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5-HYDROXY-6,2′-DIMETHOXYFLAVONE FROM *PRIMULA DENTICULATA*

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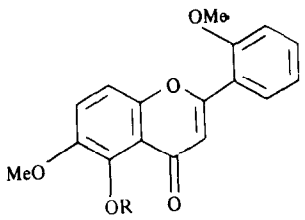
Key Word Index—*Primula denticulata*; Primulaceae; leaf exudate; 5-hydroxy-6,2′-dimethoxyflavone: 5,6,2′-trimethoxyflavone; synthesis; mass spectrum.

Abstract—A novel natural flavone, 5-hydroxy-6,2′-dimethoxyflavone, was isolated from the leaf exudate of *Primula denticulata*. Its structure was elucidated by spectral studies and confirmed by synthesis. This flavone shows unusual fragments in its mass spectrum which is caused by the stability of the molecular ion and a new fragmentation pattern is proposed. The structure of 5,6,2′-trimethoxyflavone, reported earlier from *Sargentia greggii*, is also confirmed by synthesis. All the flavones found in *Primula* exudates are surveyed.

INTRODUCTION

Within the past three years, the number of unusual flavones reported from the farinose leaf exudate of *Primula* species [1] has increased considerably, from eight to 17 [2–5]. From some fractions remaining from our recent studies, we have now isolated another minor component. Based on spectral studies, in particular on ^{1}H NMR, it was identified as 5-hydroxy-6,2′-dimethoxy flavone (**1**). This rare structure was confirmed by synthesis. The structure of its methyl ether, reported earlier from a plant in the Rutaceae, was also corroborated.



1 R = H
2 R = Me

Fig. 1.

RESULTS AND DISCUSSION

A new flavone was isolated as a trace constituent from the exudate of *Primula denticulata* Sm. after fractionation by column chromatography and repeated preparative TLC on silica. On polyamide TLC, it forms a dark spot (UV_{366}) that shows no reaction with the Naturstoff A reagent. The molecular ion at *m/z* 298 in the mass spectrum suggested a flavone with one hydroxy and two methoxy groups, as a flavone with three hydroxy and two methyl groups would exhibit a much lower R_f on TLC. This was confirmed by the ^{1}H NMR signals of methoxy groups at δ 4.02 and 3.90. Further the ^{1}H NMR spectrum showed a signal for a chelated hydroxy group at C-5 (δ 12.86, *s*, 1 H), a singlet at δ 6.96 assignable to H-3, and two one-proton doublets (J = 9.16 Hz) due to *ortho*-coupling at δ 7.43 and 7.07. These signals indicated that one of the methoxy groups was at C-6 or at C-8, that is, the oxygenation pattern of the A-ring was 5,6–$[O]_2$ or 5,8–$[O]_2$. The fact that the intensity of the $[M-15]$ peak is much lower than that of $[M]^+$ shows that the former substitution pattern is present in this product. Other complicated signals from δ 7.16 to 7.96, based on protons of the B-ring moiety, could be clearly assigned (see Experimental) by comparison with those of 5,2′-dihydroxyflavone [4], which shows that the second methoxy group must be located at C-2′. This product is thus 5-hydroxy-6,2′-dimethoxyflavone.

In order to confirm this unusual substitution, the flavone as well as its methyl derivative were synthesized. Elbs oxidation [6] of 2-hydroxy-6-methoxyacetophenone may yield two alternative compounds (by *para* or *ortho* oxidation), 2,5-dihydroxy-6-methoxyacetophenone (**3**) or 2,3-dihydroxy-6-methoxyacetophenone (**4**). (Fig. 2). In the ^{13}C NMR spectrum of the acetophenone obtained in our experiment, the chemical shift of a methoxyl carbon at δ 62.03 showed that the methoxy group was surrounded by *ortho*-substituted groups, thus indicating that only the acetophenone **3** had been obtained. By usual partial methylation, **3** gave 2-hydroxy-5,6-dimethoxyacetophenone (**5**), which was condensed with 2-methoxybenzaldehyde to yield 2′-hydroxy-2,5′,6′-trimethoxychalcone (**6**). This chalcone was isomerized to the corresponding flavanone **7** and then oxidized with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to yield 5,6,2′-trimethoxyflavone (**2**). This flavone was partially demethylated with boron trichloride to afford **1** as yellow needles. Comparison of the spectral data of this synthetic sample with those of the natural product showed good agreement. The structure of the flavone isolated from *Primula denticulata* is thus confirmed as 5-hydroxy-6,2′-dimethoxyflavone.

