Journal of Natural Products
Vol. 47, No. 4, pp. 585-591, Jul-Aug 1984

CHEMICAL CONSTITUENTS OF *CROTALARIA MADURENSIS*[1]

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ABSTRACT.—Two new compounds, crotmadine (**1**) and crotmarine (**3**); a known pyrrolizidine alkaloid, fulvine (**8**), and three other known compounds, *trans*-3,4,3′,5′-tetra methoxystilbene (**5**), dihydroalpinumisoflavone (**6**), and 4′,5,7-trihydroxy-3-methoxyflavone (**7**), have been isolated from the leaves and stems of *Crotalaria madurensis*. Both crotmadine (**1**) and crotmarine (**3**) exhibit antifungal activity against *Trichophyton mentagrophytes*.

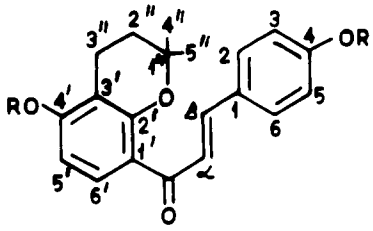
Crotalaria madurensis R. Wight (Leguminosae), an ornamental shrub that grows in the Nilgiris and Madura hills, is one of 80 *Crotalaria* species found in India (1). During a program of screening Indian plants for broad biological activity, antifungal activity was confirmed when a 50% ethanolic extract of the leaves and stems of *C. madurensis* was screened (2). In the follow-up studies, activity was concentrated in the $CHCl_3$ and EtOAc soluble fractions of the EtOH extract. Column and preparative tlc of the EtOAc soluble fraction on silica gel afforded two new compounds, crotmadine (**1**) and crotmarine (**3**), and the known compounds *trans*-3,4,3′,5′-tetramethoxystilbene (**5**), dihydroalpinumisoflavone (**6**), and 4′,5,7-trihydroxy-3-methoxyflavone (**7**). Compounds **5, 6,** and **7**, although known previously, were isolated for the first time from this plant. Fulvine (**8**), a known pyrrolizidine alkaloid, was isolated from the alkaloid fraction of the EtOH extract of the plant.

The molecular formula, $C_{20}H_{20}O_4$, for compound **1** was confirmed by ms (M^+, *m/z* 324), and its chalcone structure was indicated by its uv and ir spectra. An absorption band at 372 nm, which showed a bathochromic shift of 64 nm with an increase in intensity in the presence of sodium methoxide, indicated the presence of a hydroxy group at position 4. In its ir spectrum, an absorption at 1630 cm^{-1} was attributed to a chalcone carbonyl and an absorption at 1370 cm^{-1} to a *gem*-dimethyl group. In the pmr spectrum of compound **1**, six protons of a *gem*-dimethyl group appeared as a singlet at δ 1.36. Two apparent triplets for the methylene protons were centered at δ 1.82 and 2.71, respectively, as in the spectra of other compounds containing chroman ring systems (3,4). Two *ortho*-coupled doublets centered at δ 6.89 and 7.52, each integrating for two protons, were attributed to two sets of protons at C-3, C-5 and C-2, C-6 (A_2B_2 system of a *para*-substituted B-ring). An *ortho*-coupled doublet at δ 6.37, integrating for one proton, could be assigned to the C-5 proton. The corresponding downfield doublet for the C-6′ proton appeared at δ 7.6 due to the deshielding effects of a carbonyl group. The α- and β-protons of the chalcone moiety appeared as two doublets centered at δ 7.43 and 7.82, respectively (5).

The mass spectrum of compound **1** showed a molecular ion *m/z* 324 (M^+). Other significant ions in the spectrum were at *m/z* 323 (M^+-1), 281 (M^+-43), 269 (M^+-55), 268 (M^+-56), 205, 175, 149, and 120. Ions at *m/z* 205, 176, and 149 supported the presence of one hydroxyl group and a dimethylchroman ring in ring A, and the ion at *m/z* 120 confirmed the presence of a second hydroxy group in ring B (Scheme 1). Ions at *m/z* 281, 269, and 268 were characteristic of the presence of a dimethylchroman ring (6).

