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# ISOFLAVANOIDS OF *CROTALARIA MADURENSIS*[1]

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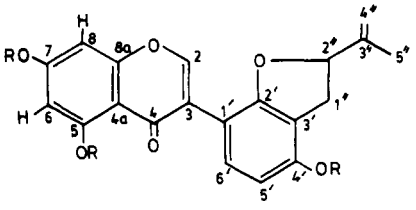
In continuation of our earlier studies (1,2) on the chemistry of the stem and leaves of *Crotalaria madurensis* R. Wight (Leguminosae), we now report the isolation and structure elucidation of two new isoflavones named crotarin [**1**] and crotalarin [**3**]. These isoflavones were obtained by repeated silica gel chromatography of the $CHCl_3$-soluble fraction of the EtOH extract followed by purification through preparative tlc.

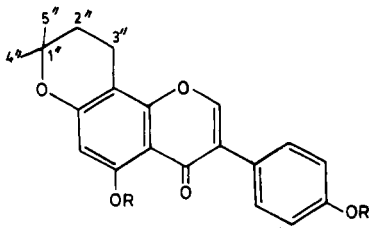
The molecular formula of crotarin [**1**], $C_{20}H_{16}O_6$, was determined by ms ($M^+$, *m/z* 352). Its ir spectrum showed strong bands at 3225, 1660, and 1640 $cm^{-1}$ suggesting the presence of an hydroxyl group and an α, β-unsaturated keto moiety. The uv absorption maximum at 262 and 324 (sh) nm of **1** and shifts with $AlCl_3$ and NaOMe indicated the presence of an isoflavone nucleus (3). The $^1$H-nmr spectrum of **1** revealed the presence of a dihydrofuran ring having an isopropenyl group (4). Methyl protons of the isopropenyl group at C-5″ appeared as a singlet at δ 1.70. Two singlets integrating for one proton each at δ 4.8 and 4.98 were assigned to the exocyclic methylene protons at C-4″. A triplet at δ 5.1 integrating for one proton accounted for the C-2″ proton. In the aromatic region, a singlet at δ 7.8 (1H) was characteristic of the C-2 proton (5) of an isoflavone nucleus, while a three-proton multiplet at δ 6.2 corresponded to aromatic protons at C-6, C-8, and C-5′. The *ortho* coupled doublet at δ 6.84 ($J$=12 Hz) was due to the C-6′ proton.

The retro Diels-Alder fragmentation of **1** ($M^+$, *m/z* 352) in eims gave an ion at *m/z* 200 ($C_{13}H_{12}O_2$) comprised of ring B, while the ion constituting ring A appeared at *m/z* 153. The ion of *m/z* 200 further showed the loss of a methyl group to give the fragment at *m/z* 185.

The $^1$H-nmr pattern coupled with the mass fragmentation confirmed the presence of a dihydrofuran ring having an isopropenyl group in ring B of crotarin.



**1** R=H
**2** R=Ac



**3** R=H
**4** R=Ac

Crotarin [**1**] formed a triacetate **2** ($M^+$, *m/z* 478). The $^1$H-nmr spectrum of **2** exhibited three peaks for acetoxy methyls at δ 2.08, 2.24, and 2.30, confirming the presence of three hydroxyl groups. In the aromatic region, the position of *ortho*-coupled proton of ring B at C-6′ remained unaffected, while the C-5′ proton showed a paramagnetic shift to δ 6.9 and appeared as a doublet. The *meta*-coupled C-6 and C-8 protons shifted downfield to δ 6.78 and 7.14, respectively, inferring the *ortho*-relationship of these protons to the site of acetylation. Thus, the three hydroxyl groups

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in crotarin were placed at C-5, C-7, and C-4′.

The structure of crotarin as **1** was further supported by $^{13}$C-nmr spectral studies (Table 1). The broad band de-