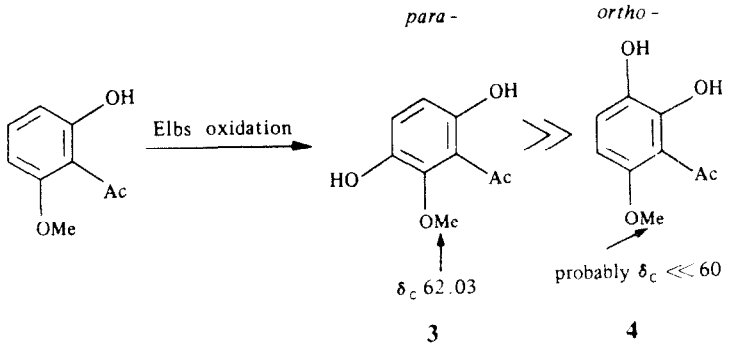


Fig. 2.

Further the synthetic flavone **2** was compared directly with an authentic sample of natural 5,6,2′-trimethoxyflavone, isolated from *Sargentia greggii* (Rutaceae) [7] and the structure of the latter product was thus also confirmed. This is important insofar as these two products are as yet the only natural flavones with 5,6,2′-trioxygenation.

In general, the mass spectrum of a flavone provides useful information on the number of hydroxy and methoxy groups on the molecule by means of fragment ions designated as $[A_1]^{+\cdot}$, $[B_1]^{+\cdot}$ and so on. However, a fragmentation sequence observed in the mass spectrum of 5-hydroxy-6,2′-dimethoxyflavone was different from that of common flavones (Fig. 3). Due to the stability of its molecular ion, the intensity of $[A_1]^{+\cdot}$ and $[B_1]^{+\cdot}$ was very low. In the case of **1**, elimination of the methyl group at C-6 occurred predominantly to yield a quinoid ion at *m/z* 283, which gave rise to further fragments at *m/z* 255, 151 etc. Thus a new fragmentation pattern is proposed for **1**.

In leaf washes of several other *Primula* species and in particular in some remaining fractions, a few further trace constituents have been observed. However, the quantities are so minute that isolation in pure state for spectral analysis is not possible so these minor components remain unidentified. The time has come, therefore, to present a survey on all *Primula* flavones up to the present and this is shown in Fig. 4, starting with unsubstituted flavone itself and then with flavones having an increasing number of hydroxyl (or methoxyl) substituents. The most interesting product is 2′,β-dihydroxychalcone, a novel representative of the rare group of β-diketones or dibenzoylmethanes. Attention is also drawn to 8,2′-dihydroxyflavone for its unusual oxygenation pattern.

According to Harborne's earlier extensive studies on leaf and flower flavonoids in *Primula* [8], these organs accumulate flavonol glycosides based on kaempferol and quercetin, sometimes also herbacetin and gossypetin. The tissue flavonoids thus are 'normal' polyoxygenated flavonols, whereas the exudate flavonoids have a remarkably low degree of oxygenation. The dominating component of the farina, flavone itself, completely lacks *O*-substitution. It is striking that none of these flavones exhibits 7-*O*-substitution and seven do not exhibit 5-*O*-substitution either. The glandular cells obviously have their own particular biosynthetic capacity, i.e. their own set of special enzymes. It is suggested, therefore, that the biosynthesis of these specific exudate flavones in *Primula* requires a special, so-far unknown pathway.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 270 MHz, MS were obtained at 70 eV by direct inlet.

