavone, accompanied in many species by 5,8-dihydroxy flavone (primetin) and 5,2′-dihydroxyflavone. Further components with scattered distribution are 5,8,2′-trihydroxy flavone and 3′,4′-dihydroxy flavone, the latter always occurring only as a trace constituent [3]. Recently we identified 2′-methoxyflavone, 5-hydroxy-2′-methoxyflavone and 2′,4′-dihydroxy chalcone as new constituents of *Primula* exudate and mentioned the existence of further compounds [4]. Now we wish to report on the identification of a novel flavone and its natural acetate from this material as well as on the synthesis of this flavone.

Materials and Methods

*Isolation of products **1** and **2***

Material of *Primula japonica* and *P. pulverulenta* was obtained from the Botanischer Garten der TH Darmstadt. The farinose exudate from leaves and inflorescences was recovered and prepared as described previously [4]. From the farina of *P. pulverulenta* we isolated compound **1** which occurs on polyamide TLC (toluene/dioxane/MeOH 80:10:10) as a spot with light turquoise fluorescence (UV_{366}). This is similar to 2′-hydroxy flavone but with a slightly higher R_f and hence is partly concealed by the latter. Separation is improved on silica (toluene/dioxane/glac. acetic acid 90/25/4), where the unknown product **1** exhibits lower R_f than 2′-hydroxy flavone. Preparative TLC on silica was used, therefore, to isolate this product. A more polar component **2** which showed a spot of similar colour to **1** was also isolated by preparative TLC on silica from relevant fractions of *P. pulverulenta* as well as from *P. japonica*.



Compound **1** crystallized from ethyl acetate as colourless needles, m.p. 221–222 °C. It exhibits the following spectral properties: UV λ_{max}^{MeOH} (nm) 332, 308, 286, 241; unchanged with $AlCl_3$; + NaOH 428, 303, MS *m/z* (rel.int.) 298 (14, M^+), 254 (100, MOAc), 237 (8), 226 (9), 197 (4), 134 (10), 121 (42), 105 (5), 42 (34). For ^1H NMR data see Table I.

Compound **2** could not be crystallized, due to lack of material. UV λ_{max}^{MeOH} (nm) 360, 296, 247. MS *m/z* (rel.int.) 254 (88, M^+), 238 (10), 237 (13), 226 (13), 197 (9), 134 (32), 121 (100), 105 (15).

Compound **1** was hydrolyzed by adding a few drops of conc. HCl to a solution of **1** in boiling glac. acetic acid. Methylation of compound **2** was done with dimethyl sulphate to yield **5**.

*Synthesis of **1** and its diacetate*

2-Hydroxyacetophenone (700 mg, 5 mmol) was condensed with 2,5-diisopropyloxybenzaldehyde (1.1 g, 5 mmol) in the presence of KOH (3 g) to give 2′-hydroxy-2,5-diisopropyloxychalcone as yellow needles (1.4 g), m.p. 94–95 °C (MeOH). A dry dioxane solution containing the chalcone (1.0 g, 3 mmol) and 2,3-dichloro-5,6-dicyanobenzoquinone (1.36 g, 6 mmol) was heated under reflux for 9 h.

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Table I. ^{1}H NMR spectra of compounds **1**–**5** (in $CDCl_3$, **2** in d_6-DMSO; in ppm/TMS; J in Hz. JEOL GX 270).*

	1	**2**	**3**	**4**	**5**
H-3	7.30 s	7.17 s	7.13 s	6.72 s	7.26 s
H-5	8.30 dd (1.2, 7.7)	8.40 dd (1.5, 7.69)	8.25 br d (7.55)	8.23 br d (8.11)	8.24 dd (1.5, 8.11)
H-6	7.47 br t (7.7)	7.49 t (7.69)	7.44 t (7.55)	7.45 t (8.11)	7.42 t (8.11)
H-7	7.77 dt (7.7)	7.83 dt (1.5, 7.69)	7.67 br t (7.55)	7.63 br t (8.11)	7.68 dt (1.5, 8.11)
H-8	7.65 br d (7.7)	7.71 d (7.69)	7.52 d (7.55)	7.52 d (8.11)	7.53 d (8.11)
H-3′	7.28 d (8.9) m	6.89 d (8.60)	6.97–7.02 m	7.23 d (8.97)	6.98 d (8.98)
H-4′	7.16 d (2.3, 8.9)	6.85 d (2.5, 8.60)		7.29 dd (2.57, 8.97)	7.04 dd 2.5, 8.89
H-6′	7.74 d (2.3)	7.32 d (2.5)	7.44 d (1.5)	7.57 d (8.97)	7.46 d (2.5)
	8.07 (OH) 2.35 (Ac)	9.13, 10.02 (OH)	1.36, 1.38 ($-CH(CH_3)_2$) 4.55 (CH)	2.31, 2.35 (Ac)	3.86, 3.98 (OMe)

