# 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

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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  $R_{\rm f}$  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  $\lambda_{max}^{MeOH}$  (nm) 332, 308, 286, 241; unchanged with AlCl<sub>3</sub>; + NaOH 428, 303, MS m/z (rel.int.) 298 (14, M<sup>+</sup>), 254 (100, MOAc), 237 (8), 226 (9), 197 (4), 134 (10), 121 (42), 105 (5), 42 (34). For <sup>1</sup>H NMR data see Table I.

Compound **2** could not be crystallized, due to lack of material. UV  $\lambda_{\text{max}}^{\text{MeOH}}$  (nm) 360, 296, 247. MS m/z (rel.int.) 254 (88, M<sup>+</sup>), 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.



Table I. <sup>1</sup>H NMR spectra of compounds **1–5** (in CDCl<sub>3</sub>, **2** in d<sub>6</sub>-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	8.40 dd	8.25 br d	8.23 br d	8.24 dd
	(1.2, 7.7)	(1.5, 7.69)	(7.55)	(8.11)	(1.5, 8.11)
H-6	7.47 br t	7.49 t	7.44 t	7.45 t	7.42 t
	(7.7)	(7.69)	(7.55)	(8.11)	(8.11)
H-7	7.77 dt	7.83 dt	7.67 br t	7.63 br t	7.68 dt
	(7.7)	(1.5, 7.69)	(7.55)	(8.11)	(1.5, 8.11)
H-8	7.65 br d	7.71 d	7.52 d	7.52 d	7.53 d
	(7.7)	(7.69)	(7.55)	(8.11)	(8.11)
H-3'	7.28 d	6.89 d		7.23 d	6.98 d
	(8.9) m	(8.60)		(8.97)	(8.98)
			6.97-7.02 m		
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	7.32 d	7.44 d	7.57 d	7.46 d
	(2.3)	(2.5)	(1.5)	(8.97)	(2.5)
	8.07 (OH) 2.35 (Ac)	9.13, 10.02 (OH)	1.36, 1.38 (-CH(CH <sub>3</sub> ) <sub>2</sub> ) 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<sub>3</sub>. From the early fractions, 2',5'-diisopropyloxyflavone (3) was obtained as a pale yellow oil (570 mg). - BCl<sub>3</sub> (1 ml) was added to a CH<sub>2</sub>Cl<sub>2</sub> 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<sub>6</sub>H<sub>12</sub>). UV λ<sub>max</sub> (nm) 364, 295, 246; unchanged with AlCl<sub>3</sub>; + NaOH 440, 303  $\rightarrow$  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 <sup>1</sup>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 Aring. 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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