Plant material and flavone isolation. Freshly collected leaves of *Primula denticulata* (Botanischer Garten der TH Darmstadt, F.R.G.) were rinsed with Me_2CO to dissolve the exudate which was concd *in vacuo* and fractionated by CC on silica and on polyamide as described previously [2]. The product studied here was isolated from a minor fraction by prep. TLC on silica (toluene–MeCOEt 4:1) and purified by repeated TLC on silica (*n*-hexane–Me_2CO 5:1). TLC control was on polyamide DC-11 (petrol, bp 100–140°–toluene–MeCOEt–MeOH, 12:6:1:1).

5-Hydroxy-6,2′-trimethoxyflavone (**1**). UV λ_{max}^{MeOH} nm: (236) 276, 329; +NaOMe 278, 314, 395; +$AlCl_3$ 232, (250), 292, 351; +$AlCl_3$/HCl 232, (250), 290, 346; +NaOAc (236), 276, 330; +NaOAc/H_3BO_3 (236), 276, 330. MS *m/z* (rel. int.): 298 ($[M]^+$, 100), 297 (29), 293 (31), 269 (22), 255 (26), 252 (17), 151 (21), 149 (10), 123 (31), 105 (13). ^{1}H NMR (Me_2CO-d_6) δ (12.86 (1H, *s*, OH-5), 7.96 (1H, *dd*, *J* = 1.84, 7.69 Hz, H-6′), 7.58 (1H, *ddd*, *J* = 1.83, 7.69, 8.34 Hz, H-4′), 7.43 (1H, *d*, *J* = 9.16 Hz, H-7), 7.26 (1H, *dd*, *J* = 1.10, 8.43 Hz, H-3′), 7.16 (1H, *td*, *J* = 1.10 Hz, 7.69, H-5′), 7.07 (1H, *d*, *J* = 9.15 Hz, H-8), 6.96 (1H, *s*, H-3), 4.02 (3H, *s*, OMe), 3.90 (3H, *s*, OMe).

Synthesis of 5,6,2′-trimethoxy- (**2**) *and 5-hydroxy-6,2′-dimethoxyflavone* (**1**). 2-Hydroxy-6-methoxyacetophenone (2.8 g, 17 mmol) was subjected to Elbs oxidation, as described in our previous paper [6], to give 2,5-dihydroxy-6-methoxyacetophenone (**3**) (1.2 g) after purification by CC on silica (eluent: C_6H_6), mp 94–96° (C_6H_6), pale greenish-yellow plates. EIMS *m/z* (rel. int.): 182 ($[M]^+$, 100), 167 ($[M-Me]^+$, 91), 164 (20), 152 (22). ^{1}H NMR ($CDCl_3$) δ: 2.74 (3H, *s*, Ac), 3.84 (3H, *s*, OMe), 5.24 (1H, *s*, C_3–OH), 6.70, 7.14 (each 1H, *d*, *J* = 9.2 Hz, H-4 and H-5), 12.01 (1H, *s*, C_6–OH). ^{13}C NMR ($CDCl_3$) δ: 31.35 (COMe), 62.03 (OMe), 204.32 (CO). Using Me_2SO_4 and K_2CO_3 in dry Me_2CO, **3** (700 mg) was partially methylated to give **5** as a yellow oil (620 mg). EIMS *m/z* (rel. int.): 196 ($[M]^+$, 100), 181 (72), 166 (19), 163 (27). To a 70% methanolic soln (100 ml) containing KOH (8 g), the acetophenone (**5**) (400 mg, 2 mmol) and 2-methoxybenzaldehyde (300 mg, 2.2 mmol) were added. The reaction mixture was stirred overnight, and acidified with 10% HCl to precipitate 2′-hydroxy-2,5′,6′-trimethoxychalcone (**6**) (430 mg), which was recrystallized from MeOH, mp 90–91°, red needles. EIMS *m/z* (rel int.): 314 ($[M]^+$, 29), 207 (7), 180 (100), 165 (56), 137 (13). ^{1}H NMR (Me_2CO -d_6) δ: 3.86, 3.91, 3.96 (each 3H, *s*, OMe), 6.97, 7.29 (each 1H, *d*, *J* = 9.1 Hz, H-4′ and H-5′), 7.04 (1H, *br dd*, *J* = 7.7, 7.3 Hz, H-5), 7.12 (1H, *br d*, *J* = 8.4 Hz, H-3), 7.45 (1H, *ddd*, *J* = 8.4, 7.3, 1.5 Hz, H-4), 7.76 (1H, *dd*, *J* = 7.7,

