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5,2′,5′-TRIHYDROXYFLAVONE AND 2′,β-DIHYDROXYCHALCONE FROM *PRIMULA PULVERULENTA*

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Key Word Index—*Primula pulverulenta*; Primulaceae; farinose exudate; 5,2′,5′-trihydroxyflavone; 2′,β-dihydroxychalcone; conversion of β-diketone into flavone.

Abstract—A new flavone, 5,2′,5′-trihydroxyflavone and 2′,β-dihydroxychalcone (β-diketone) were isolated from the farinose exudate of *Primula pulverulenta*. These structures were established on the basis of spectral data and confirmed by total synthesis. A transformation experiment suggests that the β-diketone is converted into flavone during isolation procedures.

INTRODUCTION

In recent communications, we reported two novel flavones, namely, 8,2′-dihydroxyflavone and 5,2′-dihydroxyflavone, as well as the natural 5′-acetate of the latter, as constituents of the farinose exudate of *Primula pulverulenta* and *P. japonica*, respectively [1, 2]. Investigation of the remaining minor fractions led to the isolation of two further products. In the present paper we report their structural elucidation as a novel flavone and a novel β-hydroxychalcone (β-diketone).

RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated from the farinose exudate of *Primula pulverulenta* Duthie (Primulaceae) by preparative TLC of some minor fractions on silica gel and finally obtained in pure form by recrystallization. In the MS of compound **1**, three prominent fragments at *m/z* 137, 136 and 134 due to $[A_1+H]^+$ and/or B_2^+, $A_1^{+\cdot}$, and $B_1^{+\cdot}$ were observed besides a molecular ion at *m/z* 270 ($C_{15}H_{10}O_5$), which indicated that **1** was flavone bearing one hydroxyl group in the A ring, and two hydroxyl groups in the B ring. The ^{1}H NMR spectrum exhibited seven aromatic protons. A one-proton singlet at 7.32 ppm was readily assigned to the C-3 proton. Of the remaining six protons, two one-proton double-doublets at 6.83 ($J=1.0$, 8.3 Hz), 7.13 ($J=1.0$, 8.3 Hz) and one-proton triplet at 7.67 ppm ($J=8.3$ Hz) were attributable to the protons in the A ring at C-6, C-8 and C-7 due to $J_{ortho}=8.3$, $J_{meta}=1.0$ Hz. In contrast, three one-proton double–doublets at 6.89 ($J=2.8$, 8.3 Hz), 6.91 ($J=1.0$, 8.3 Hz) and 7.32 ($J=1.0$, 2.8 Hz) were assignable to the protons in the B ring at C-4′, C-3′ and C-6′ due to $J_{ortho}=8.3$, $J_{meta}=2.8$ and $J_{para}=1.0$ Hz. Hence the positions of hydroxyl groups were deduced to be at C-5, C-2′ and C-5′, respectively. Taking into account only the coupling constants mentioned above, further substitutions can be considered, e.g. 8-hydroxy instead of 5-hydroxy for the A ring, 3′,4′-dihydroxy- or 2′,4′-dihydroxy instead of 2′,5′-dihydroxy for the B ring. These substitution patterns were, however, rejected on the basis of the chemical shifts observed in the NMR spectrum. The bathochromic shifts of band I in the UV spectrum (+38 by sodium methoxide; +40 nm by aluminium chloride) well supported the structure of 5,2′,5′-trihydroxyflavone for compound **1**. The structure was confirmed by direct comparison with the synthetic product prepared by condensation of 2-hydroxy-6-methoxyacetophenone with 2,5-dibenzyloxybenzaldehyde followed by the usual treatments (see Experimental).

Compound **2**, mp 114–117°, was obtained as yellow prisms from MeOH. The MS showed a parent peak at *m/z* 240, an empirical formula of which corresponds to $C_{15}H_{12}O_3$. Characteristic absorption bands of the UV spectrum (254 and 364 nm) indicated that **2** had the same β-diketone skeleton as found for licodione isolated from the cell cultures of *Glycyrrhiza echinata* [3]. In the ^{1}H NMR spectrum two one-proton double doublets at 6.92 ($J=1.10$, 8.06, 8.43 Hz) assigned H-3′ and 7.46 ($J=1.47$, 8.06, 8.43 Hz) (H-4′) and two one-proton double doublets at 7.00 ($J=1.10$, 8.43 Hz) (H-5′) and 7.76 ppm ($J=1.47$, 8.06 Hz) (H-6′) were observed, which led us to place a hydroxyl group at C-2′ in the A ring. A two-protons double doublet at 8.06 ppm ($J=1.46$ and 8.06 Hz) assigned to C-2 and C-6 and three-protons multiplet showed the B ring was unsubstituted. The mass fragments at *m/z* 120, 121, and 105 supported the above substitution pattern. Hence the structure of **2** was deduced to be 2′,β-dihydroxychalcone and confirmed by comparison with the synthetic sample prepared by a standard method.

