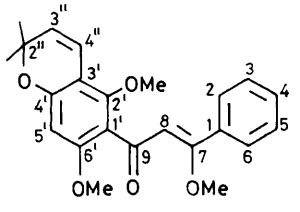
Flavonoids of *Tephrosia procumbens*—Revised Structure for Praecansone A and Conformation of Praecansone B

G. Venkataratnam and Erraguntla Venkata Rao *
Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam 530 003, India
C. Vilain
Université de Liège, Institut de Chimie Organique, Sart-Tilman par 4000 Liège 1, Belgium

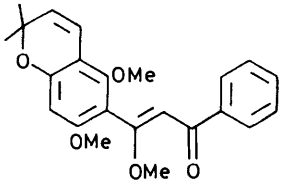
Two rotenoids (rotenone and sumatrol), a flavanone (obovatin), a β-diketone (praecansone B), and its isomeric methyl ether (praecansone A) have been isolated from the chloroform extract of roots of *Tephrosia procumbens*. Furthermore, an unusual flavonol (7-ethoxy-3,3',4'-trihydroxyflavone; fisetin 7-ethyl ether) and an isoflavone (7,4'-dihydroxy-3'-methoxyisoflavone) have been isolated from an ethyl acetate fraction of the alcoholic extract of the roots. The structures of all the compounds were established spectroscopically.

The structure of praecansone A has been revised as (**3**) on the basis of ^{13}C and ^{1}H n.m.r. data, and confirmed by an n.O.e. experiment. The possible conformations of praecansone B in $CDCl_3$ are discussed on the basis of ^{1}H n.m.r. data and D_2O-exchange studies.

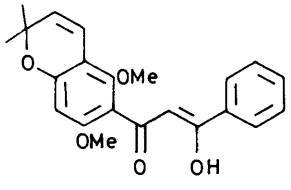
In continuation of our studies[1] on the flavonoids of *Tephrosia* species, we have examined *T. procumbens*, a species which has apparently not been investigated previously. The chloroform extract of root powder on column chromatography on silica gel yielded rotenone, sumatrol, praecansone A (**3**), and praecansone B (**2**). The ether–ethyl acetate fraction of an alcoholic extract afforded a new flavonol and an isoflavone. Praecansone A and praecansone B were previously isolated from a chloroform extract of seeds of *T. praecans*.[2] Previously, the structure of praecansone A was established as (**1**) by ^{1}H n.m.r. data, but it was recently suggested that this structure needs to be revised.[3] We now present ^{13}C and ^{1}H n.m.r. evidence in support of the revised structure (**3**) for praecansone A.



(**1**)



(**3**)



(**2**)

Results and Discussion

Rotenone, sumatrol, and obovatin were identified by comparison of their ^{1}H n.m.r. data with literature data.[4–6] Rotenone, praecansone A, and praecansone B were compared on t.l.c. and co-t.l.c. with authentic samples.

The ^{1}H n.m.r. spectrum of praecansone A in $CDCl_3$ (300 MHz) is similar to that reported earlier for this compound praecansone A.[2] We have noticed a well separated low-field multiplet at δ 7.86—7.82 which accounted for 2- and 6-H *ortho* to a CO group as in synthetic pongamol 9-methyl ether.[3] Another multiplet at δ 7.45—7.37 accounted for 3-, 4-, and 5-H. In derribtusone A,[7] an auronol having a PhCOC=CO system, the side-chain phenyl nucleus proton signals appear as two separate multiplets at δ 7.99 (2 H) and δ 7.56 (3 H). These facts suggest that praecansone A is the 9-methyl ether rather than the 7-methyl ether proposed earlier.[2] This conclusion was confirmed on comparing the ^{13}C chemical shifts of the side-chain phenyl ring of praecansone A with those of pongamol 9-methyl ether and pongamol 7-methyl ether[3] (Table). The assignment of carbons C-1 to C-9 was readily effected using the data for pongamol 7- and 9-methyl ethers.[3]