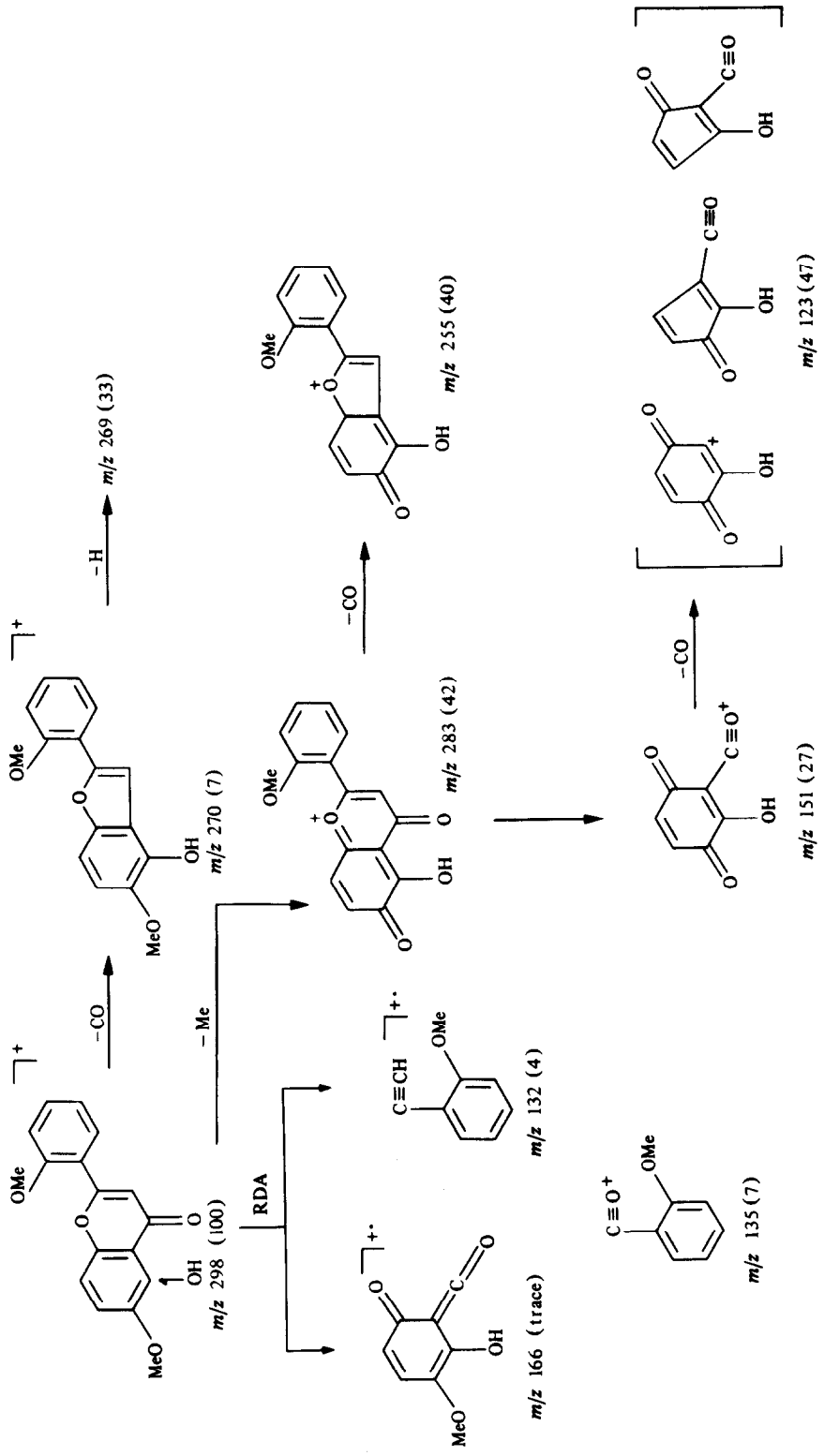

m/z 298 (100)
m/z 270 (7)
m/z 269 (33)
m/z 283 (42)
m/z 255 (40)
m/z 166 (trace)
m/z 132 (4)
m/z 135 (7)
m/z 151 (27)
m/z 123 (47)
RDA
-CO
-Me
-H
-CO
-CO

Fig. 3.