Compound **1** formed a diacetate **2** (M^+, *m/z* 408) whose ir spectrum exhibited intense bands due to the acetoxyl carbonyl (1760 and 1190 cm^{-1}). In the pmr spectrum of the diacetate, the two acetate methyl groups appeared as a singlet at δ 2.24, and a

[1]CDRI Communication No. 3245.



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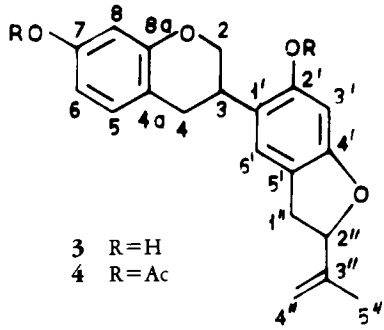

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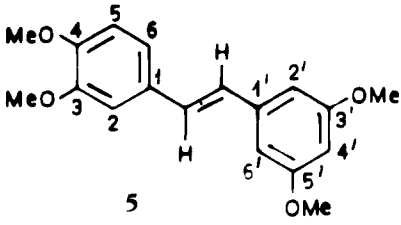

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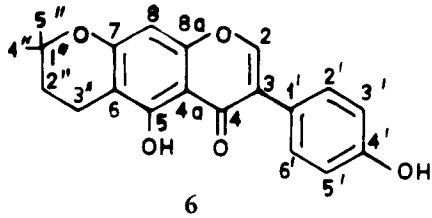

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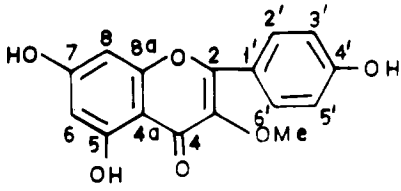

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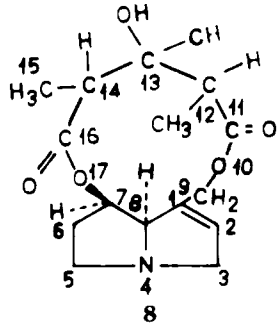

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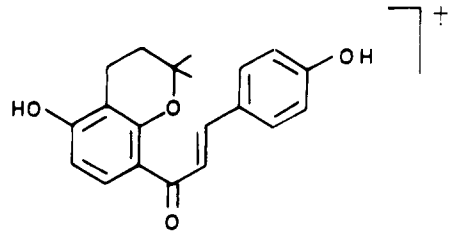
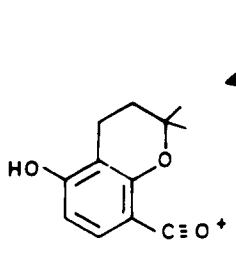
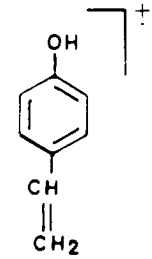
MASS FRAGMENTATION OF (**1**)



m/z 324



m/z 205



m/z 120

Scheme 1

paramagnetic shift (0.27 ppm) of the aryl methine (δ 7.16) of ring B was observed, inferring its *ortho*-relationship to the site of acetylation. The doublet at δ 7.52 remained unaffected, confirming the presence of a hydroxyl group at the C-4 position. The location of one of the two *ortho*-coupled protons of ring A was also shifted downfield by 0.35 ppm (δ 6.62), again indicating the *ortho*-relationship of the acetylation site. The counterpart at δ 7.61, however, remained unaffected, confirming the position of second hydroxyl group at the C-4′ position as well as the positioning of the dimethylchroman ring at the C-2′ and C-3′ position. The spectroscopic data discussed above suggested structure **1** for crotmadine.

The cmr spectrum of the crotmadine fully supported the proposed structure **1** (Table 1). The broad band decoupled spectrum of the compound exhibited 17 signals for the 20 carbon atoms, and the SFORD spectrum revealed the presence of eight quaternary, eight aromatic CH, two CH_2 and two CH_3 carbons. The characteristic signal of carbonyl carbon of the chalcone was observed at δ 192.09 (7). Three downfield signals at δ 160.22, 160.68, and 164.04 accounted for oxygen-substituted carbons C-2′, C-4, and C-4′, respectively (8). The β- and α-carbons of the chalcone moiety were identified by their typical appearance in the SFORD spectrum of δ 144.33 and 117.34, respectively (9). The carbons of the dimethylchroman ring appeared at δ 75.68, 32.02, 16.41, and 26.77, accounting for the C-1″, C-2″, C-3″, C-4″, and C-5″ positions, respectively (10). Two upfield carbons of ring A at δ 109.31 and 109.07 were attributed to C-3′ and C-5′ confirming the position of an hydroxyl group at the C-4′ position. The signals for carbons C-1′ and C-6′ were observed at δ 112.7 and 128.47, respectively. The carbons of ring B were centered at δ 126.53 (C-1), 130.47 (C-2, C-6), 116.31 (C-3, C-5), and 160.22 (C-4). The upfield shift of two carbons to 116.31 was attributed to the shielding effect of the oxygen-substituted *ortho*-carbon, confirming the position of second hydroxyl group at the C-4 position.