It is interesting to examine the signals of the side-chain phenyl group, the chemical shifts of C-1 to C-6 differ by less than 0.5 p.p.m. from the corresponding values given for synthetic pongamol 9-methyl ether[3] (Table). This clearly demonstrates that praecansone A possesses the same acetophenone moiety as in pongamol 9-methyl ether,[3] and not the styrene moiety present in natural pongamol 7-methyl ether,[3] of which the δ_C values for C-1 to C-6 are in poor agreement with those of praecansone A (Table). The resonance of C-8 at δ_C 101.54 is not significantly affected by methylation at C-9 (see praecansone B). The resonance of C-7 at δ_C 190.11 (C=O) and of C-9 at δ_C 165.86 (C–OMe) are in good agreement with those of the 9-methyl ether of pongamol.[3]

Irradiation of the C-9 methoxy group resulted, as expected, in an appreciable increase (15%) in the intensity of the signal for 8-H (Figure), but the intensities of the signals of the two remaining methoxy groups were also noticeably increased (+4% at δ 3.68 and +1% at δ 3.73). There was no change in the intensity of signals for 2- and 6-H of the side-chain phenyl ring. This indicates that the methoxy group is at C-9 and not on C-7. The mass spectrum of praecansone A is in conformity with the 9-methylated structure (**3**) as proposed earlier.[3]

Praecansone B (**2**) was obtained as a yellow oil. Its ^{1}H n.m.r. spectrum showed, similarly to that of praecansone A, a well separated multiplet at δ 7.96—7.92 for 2- and 6-H and another set at δ 7.45—7.43 for 3-, 4-, and 5-H. This situation created doubt as to whether enolization takes place at C-9 or at C-7. Furthermore, in the ^{1}H n.m.r. ($CDCl_3$) spectrum we have noticed an exchange of 8-H with D_2O along with exchange of enolic OH. These facts are not reported in the literature for

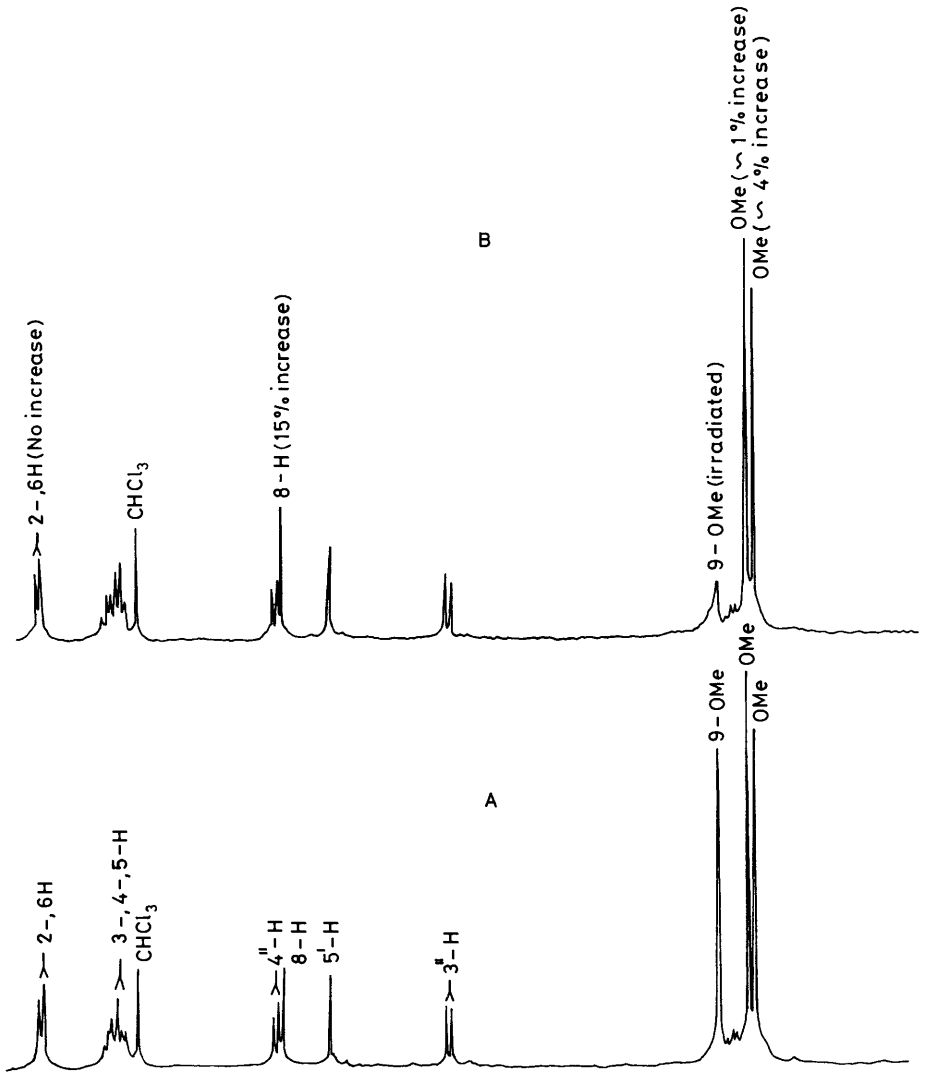
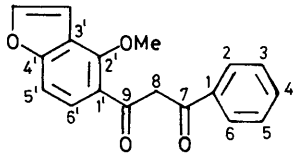