The farinose of *Primula* species are well known for the biosynthesis of unsubstituted flavone, the precursor of which was isolated for the first time in the present study. 2′,β-Dihydrochalcone is a new natural representative of the rare group of β-diketones or dibenzoylmethanes and 5,2′5′-trihydroxyflavone is a novel flavone. The latter can not be detected in crude solutions of *Primula* exudate nor in major fractions, as the amount present is extremely low and the relevant spot is concealed, on polyamide TLC, by both 5,8,2′-trihydroxyflavone and particularly by the turquoise fluorescent spot of 5′-acetoxy-2′-hydroxyflavone. Therefore, we can not yet tell whether this new

flavone is also present in further *Primula* species. However, traces of the diketone were observed in the farinose exudate of *P. japonica*.

On purification of **2** by preparative TLC, a new band appeared, which was shown to be unsubstituted flavone. This observation suggested that a certain portion of **2** was converted into flavone during this procedure. Quantitative analysis by HPLC was performed in order to investigate the stability of **2**. The result is shown in Fig. 1. Compound **2** was linearly converted into flavone by dehydration, and *ca* 65% hr. Under the same conditions 2,2′,β-trihydroxychalcone was not converted into 2′-hydroxyflavone, which has also been reported as constituent of *P. farina*.

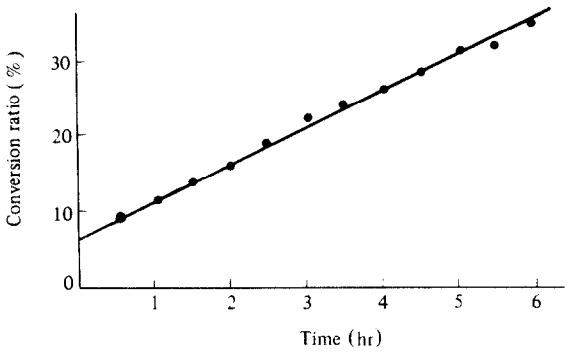

Fig. 1 Conversion of 2′,β-dihydroxychalcone into flavone in the presence of silica gel.

EXPERIMENTAL

Chemical shifts are given in δ (ppm). Mps: uncorr.

Fractions obtained from the farinose exudate of *Primula pulverulenta* by CC on silica gel, as reported earlier [1, 2], were further analysed by prep. TLC on silica gel (toluene–dioxane–HOAc 19:5:1) and the products of interest were purified by TLC on silica gel (ETOAc–hexane 1:1) to yield **1** (2 mg) and **2** (4 mg). The latter was recryst. from MeOH.

Compound **1**. Mp was not measured because of the small quantity available. MS *m/z* (rel. int.): 270 [M^+] (100), 253 (7.8), 242 (5.6), 213 (2.4), 197 (0.9), 187 (1.0), 161 (0.7), 149 (2.4), 138 (15.3), 137 (67.8), 136 (17.8), 135 (12.2), 134 (21.8), 121 (4.0), 108 (13.8), 84 (20.0). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.83 (1H, *dd*, J = 1.0, 8.3 Hz, H-6), 6.89 (1H, *dd*, J = 2.8, 8.3 Hz, H-4′), 6.91 (1H, *dd*, J = 1.0, 8.3 Hz, H-3′), 7.13 (1H, *dd*, J = 1.0, 2.8 Hz, H-6′), 7.67 (1H, *t*, J = 8.3 Hz, H-7), 9.18 (2H, *br s*, 2 × OH), 12.72 (1H, *br s*, OH). UV λ_{max}^{MeOH} (log ε) nm: 266 (4.36), 295 sh (4.04), 366 (4.08); + NaOMe: 262 sh, 354, 404 (dec.); + $AlCl_3$: 276, 290 sh, 310 sh, 406; + $AlCl_3$/HCl: 276, 290 sh, 308 sh, 400; NaOAc: 266, 295 sh, 366; + NaOAc/H_3BO_3: 266, 295 sh, 366.