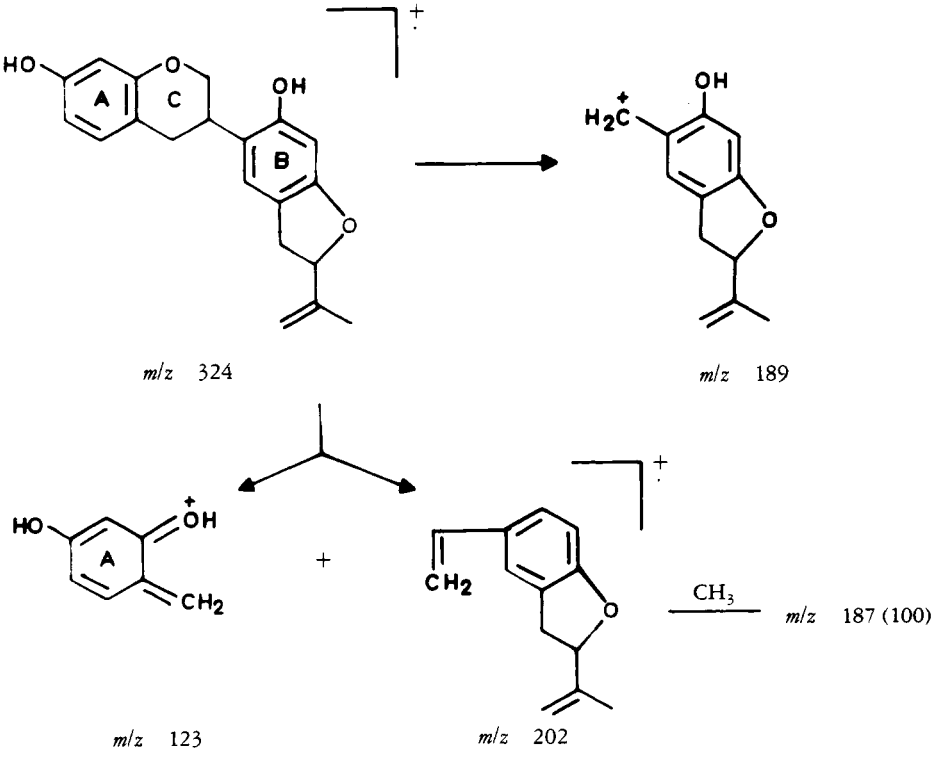
TABLE 1. Cmr Chemical Shift Assignment for Compound **1** ($CDCl_3$, TMS as internal standard)

Carbon atom	Chemical shifts in δ ppm
C-1	126.53
C-2	130.47
C-3	116.31
C-4	160.22
C-5	116.31
C-6	130.47
C-α	117.34
C-β	144.33
CO	192.07
C-1′	112.7
C-2′	160.68
C-3′	109.31
C-4′	164.04
C-5′	109.07
C-6′	128.47
C-1″	75.68
C-2″	32.02
C-3″	16.41
C-4″, C-5″	26.77