Figure. A original spectrum of praecansone A. B spectrum of praecansone A after irradiation of 9-OMe

praecansone B.[2] We report here that enolization takes place at C-7 rather than at C-9, based on our interpretation of the ^{13}C n.m.r. data of praecansone B and comparison with the carbon resonances of pongamol[3] (**4**), and the enol and keto tautomers of demethylpraecansone B.[8] In the ^{13}C n.m.r. spectrum of praecansone B assignment of carbons C-1 to C-9 was readily effected using the data for pongamol[3] (**4**), which possesses the same β-hydroxychalcone moiety. A rough calculation of the resonances of C-1′ to C-6′ was made on the basis of those of pongamol (**4**) and using the increments of monosubstituted phenyl groups [$\delta_{calc.}$ = δ(pongamol) + Δ(6′-OMe)].[9] The carbons C-2″, C-3″, and C-4″, and gem dimethyl groups were assigned according to the data previously published for isoflavones possessing a 5,7-dioxygenated 2,2-dimethylchromene ring system[10] or for 5-hydroxy-6-(*p*-methoxyphenyl)acetyl-2,2-dimethylchromene.[11] The remaining signals are those of the two non-equivalent aromatic 2′- and 6′-OMe groups.

It is worth comparing the carbon resonances observed for the enol and keto tautomers of demethylpraecansone B[8] (Table). The signal of C-1 appears noticeably upfield when position 7 is enolized (δ_C 135.0) relative to its resonance when there is a keto function (δ_C 137.9) in demethylpraecansone B. The C-1 of praecansone B exhibited a signal at δ_C 135.16, which correlated well with the C-1 resonance of both pongamol (δ_C 135.70) and



(**4**)