TABLE 1. $^{13}$C nmr of Crotarin [**1**] and Crotalarin [**3**] in DMSO-$d_6$, TMS as Internal Standard

| Carbon | Compounds | |
|---|---|---|
| | **1** | **3** |
| C-2 | 155.16, d | 151.1, d |
| C-3 | 120.60, s | 124.0, s |
| C-4 | 180.46, s | 174.5, s |
| C-4a | 104.48 | 106.4, s |
| C-5 | 161.17, s | 158.1, s |
| C-6 | 98.79, d | 94.19, d |
| C-7 | 164.06, s | 157.7, s |
| C-8 | 93.53, d | 109.12, s |
| C-8a | 157.60, s | 155.9, s |
| C-1′ | 112.6, s | 125.5, s |
| C-2′ | 152.08, s | 131.1, d |
| C-3′ | 111.32, s | 115.7, d |
| C-4′ | 160.84, s | 160.7, s |
| C-5′ | 100.16, d | 115.7, d |
| C-6′ | 131.76, d | 131.1, d |
| C-1″ | 31.97, t | 75.74, s |
| C-2″ | 85.28, d | 31.74, t |
| C-3″ | 143.94, s | 17.66, t |
| C-4″ | 111.01, t | 27.30, q |
| C-5″ | 16.91, q | 27.30, q |

coupled spectrum of the compound exhibited signals for 20 carbon atoms, and the SFORD spectrum revealed the presence of 11 quaternary, 6 methine, 2 methylene, and 1 methyl carbons. The signal at δ 180.4 was assigned to the carbonyl carbon (C-4) of the isoflavone nucleus (6). The characteristic C-2 carbon (7) of **1** appeared as a doublet at δ 155.2. The five oxygen bearing carbons in the aromatic ring (8) appeared between δ 164.06 and 152.08. The downfield carbons at δ 164.16 and 161.17 could be assigned to the C-7 and C-5 carbons, while C-8a appeared at δ 157.6 (9). The chemical shifts of the oxygen bearing aromatic carbons were in agreement with the *meta*-substitution rather than occupying *ortho*-positions. The chemical shifts for *ortho*-substitued oxygen-bearing carbons are reported (10) to be between δ 144.0 to δ 149.0, and, hence, isoprenyl group in ring B, constituting the dihydrofuran ring, is attached through C-3′ to oxygen at C-2′. The alternate cyclization of the isoprenyl group attached at C-2′ to oxygen at C-3′ is ruled out as the latter would place the C-3′ and C-4′ oxygens *ortho* to each other and would have influenced the $^{13}$C chemical shifts of C-3′ and C-4′ (10). The doublet at δ 85.28 was assigned to C-2″ and the triplet at δ 31.97 to C-1″ of the dihydrofuran ring (11). The quaternary carbon of the isopropenyl group appeared at δ 143.94 and the methyl at δ 16.91, while the methylene carbon was observed as a triplet at δ 111.01. Due to the shielding effect of the *ortho*-substituted hydroxyl function, C-6 and C-8 in ring A and C-5′ in ring B appeared at δ 98.79, 93.53, and 100.16, respectively. This fully supported the structure of crotarin as **1**.

Crotalarin [**3**] was obtained as colorless needles, mp 281-85°, and the molecular formula $C_{20}H_{18}O_5$ was confirmed by ms ($M^+$, *m/z* 338). The ir spectrum of **3** has absorption bands at 3200 (OH), 1620, and 1560 $cm^{-1}$ characteristic of an isoflavone carbonyl. The uv absorption at λ max 262 and 320 (sh) nm was supportive of the isoflavone structure (3) for crotalarin **3**. The $^1$H-nmr spectrum of **3** suggested the presence of a dimethyl chroman ring (12,13). The methyl protons of the dimethyl chroman ring at C-4″ and C-5″ appeared as a singlet at δ 1.3, and two multiplets at δ 1.74 and 2.6, each integrating for two protons accounted for the methylene protons at C-2″ and C-3″, respectively. A characteristic singlet of the C-2 proton of the isoflavone nucleus in **3** appeared at δ 7.62. The pair of *ortho* coupled C-2′, C-3′ and C-5′, C-6′ protons appeared at δ 6.75 and 7.32, respectively, while a singlet at δ 6.36 was assigned to C-6 proton indicating the C-alkylation at C-8.

The mass spectrum of crotalarin [**3**] showed the molecular ion peak $M^+$ at

*m/z* 338. The other significant peaks at *m/z* 323 ($M^+$-$CH_3$), 295 ($M^+$-43), 283 ($M^+$-55), 282 ($M^+$-56) were characteristic of fragmentation of the dimethyl chroman ring (14). The peak at *m/z* 118 supported the presence of the monohydroxy (4′-OH) ring B.

Crotalarin [**3**] formed a diacetate **4** ($M^+$, *m/z* 422). The $^1$H-nmr spectrum of the acetate **4** showed the presence of two acetoxy methyls at δ 2.22 and 2.3. The C-6 protons were shifted downfield by 0.3 ppm to δ 6.66 indicating its *ortho*-relationship to the site of acetylation (C-5). The C-3′ and C-5′ protons of ring B were also shifted downfield to δ 7.05 confirming the position of the hydroxyl group at C-4′ in **3**.