The molecular formula, $C_{20}H_{20}O_4$, for crotmarine (**3**) was confirmed by ms (M^+, *m/z* 324). In the ir spectrum of the compound, an absorption at 3300 cm^{-1} indicated the presence of an hydroxyl group in the molecule whereas the uv absorption maximum at 285 nm suggested the aromatic nature of the compound. The integrated pmr spectrum confirmed the presence of 20 protons in the molecule. A singlet at δ 1.76 for a CH_3 group at C-5″ and two broad singlets at δ 4.91 and 5.08 for C-4″ methylene protons suggested the presence of an isoprenyl group. One-proton multiplets at δ 3.22 and at 3.4 accounted for two protons at the C-1″ position. A triplet for a methine proton appearing at δ 5.19 was characteristic of dihydrofuran ring substituted at position 2″ (11). The data presented above confirmed the presence of 2″ (isoprenyl)-dihydrofuran ring system in the molecule. The C-2-axial proton appeared as a triplet at δ 3.97 (J=12 Hz), and the C-2-equatorial proton as a doublet of doublets (J=2; 12 Hz) centered at δ 4.30 (12,13). The aromatic region was not well resolved, appearing as two multiplets integrating for two and three protons centered at δ6.35 and 6.91, respectively. In the mass spectrum of crotmarine (**3**), fragment ions at *m/z* 123 and 202 were thought to arise by retro-Diels-Alder cleavage and ion *m/z* 189 from retro-Diels-Alder cleavage with H-transfer. The fragment ion at *m/z* 187 resulted from loss of a methyl group from the ion *m/z* 202 (12). The ions at *m/z* 202 and 187 confirmed the presence of the substituted dihydrofuran ring with one hydroxyl group in ring B. The presence of a second hydroxyl group in ring A was confirmed by the formation of an ion at *m/z* 123 (Scheme 2).

Crotmarine (**3**) formed a diacetate (**4**) in the pmr spectrum of which signals for the acetoxy methyl groups appeared at δ 2.22 and 2.26. The mass spectrum of the diacetate displayed a M^+ ion at *m/z* 408. Other important ions in the spectrum were at *m/z* 366

MASS FRAGMENTATION OF (**3**)



Scheme 2

(M^+-42), 324 (366-42), 244, 202 (244-42), 189, 187, and 165. The spectroscopic data discussed above suggested structure **3** for crotmarine.

The cmr spectrum of crotmarine (**3**) (Table 2) was in complete agreement with the proposed structure, there being 20 signals for the 20 carbon atoms observed in the broad band decoupled spectrum. The signals were assigned with the aid of the SFORD spectrum and by comparison with the reported data on related compounds (14-16). The presence of a doublet at δ 86.35 accounted for C-2″ and a triplet at δ 31.93 was assigned to the 1″ carbon. The quartet at δ 17.21 was assigned to C-5″ while C-4″ was observed as a triplet at δ 112.29. The singlet at δ 143 was ascribed to C-3″.

TABLE 2. Cmr Chemical Shift Assignment for Compound (**3**)

Carbon atom	Chemical shifts in δ ppm
C-2	70.21
C-3	31.16
C-4	30.96
C-4a	114.79
C-5	130.45
C-6	108.19
C-7	159.07
C-8	103.30
C-8a	150.23
C-1′	119.00
C-2′	155.07
C-3′	102.26
C-4′	154.23
C-5′	112.46
C-6′	127.18
C-1″	31.93
C-2″	86.35
C-3″	143.00
C-4″	112.29
C-5″	17.21

Elution of the silica gel column with C_6H_6 gave *trans*-3,4,3′,5′-tetramethoxystilbene (**5**) (17), a known compound whose structure was confirmed previously by synthesis (18). Continued elution of the column with C_6H_6-MeOH (98:2) afforded dihydroalpinumisoflavone (**6**). This compound had been prepared earlier by hydrogenation of alpinumisoflavone (19) as well as by cyclization of isopentenylgenistein (20). Further elution of the column with C_6H_6-MeOH (92.5:7.5) yielded 4′,5,7-trihydroxy-3-methoxyflavone (**7**) (21).

Crotmadine (**1**) and crotmarine (**3**) were tested against *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, *Cryptococcus neoformans*, *Sporotrichium schenckii*, *Trichophyton mentagrophytes*, and *Aspergillus fumigatus* as described earlier (22) and were found inactive except against the fungus *T. mentagrophytes*. Both crotmadine (**1**) and crotmarine (**3**) inhibited the growth of the fungus at a concentration of 62.5 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Unless otherwise stated, uv spectra were obtained in MeOH solution, ir spectra were run in KBr discs, nmr spectra were obtained in $CDCl_3$ with a Varian F-90 spectrometer. Tlc was carried out on silica GF 254 and column chromatography over silica gel (BDH).