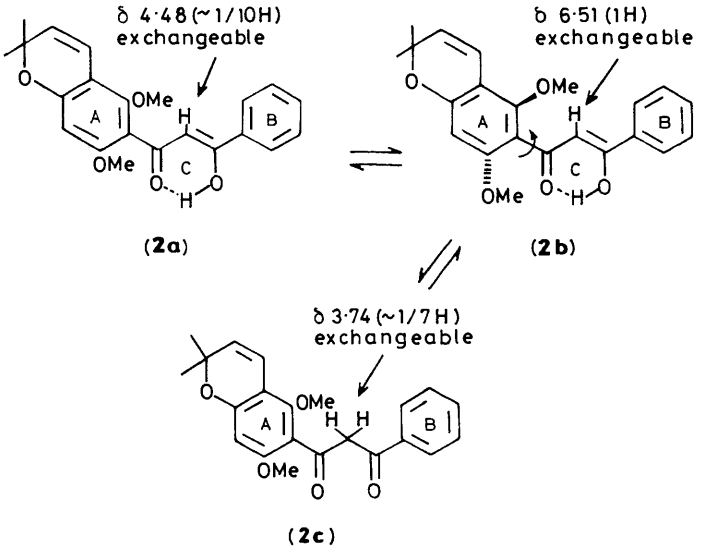
pongamol 7-methyl ether (δ_C 135.48). This therefore confirms the 7-enolized structure proposed for praecansone B. On the other hand, pongamol 9-methyl ether and praecansone A exhibit a signal assigned to C-1 at δ_C 139.76 and 140.00 respectively, in agreement with a 7-keto group. Thus enolization takes place at C-7 in praecansone B (**2**) as in pongamol (**4**), rather than at C-9.

We have noticed in the ^1H n.m.r. spectrum of praecansone B signals which, similar to milletenone,[12] and ovalitenone,[13,14] may be assigned to exchangeable methylene protons of dibenzoylmethane form (**2c**) and the olefinic protons of either enolic form (**2a**) and (**2b**) (Scheme). The most stable conformer of praecansone B could be represented as having the A-ring

Table. ^{13}C N.m.r. data

Carbon	Pongamol[3] (4)	$\delta_{calc.}$ = δ(pongamol) + Δ(6′-OMe)	Praecansone B (2)	Pongamol 7-methyl ether[3]	Demethylpraecanson B[8] Enol form	Demethylpraecanson B[8] Keto form	Pongamol 9-methyl ether[3]	Praecansone A (3)
C-1	135.70		135.16	135.48	135.0	137.9	139.76	140.00
C-2/6	128.63		128.42	128.95	129.7	129.6	128.19	127.92[a]
C-3/5	127.11		126.97	127.63	127.5	128.9	127.99	127.75[a]
C-4	132.14		131.91	129.61	134.0	132.9	131.84	131.37
C-7	184.28		181.95	170.57	176.5	200.0	189.95	190.11
C-8	97.90		100.49	103.21	98.9	55.3	99.72	101.54
C-9	186.10		187.89	191.04	195.0	195.2	169.33	165.86
C-1′	119.58	105.2	108.05	119.13	103.4	103.8	118.48	107.69
C-2′	158.73	159.7	158.39	158.22	116.9[a]	162.7[a]	157.40	157.86
C-3′	122.18	114.5	114.35	**	104.9	106.1	121.13	111.61
C-4′	152.78	153.8	155.21	152.81	163.4	163.4	151.22	154.68
C-5′	105.28	90.9	96.20	105.13	92.3	93.0	105.14	96.09
C-6′	126.50	157.9	156.37	126.76	161.0[a]	161.8[a]	126.08	155.69
C-2″			76.88*		79.0	78.8		77.10
C-3″	144.83		127.61	144.63	126.6	126.6	144.10	126.83
C-4″	107.03		116.49	106.45	116.2	116.5	105.98	166.85
2″-Me_2			27.86		28.6	28.6		27.93
RO*Me*				59.39			56.36	55.78[b]
ArO*Me*	61.10		62.97, 55.94	61.28	56.7	56.2	60.22	62.04, 55.93[b]

[a,b] Attributions having the same superscript are interchangeable. * Resonance accidentally hidden by the central peak of $CDCl_3$, whose intensity was slightly greater than that of the two external peaks. ** No value given in ref. 3. R = alkyl; Ar = Aromatic.



Scheme.