Synthesis of 5,2′,5′-*trihydroxyflavone*. To a soln (60 ml) of 2-hydroxy-6-methoxyacetophenone (0.7 g, 4.2 mmol) and 2,6-dibenzyloxybenzaldehyde (1.4 g, 4.2 mmol) in EtOH, 25% KOH (20 ml) was added. The reaction mixture was stirred at room temp. overnight. By usual work-up procedure, 2,5-dibenzyloxy-2′-hydroxy-6′-methoxychalcone (1.8 g) was obtained as yellow needles, mp 117–119° (MeOH). MS *m/z* (rel. int.): 466 [M^+] (4.0), 448 (2.4), 375 (6.2). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 3.74 (3H, *s*, OMe), 5.06, 5.14 (2H, each *s*, $OC\underline{H}_2Ph$), 6.36 (1H, *d*, J = 8.06 Hz, H-5′), 6.60 (1H, *d*, J = 8.06 Hz, H-3′), 6.89 (1H, *d*, J = 8.16 Hz, H-3), 6.95 (1H, *dd*, J = 2.57, 8.16 Hz, H-4), 7.22–7.45 (12H, H-6, 4′ and 2 × $OCH_2\underline{Ph}$), 7.90 (1H, *d*, J = 15.76 Hz, H-β), 8.14 (1H, *d*, J = 15.76 Hz, H-α), 13.11 (1H, *s*, OH). The chalcone (1.1 g, 2.3 mmol) was refluxed with 2,3-dichloro-5,6-dicyanobenzoquinone (1.1 g, 4.7 mmol) in dry dioxane (40 ml) for 6 hr. After CC of the reaction product on silica gel (eluent: EtOAc–hexane 1:1), 2′,6′-dibenzyloxy-5-methoxyflavone (0.65 g) was obtained as pale brown prisms, mp 149–150° (EtOAc). MS *m/z* (rel. int.): 464 [M^+] (14.9), 373 (100), 281 (3.6), 253 (2.3), 236 (3.1), 91 (100). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 3.96 (3H, *s*, OMe), 5.04, 5.12 (2H, each *s*, 2 × $OC\underline{H}_2Ph$), 6.77 (1H, *br d*, J = 8.42 Hz, H-6), 6.93 (1H, *br d*, J = 8.42 Hz, H-8), 7.03 (1H, *s*, H-3), 6.96–7.45 (13H, *m*, H-3′, 4′, 6′ and 2 × $OCH_2\underline{Ph}$), 7.50 (1H, *t*, J = 8.47 Hz, H-7). To a soln of the above flavone (0.5 g) in CH_2Cl_2, BCl_3 (1 ml) was added at −60°. The soln was left at room temp. for 1 hr and then poured into H_2O. After extraction with EtOAc and concn, 5,2′,5′-trihydroxyflavone (0.15 g) was obtained as a yellow powder, mp > 270° (dec.). MS *m/z* (rel. int.): 270 [M^+] (100), 253 (7.1), 242 (6.0), 213 (2.2), 137 (62.2), 134 (20.4), 121 (4.0), 111 (6.4), 108 (13.3), 97 (11.3). ^{1}H NMR (DMSO-d_6, 270 MHz) δ: 6.79 (1H, *d*, J = 8.06 Hz, H-6), 6.86 (1H, *dd*, J = 2.57, 8.79 Hz, H-4′), 6.90 (1H, *d*, J = 8.79 Hz, H-3′), 7.12 (1H, *d*, J = 8.43 Hz, H-8), 7.22 (1H, *s*, H-3), 7.30 (1H, *d*, J = 2.57 Hz, H-6′), 7.65 (1H, *dd*, J = 8.06, 8.43 Hz, H-7), 9.13, 10.16 (1H, each *s*, $C_{2'}$,$_{5'}$–OH), 12.75 (1H, *s*, C_5–OH). The synthetic sample was identical with **1** (co-TLC, MS and ^{1}H NMR spectral data).

Compound **2**. Mp 114–117° (MeOH), yellow prisms. MS *m/z* (rel. int.): 240 [M^+] (20.7), 233 (6.9), 194 (2.2), 184 (3.1), 153 (4.2), 149 (4.9), 141 (4.4), 120 (11.1), 121 (19.3), 105 (100), 77 (28.7). UV λ_{max}^{MeOH} (log ε) nm: 254 (3.93), 364 (4.23); + MeONa: 236 sh, 368; + $AlCl_3$: 276, 346 sh, 362, 394; 362, 394; + $AlCl_3$/HCl: 256, 340 sh, 366; + NaOAc: 250, 363; + NaOAc/H_3BO_3: 250, 322 sh, 363. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 6.84 (1H, *s*, H-α), 6.92 (1H, *ddd*, J = 1.10, 8.06, 8.43 Hz, H-3′), 7.00 (1H, *dd*, J = 1.10, 8.43, H-5′), 7.46 (1H, *ddd*, J = 1.47, 8.06, 8.43, H-4′), 7.48–7.56 (3H, *m*, H-3, 4, 5), 7.78 (1H, *dd*, J = 1.47, 8.06 Hz, H-6′), 8.06 (2H, *br dd*, J = 1.46, 8.06 Hz, H-2 and 6), 12.09 (1H, *s*, C_2–OH), a small peak at 4.46 ppm based on $COCH_2CO$ was observed.

Conversion 2′,β-*dihydroxychalcone into flavone*. An Me_2CO soln containing 2′,β-dihydroxychalcone (2 mg) and 5,6,7-trimethoxyflavone (2 mg) as int. standard was mixed with silica gel (1.0 g). After evapn of the Me_2CO *in vacuo* at room temp., the dried silica gel was kept in an oven at 100°. At intervals, a small amount of silica gel was taken out and extracted with acetonitrile (5 ml). The extract (20 μl) was injected into an HPLC system (column: Cosmosil $_5C_{18}$, mobile phase: 70% acetonitrile). The amount of flavone was calculated by ratio from the internal standard (Fig. 1.).

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