PLANT MATERIAL.—The leaves and stems of *C. madurensis* were collected in Karnol, Andra Pradesh, India, by Dr. B.N. Mehrotra of the Botany section, CDRI, and a herbarium sample is retained in the herbarium of Central Drug Research Institute, Lucknow, India.

EXTRACTION.—The air-dried plant material (5 kg) was pulverized and percolated five times with 95% EtOH (12 liters) at room temperature. The solvent from the percolate was removed under reduced pressure below 40° to give a greenish viscous mass which was extracted five times with 5% aqueous HCl (400 ml). The aqueous acidic extract was defatted with petroleum ether five times, 300 ml, and basified with an aqueous solution of Na_2CO_3 to pH 8.5. The liberated bases were extracted five times with $CHCl_3$ (200 ml) to give an alkaloid mixture (4 g). The acid insoluble greenish viscous mass from the EtOH extract was successively extracted five times each with petroleum ether (300 ml), $CHCl_3$ (300 ml) and EtOAc (300 ml) to give petroleum ether (50 g), $CHCl_3$ (40 g), and EtOAc (30 g) soluble fractions, respectively.

CHROMATOGRAPHY OF THE ALKALOID MIXTURE.—The alkaloid mixture (4 g) was chromatographed over a column of silica gel (150 g), which was successively eluted with $CHCl_3$, $CHCl_3$-MeOH (99:1), $CHCl_3$-MeOH (98:2), $CHCl_3$-MeOH (96:4), and $CHCl_3$-MeOH (92:8). Elution was monitored by tlc. A total of 50 fractions, 100 ml each, were collected.

FULVINE (**8**).—The product from fractions 34-49, eluted with $CHCl_3$MeOH (96:4), was crystallized from MeOH to afford fulvine (**8**) (2.2 g, 0.044%), mp 210°. The physical constants (mp, [α]D) and spectroscopic data (ir, uv, nmr, and ms) of the base were identical with those of fulvine (**8** (23).

CHROMATOGRAPHY OF EtOAc SOLUBLE FRACTION.—The EtOAc-soluble fraction (30 g) was chromatographed over a column of silica gel (1 kg), which was successively eluted with C_6H_6, C_6H_6-MeOH (99:1), C_6H_6-MeOH (98:2), C_6H_6-MeOH (95:5), C_6H_6-MeOH (92.5:7.5), and C_6H_6-MeOH (90:10). Elution was monitored by tlc. A total of 100 fractions, each of 250 ml, was collected.

TRANS-3,4,3′,5′-TETRAMETHOXYSTILBENE (**5**).—Fractions 5 to 15 eluted with C_6H_6 were combined and the solvent removed. The residue was crystallized from MeOH to give *trans*-3,4,3′,5′-tetramethoxystilbene (**5**) (7g, 0.14%) mp 66-68°. The physical constant (mp) and spectroscopic data (ir, uv, nmr, and ms) of the compound were identical to those of *trans*-3,4,3′,5′-tetramethoxystilbene (**5**) (17).

CROTMADINE (**1**).—The product from Fractions 28 to 44, eluted with C_6H_6-MeOH (99:1), was crystallized from $CHCl_3$-MeOH to give crotmadine (**1**) (280 mg, 0.006%) mp 191°; uv λ max (MeOH) 372 nm (log ε 5.3) and λ max (MeOH-NaOMe) 435 nm; ir ν max (KBr) 3200 (OH), 1630 (C=O), 1580, 1570, 1480, 1370, 1280, 1220, 1165, 1110, 1040, and 830 cm^{-1}; pmr δ ($CDCl_3$-DMSO-d_6) 1.36 (s, 6H, C-4″, C-5″ (CH_3)$_2$), 1.82 (t, J=7 Hz, 2H, C-2″-H_2), 2.71 (t, J=7 Hz, 2H, C-3″-H_2), 6.37 (d, J=9 Hz, 1H, C-5′-*H*), 6.89 (d, J=9 Hz, 2H, c-3, C-5-*H*), 7.43 (d, J=14 Hz, 1H, C_α-*H*), 7.61 (d, J=9 Hz, 1H, C-6′-*H*), 7.52 (d, J=9 Hz, 2H, C-2, C-6-*H*), and 7.82 (d, J=14 Hz, 1H, C_β-*H*); cmr see Table 1; ms *m/z* (rel. int.) 324 (24), 323 (M^+-1,12), 281 (M^+-43,18), 269 (M^+-55,14), 268 (M^+-56,10), 205 (22), 176 (14), 149 (81), 120 (42), and 55 (100).