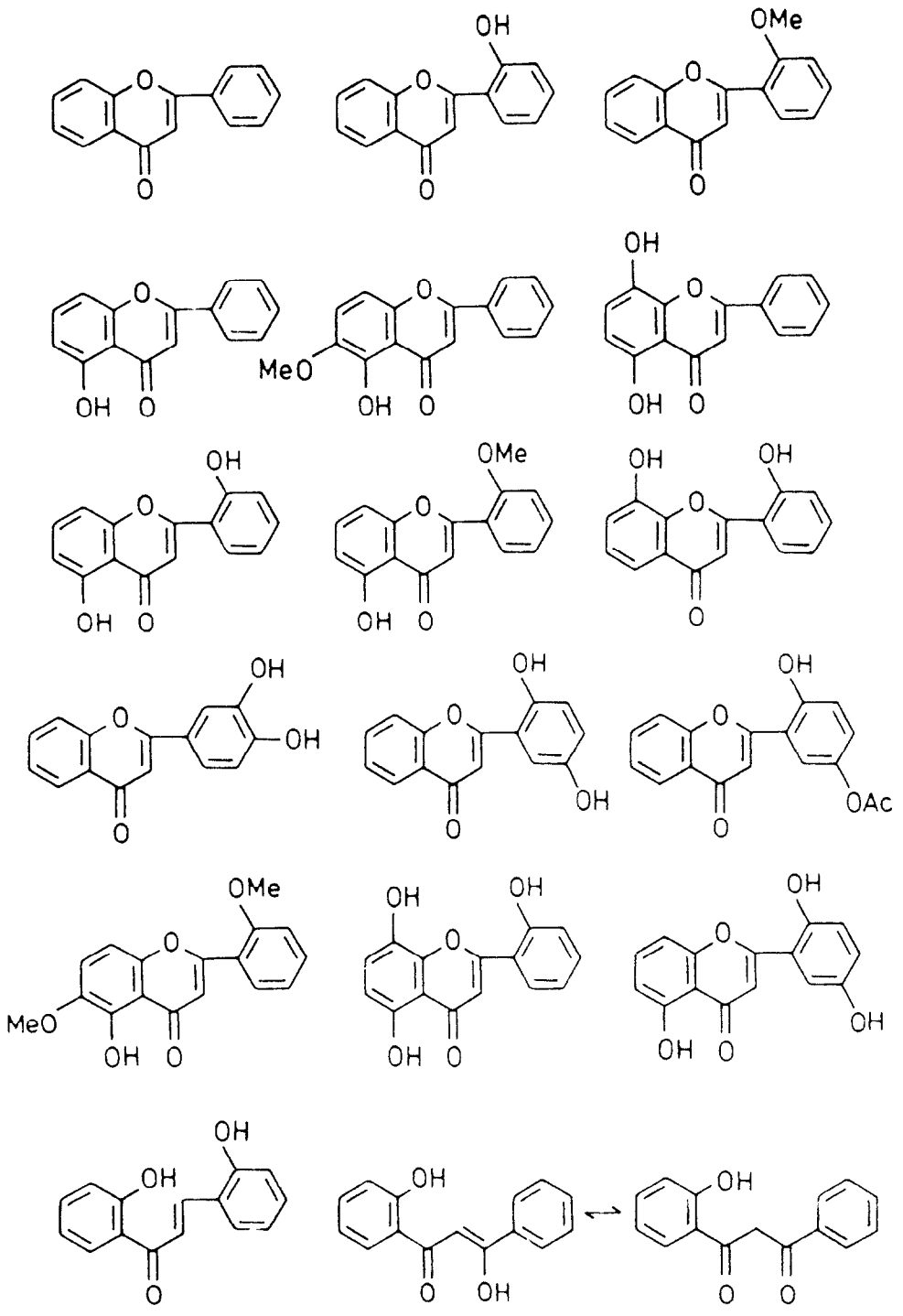


Fig. 4.