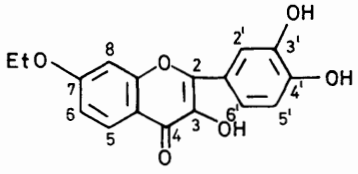
perpendicular to the C-ring as shown in (**2b**). The olefinic hydrogen should therefore be strongly deshielded (from δ 6.27 in CCl_4 spectrum[2]) by the aromatic ring A, in agreement with the signal, 1 H, at δ 6.51 which exhibited somewhat reduced intensity and a slight shift to δ 6.49 after addition of D_2O. The much less stable conformer could be assigned the nearly planar structure (**2a**) and the tautomer can be assigned a dibenzoylmethane structure (**2c**).

A variable-temperature study on praecansone B at 2, 18, and 32 °C in $CDCl_3$ showed no significant differences in the ratio of the keto–enol tautomers. Only a slight upfield shift (from δ 6.70 to 6.50) of the 8-H signal was noticed when the temperature increased. This may be connected with a change in the restricted rotation of the A-ring about the 1′–9 bond which could place the 2′-,6′-OMe groups in different spatial positions relative to 8-H.

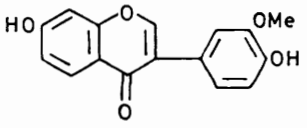
The flavonol was obtained as a yellow powder, m.p. 192 °C. The ^1H n.m.r. ([$^2H_6$]acetone) spectrum clearly revealed a 7-oxygenated A-ring: δ 8.14 (1 H, d, $J_{5,6}$ 8.9 Hz, 5-H), 6.55 (1 H, dd, $J_{5,6}$ 8.9, $J_{6,8}$ 2.4 Hz, 6-H), and 6.39 (1 H, d, $J_{8,6}$ 2.4 Hz, 8-H) and a CH_3CH_2OAr group: δ 4.35 (2 H, q, J 7.1 Hz, Me*C*H_2OAr) and 1.38 (3 H, t, *Me*CH_2OAr), with EtO on the A ring. The spectrum also revealed a set of peaks characteristic of a 3′,4′-dioxygenated B-ring: δ 6.96 and 6.95 (1 H, $J_{5',6'}$ 8.74 Hz, 5′-H), 7.68—7.64 (1 H, dd, $J_{5',6'}$ 8.74, $J_{2',6'}$ 1.94 Hz, 6′-H), and 7.79 (1 H, $J_{2',6'}$ 1.94 Hz, 2′-H). The presence of a 3,3′,4′-trihydroxy system was clearly evident in the u.v. spectrum. After the addition of sodium methoxide, the band I quickly collapsed as in fisetin.[15] The mass spectrum of the flavonol showed $M^{+\bullet}$ at 314 which lost either $CH_3CH_2^{\bullet}$ or CHO$^{\bullet}$ to give a weak $[M - 29]^+$ peak, which further lost either CHO$^{\bullet}$ or $CH_3CH_2^{\bullet}$ to give a strong

$[M - 58]^+$ peak. Thus the structure of the flavonol was established as 7-ethoxy-3,3′,4′-trihydroxyflavone (**5**) (fisetin 7-ethyl ether).

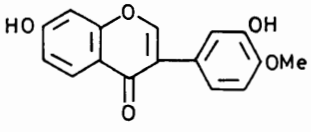
The isoflavone was obtained as white crystalline needles, m.p. 258—260 °C. The ^{1}H n.m.r. spectrum [$(CD_3)_2SO$] of the compound showed that it was one of the three mono methyl ethers of 3′,4′,7-trihydroxyisoflavone.