CROTMADINE DIACETATE (**2**).—A mixture of **1** (180 mg), Ac_2O (0.5 ml) and pyridine (0,3 ml) was kept at ambient temperature for 20 h. The resulting mixture was worked up in the usual manner to give the diacetate **2**, mp 115°; ir ν max (KBr) 1760 and 1220 cm^{-1}; pmr δ ($CDCl_3$) 2.24 (s, 6H, 2 $COCH_3$); ms, *m/z* (rel. int.) 408 (M^+, 33), 366 (M^+-42,56), 324 (366-42,63), 205 (36), 149 (100), and 120 (63).

DIHYDROALPINUMISOFLAVONE (**6**).—The product from Fractions 48 to 53, eluted with C_6H_6-MeOH (98:2), was crystallized from MeOH to give dihydroalpinumisoflavone (**6**) (100 mg, 0.002%) mp 258-262°. The physical constant (mp) and spectroscopic data (ir, uv, ms, and nmr) of the compound were almost identical to dihydroalpinumisoflavone (**6**) (19).

CROTMARINE (**3**).—The product from Fractions 54 to 67, eluted with C_6H_6-MeOH (98:2), was crystallized from MeOH-C_6H_6 to give crotmarine (**3**) (220 mg, 0.0044%), mp 120°; [α]D −47° (c, 0.91 MeOH); uv λ max (MeOH) 285 nm (log ε 5.2); λ max (MeOH+NaOMe) 295 nm; ir ν max (KBr) 3300 (OH), 1600, 1500, 1460, 1320, 1280, 1220, 1160, 1120, 1048, 1038, 860, and 805 cm^{-1}; pmr δ ($CDCl_3$) 1.76 (s, 3H, C-5″-CH_3), 2.8 to 3.1 (m, 3H, C-3, C-4-*H*), 3.22 (m, 1H, C-1″-*H*), 3.4 (m, 1H, C-1″-*H*), 3.97 (t, J=12 Hz, 1H, C-2-*Hax*), 4.30 (dd, J=2 Hz, 12 Hz, 1H, C-2-*Heq*), 4.91 (br, s, 1H, C-4″-*H*), 5.08 (br, s, 1H, C-4″-*H*), 5.19 (t, J=7 Hz, 1H, C-2″-*H*), 6.35 (m, 3H, C-6, C-8, C-3′-*H*), and 6.91 (m, 2H, C-5, C-6′-*H*); cmr see Table 2; ms, *m/z* (rel. int.) 324 (M^+, 55), 202 (68), 189 (54), 187 (100), and 123 (12).

CROTMARINE DIACETATE (**4**).—A mixture of compound **3** (85 mg), Ac_2O (0.3 ml) and pyridine (0.2 ml) was kept at ambient temperature for 20 h. The resulting mixture was worked up to give the diacetate **4** (60 mg), mp 118° (MeOH); ir ν max (KBr) 1750 and 1205 cm^{-1}; pmr δ ($CDCl_3$) 2.21 (s, 3H,

$COCH_3$), and 2.26 (s, 3H, $COCH_3$); ms *m/z* (rel. int.) 408 (M^+, 46), 366 (M^+-42, 38), 324 (366-42, 19), 244 (57), 202 (244-42, 100), 189 (60), and 187 (50%).

4′,5,7-TRIHYDROXY-3-METHOXYFLAVONE (**7**).—Fractions 81 to 92 eluted from C_6H_6-MeOH (92:5,7.5) were combined and the solvent removed to give a crude product which was subjected to preparative tlc on silica gel eluting with $CHCl_3$-MeOH, 95:5. The major band was removed, extracted with $CHCl_3$-MeOH (3:1), and the solvent removed. The product was crystallized from MeOH to give 4′,5,7-trihydroxy-3-methoxyflavone (**7**; 40 mg, 0.0008%) mp 298°. The physical constant (mp) and the spectroscopic data (uv, ir, nmr, and ms) of the compound were almost identical to those of 4′,5,7-trihydroxy-3-methoxyflavone (**7**) (kaempferol-3-methyl ether) (21).

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Received 5 July 1983