1.5 Hz, H-6), 7.85, 8.09 (each 1H, *d*, $J = 15.8$ Hz, H-α and H-β), 11.44 (1H, *s*, C_2-OH). The chalcone (**6**) was heated in an ethanolic soln (70 ml) containing H_3PO_4 (5 ml) for 50 hr under reflux. The reaction mixture was poured into water and extracted with EtOAc. The organic phase was concd *in vacuo*, and then chromatographed on silica, eluted with *n*-hexane–EtOAc (5:1) to give 5,6,2′-trimethoxyflavanone (**2**) as prisms (120 mg), mp 120–121°. EIMS *m/z* (rel. int.): 314 (M^+, 42), 180 (100), 165 (56), 137 (16). ^{1}H NMR (Me_2CO-d_6) δ: 2.77 (1H, *dd*, $J = 16.5$, 3.3 Hz, H-3), 2.91 (1H, *dd*, $J = 16.5$, 12.8 Hz, H-3), 3.84, 3.88, 3.92 (each 3H, *s*, OMe), 5.72 (1H, *dd*, $J = 12.8$, 3.3 Hz, H-2), 6.80, 7.25 (each 1H, *d*, $J = 9.2$ Hz, H-7 and H-8), 7.05–7.08 (2H, *m*, H-2′ and H-5′), 7.38 (1H, *m*, H-4′), 7.62 (1H, *dd*, $J = 7.3$, 1.5 Hz, H-6′). The flavanone (70 mg, 0.2 mmol) was refluxed with DDQ (51 mg, 0.2 mmol) in dry dioxane to afford 5,6,2′-trimethoxyflavone (**2**) (43 mg), mp 122–123° (EtOAc–*n*-hexane), needles. EIMS *m/z* (rel.

int.): 312 ([M]$^+$, 44), 297 (100), 180 (3), 165 (13). ^{1}H NMR (Me_2CO-d_6) δ: 3.86, 3.92, 3.99 (each 3H, *s*, OMe), 6.81 (1H, *s*, H-3), 7.14 (1H, *br dd*, J = 7.9, 7.5 Hz, H-5′), 7.25 (1H, *br d*, J = 8.1 Hz, H-3′), 7.40, 7.53 (each 1H, *d*, J = 9.2 Hz, H-7 and H-8), 7.56 (1H, *ddd*, J = 8.1, 7.5, 1.8 Hz, H-4′), 7.93 (1H, *dd*, J = 7.9, 1.8 Hz, H-6′). The flavone (**2**) (8 mg, 0.03 mmol) was dissolved in CH_2Cl_2. To the soln cooled at $-60°$, BCl_3 (0.5 ml) was added. The reaction mixture was left for 2 hr at room temp., and poured into H_2O. After extraction with EtOAc, the organic layer was concd *in vacuo*. Flavone (**1**) was obtained from the residue by crystallization from C_6H_6–*n*-hexane as yellow needles (5 mg), mp 150°. EIMS *m/z* (rel. int.): 298 ([M]$^+$, 100), 283 (42), 269 (33), 255 (40), 252 (24), 165 (3), 151 (26), 137 (12), 131 (11), 123 (45), 105 (22). UV λ_{max}^{MeOH} nm: 236 inf., 277, 329. ^{1}H NMR (Me_2CO-d_6) δ: 12.89 (1H, *s*, C_5–OH), 7.97 (1H, *dd*, J = 7.8, 1.8 Hz, H-6′), 7.59 (1H, *ddd*, J = 8.8 Hz, 8.1, 1.8 Hz, H-4′), 7.46 (1H, *br d*, J = 9.2 Hz, H-6), 7.28 (1H, *br d*, J = 8.8 Hz, H-3′), 7.17 (1H, *ddd*, J = 8.1, 7.8, 1.1 Hz, H-5′), 7.09 (1H, *d*, J = 9.2 Hz, H-8), 6.98 (1H, *s*, H-3), 4.02 (3H, *s*, OMe), 3.90 (3H, *s*, OMe).

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