(**5**)



(**6**)



(**7**)

The mass spectrum of this compound showed [M^+] 284, consistent with the molecular formula $C_{16}H_{12}O_5$. The main feature of this spectrum is the presence of the fragments [A_1 + H]$^+$ at *m/z* 137 and [B_1]$^{+\bullet}$ at *m/z* 148 which confirm that the methoxy group is bonded to the B-ring. Therefore, the structure of this compound may be assigned as either 7,4′-dihydroxy-3′-methoxyisoflavone (**6**) or 7,3′-dihydroxy-4′-methoxyisoflavone, calycosin[16] (**7**). The u.v. spectra of this compound in methanol and methanol + NaOMe are almost identical with those recorded for 7,4′-dihydroxyisoflavone. Furthermore, the compound showed a negative Gibbs test,[17] which confirmed the absence of a free position *para* to a hydroxy group. Thus the structure of this compound is elucidated as 7,4′-dihydroxy-3′-methoxyisoflavone (**6**); this is the first report of the compound from the *Tephrosia* genus, but it was previously reported from a different source.[18] Owing to lack of authentic sample, no direct comparison could be made.

Experimental

The plant material was collected (November, 1984) around the Zoological Park, Visakhapatnam, India. A voucher sample is deposited in the Herbarium of Botany Department, Andhra University, Visakhapatnam.

The instruments used were a Bruker AM 300 WB n.m.r. spectrometer, an AEI-MS 902 S mass spectrometer, a Varian Techtron model 635 u.v.–visible spectrophotometer, and a Shimadzu i.r. spectrometer.

The root powder (2.4 kg) was extracted with hot chloroform and methylated spirit four times each. The methylated spirit extract was fractionated with ether and ethyl acetate. T.l.c. chromatograms of the ether and ethyl acetate fractions were similar, so these fractions were combined (5 g). The chloroform extracts on concentration yielded a reddish brown syrup (95 g). A portion of this chloroform extract (25 g) on column chromatography on silica gel (100—200 mesh, Acme) and elution with solvents of increasing polarity gave the following compounds.

Obovatin (25 mg), m.p. 126—126.5 °C (lit.,[6] 123—124 °C), obtained on elution with benzene. Its n.m.r., mass, i.r., and u.v. spectra were similar to the literature data.[6] [α]$_D$ Corresponded with the literature value.[6]

Sumatrol was obtained as white needles on elution with chloroform–benzene (1:3) (10 mg), m.p. 199—201 °C (lit.,[5] 198 °C). Its spectral data were similar to the literature data.[5]

Rotenone was obtained as a white crystalline compound on elution with chloroform–benzene (1:1) (2 g), m.p. 164—166 °C (lit.,[4] 168 °C). Its spectral data correlated well with those published.[4]

Praecansone B (**2**) was obtained as a yellow oil on elution with chloroform–benzene (3:1) (2 g), δ_H (300 MHz; $CDCl_3$) 16.33 (1 H, br s, β-OH), 7.96—7.92 (2 H, m, 2- and 6-H), 7.55—7.43 (3 H, m, 3-, 4-, and 5-H), 6.55 (1 H, d, *J* 3 Hz, 4″-H), 6.51 [1 H, s, 8-H of (**2b**) conformer], 6.27 (1 H, s, 5′-H), 5.56 (1 H, d, *J* 9.94 Hz, 3″-H), 4.48 (s, 8-H in twisted conformation), 3.8 (6 H, s, 2′- and 6′-OMe), 3.74 (s, methylene protons on C-8 of keto tautomer), and 1.46 (6 H, s, 2″-Me_2); *m/z* 366 ($M^{+\bullet}$, 15%), 351 (*M* − 15, 100), 335 (*M* − 31, 72), 305 (9), 247 (*M* − $C_6H_5COCH_2$, 13) 231 (9), 217 (16), 205 (57), and 203 (7). A 4.4% w/w $CDCl_3$ solution was used to record ^{1}H n.m.r. spectra at 2, 18, and 32 °C. U.v. and i.r. data were similar to the literature data.[2]