When cool, the reaction mixture was subjected to CC and eluted with $CHCl_3$. From the early fractions, 2′,5′-diisopropyloxyflavone (**3**) was obtained as a pale yellow oil (570 mg). – BCl_3 (1 ml) was added to a CH_2Cl_2 solution (20 ml) of the flavone (**3**, 520 mg) at −60 °C. The solution was left at room temp. for 40 min, then poured into water. By the normal preparative procedure 2′,5′-dihydroxyflavone (**2**) was obtained as pale yellow needles (270 mg), m.p. 173–175 °C (AcOEt/C_6H_{12}). UV λ_{max}^{MeOH} (nm) 364, 295, 246; unchanged with $AlCl_3$; + NaOH 440, 303 → dec. 2′,5′-dihydroxyflavone (**2**, 100 mg) was acetylated by the acetic anhydride/pyridine method to give 2′,5′-diacetoxyflavone (**4**) as a colourless powder, m.p. 92–94 °C (MeOH). For ^{1}H NMR data of the synthetic products see Table I.

Results and Discussion

In the mass spectrum of compound **1**, the base peak occurred at M-42, indicating loss of an acetyl group. Acidic hydrolysis of **1** yielded a product that was shown to be identical with **2**. The M^+ of **2** at *m/z* 254 pointed to a flavone with 2 OH-groups and the base peak at *m/z* 121 indicated an unsubstituted A-ring. Hence both OH-groups should be placed on the B-ring, one of them being located at C-2′ as indicated by the fluorescence on TLC. The position of the second OH-group was deduced from comparisons of the NMR spectrum of hydrolyzed and methylated compound **1** (**2**/**5**) with the spectra of flavones with dioxygenated B-rings (2′,3′/2′,4′/2′,5′/2′,6′/3′,4′) [5], which showed that **1** and **2** must be 2′,5′-oxygenated. Synthesis of 2′,5′-dihydroxyflavone **2** and its diacetate **4** proved that hydrolyzed **1** and natural **2** were indeed identical with 2′,5′-dihydroxyflavone and the diacetate **4** was identical with acetylated **1**. In the NMR spectrum of **2**, the proton signal for H-3 appeared at 7.17 ppm, rather similar to the chemical shift of **1**. In contrast, the chemical shift for the diacetate **4** was observed at a higher field (6.27 ppm). As already reported [6], introduction of an acetyl group at 2′ causes an upper field shift of H-3 (0.4–0.7 ppm). This phenomenon proves that the acetyl group in **1** is located at C-5′. Hence the novel natural product **1** is definitively identical with 2′-hydroxy-5′-acetoxy flavone and **2** is 2′,5′-dihydroxyflavone. Both compounds are novel natural products.

2′,5′-Dihydroxyflavone (**2**) and its 5′-acetate (**1**) were also observed in the farinose exudate of several other *Primula* species such as *P. beesiana, P. bulleyana,* and *P. palinuri.* The monoacetate **1** seems normally to be produced in higher amounts than the parent compound **2**. The identification of these flavones again emphasizes the particular capacity of

Primula glandular trichomes for biosynthesis of 5,7-deoxyflavones. This contrasts with the leaf and flower tissue which, according to Harborne's earlier extensive studies [7], accumulate glycosides based on kaempferol and quercetin, and sometimes on herbacetin and quercetagetin, *i.e.* polyoxygenated flavonols with the usual 5,7-dioxy-substitution pattern.

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