Praecanson A [revised structure (**3**)] (1.6 g) was obtained as a yellow oil on elution with chloroform–MeOH (99:1), δ_H (300 MHz; $CDCl_3$) 7.86—7.82 (2 H, m, 2- and 6-H), 7.45—7.37 (3 H, m, 3-, 4-, and 5-H), 6.48 (1 H, d, *J* 9.90 Hz, 4″-H), 6.43 (1 H, s, 8-H), 6.16 (1 H, s, 5′-H), 5.47 (1 H, d, *J* 9.95 Hz, 3″-H), 3.90 (3 H, s, 9-OMe), 3.73 and 3.68 (6 H, 2 s, 2′- and 6′-OMe), and 1.43 (6 H, s, 2″-Me_2); *m/z* 380 ($M^{+\bullet}$, 7%), 365 (*M* − 15, 40), 351 (20), 349 (*M* − OMe, 100), 336 (17), 319 (20), 255 (6), 245 (8), 217 (10), 167 (22), 105 ($C_6H_5C{=}O^+$, 48), 91 (4), and 77 (48). A 6% w/w $CDCl_3$ solution was used for the irradiation of the 9-OMe of praecansone A. Its u.v. and i.r. spectral data were similar to those published.[2]

Fisetin 7-ethyl ether (**5**) was obtained as a yellow powder on elution with 5% MeOH in $CHCl_3$ (5 mg), m.p. 192 °C; *m/z* 314 ($M^{+\bullet}$, 8%), 285 (*M* − 29, 10), 256 (*M* − 58, 80), 150 [(A_1 + H)$^{+\bullet}$, 30], 149 ($A^{+\bullet}$, 20), 137 (B_2^+, 100), and 121 [(A_1 − 28)$^+$, 50]; $\nu_{max.}$(KBr) 3 300br (OH), 1 650 (C=O), 1 600, 1 580, 1 520, 1 480, and 1 400 cm^{-1} (ArH); $\lambda_{max.}$(MeOH) 238sh, 306sh, and 368 nm; $\lambda_{max.}$(MeOH + NaOMe) 283sh, 318sh, 348sh, and 430 nm; $\lambda_{max.}$(MeOH + $AlCl_3$) 282sh, 317, 380sh, and 425 nm; $\lambda_{max.}$ (MeOH + $AlCl_3$ + HCl) 282sh, 313, 373, and 417 nm.

The isoflavone (**6**) (10 mg), on elution with 1% MeOH in $CHCl_3$, was obtained as a white crystalline compound, m.p. 258—260 °C (lit.,[18] 255—256 °C); δ_H [300 MHz; $(CD_3)_2SO$] 9.14 (2 H, br s, 7- and 4′-OH), 8.32 (1 H, s, 2-H), 7.96 (1 H, d, *J* 8.7 Hz, 5-H), 6.93 (1 H, *J* 8.7 and 2.1 Hz, 6-H), 6.85 (1 H, *J* 2.1 Hz, 8-H), 7.15 (1 H, *J* 1.7 Hz, 2′-H), 6.98 (1 H, *J* 8.2 and 1.7 Hz, 6′-H), 6.81 (1 H, *J* 8.2, 5′-H), and 3.78 (3 H, s, 3′-OMe); *m/z* 285 [(M^+ + 1), 19%], 284 (*M*, 100), 283 [(*M* − 1)$^+$, 22], 269 [(*M* − 15)$^+$, 8], 255 (5), 251 (3), 241 (11), 237 (7), 213 (12), 148 (B_1^+, 10), 137 [(A_1 + H)$^+$, 20], 133 [(B_1 − 15)$^+$, 11], 126 (6), 112 (10), and 105 (7); $\nu_{max.}$(KBr) 3 340br (OH), 1 660 (C=O), 1 580 and 1 480 cm^{-1} (ArH); $\lambda_{max.}$(MeOH) 283sh, 248, 260sh, 287sh, and 306sh nm; $\lambda_{max.}$ (MeOH + NaOMe), 254, 295, and 328sh.

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