The structure **3** of crotalarin was further supported by the $^{13}$C-nmr spectrum (Table 1). The C-4 carbonyl in **3** appeared at δ 174.5. The characteristic doublet at δ 151.1 accounted for the C-2 carbon of the isoflavone nucleus in [**3**]. The carbons bearing hydroxyl groups appeared at δ 160.7 (C-4′) and 158.1 (C-5). Carbons of the dimethyl chroman ring resonated at δ 75.74 (C-1″), 31.74 (C-2″), 17.66 (C-3″) and two methyl carbons at δ 27.30 (C-4″, C-5″) (14). The doublet at δ 94.19 was attributed to C-6 experiencing an α-inductive effect due to the oxygen functions at C-5 and C-7 (10). Singlets at δ 157.7 and 109.12 were assigned to the C-7 and C-8 carbons, respectively. The carbons of ring B were centered at δ 125.5 (C-1′), 131.1 (C-2′, 6′), 115.7 (C-3′, 5′), and 160.7 (C-4′) supporting the position of the C-4′ hydroxyl group in crotalarin **3**. Neither **1** nor **3** showed any antifungal activity.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Unless otherwise stated, uv spectra were obtained in MeOH, ir spectra were run KBr discs, and nmr spectra were obtained in $CDCl_3$ or a mixture of $CDCl_3$ and DMSO-$d_6$ with a Varian F-90 spectrometer. Tlc was carried out on Si GF 254 and column chromatography over Si gel (BDH).

PLANT MATERIAL.—The leaves and stems of *C. madurensis* were collected in November-December 1978, from Kurnool, Andhra Pradesh, India (1), and a voucher specimen No. 110601 of the same is preserved in the herbarium of the Institute.

EXTRACTION.—The air dried plant material (5 kg) was pulverized and extracted with 95% EtOH (3×4 liters) at room temperature. The solvent from the percolate was removed under reduced pressure below 40°, and the resulting residue was fractionated as reported earlier (1) to yield the following fractions, petroleum ether (50 g), $CHCl_3$ (40 g), and EtOAC (30 g) from the non-alkaloidal part of the EtOH extract.

CHROMATOGRAPHY OF $CHCl_3$-SOLUBLE FRACTION.—The $CHCl_3$-soluble fraction (40 g) was chromatographed on a column of Si gel (1.5 kg). The column 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), and $C_6H_6$-MeOH (90:10), and 75 fractions (500 ml each) were collected.

CROTARIN [**1**].—Elution of column with $C_6H_6$-MeOH (99:1) (fractions 21-35) gave a mixture which was further resolved by preparative tlc using $C_6H_6$-MeOH (96:4) as eluent. The major compound thus obtained was crystallized from MeOH to give crotarin [**1**] (70 mg); (mp 260° dec); yield 0.0014%; λ max (MeOH) 262 nm (log ε, 5.4687), 324 (sh) (log ε, 4.1236) nm; λ max (MeOH)+$AlCl_3$ 270 and 360 (sh) nm; λ max (MeOH) +NaOMe 262 and 324 nm; ir ν max (KBr) 3225, 1660, 1640, 1620, 1560, 1500, 1460, 1365, 1300, 1260, 1180, 1100, 1050, 975, 918, 825, 800, 740 $cm^{-1}$; $^1$H nmr δ ($CDCl_3$+DMSO-$d_6$) 1.70 (s, 3H, C-5″-$CH_3$), 2.7-3.5 (m, 2H, C-1″-$H_2$), 4.8 (s, 1H, C-4″-*H*), 4.98 (s, 1H, C-4″-*H*), 5.12 (t, *J*=7 Hz, 1H, C-2″-*H*), 6.14 (d, *J*=2 Hz, 1H, C-6-*H*), 6.26 (d, *J*=2 Hz, 1H, C-8-*H*), 6.28 (d, *J*=7 Hz, 1H, C-5′-*H*), 6.84 (d, *J*=9 Hz, 1H, C-6′-H), 7.8 (s, 1H, C-2-*H*); $^{13}$C nmr (Table 1); eims *m/z* (rel. int.) 352 ($M^+$, 52), 337 ($M^+$, 52), ($M^+$-15, 100), 200 (8), 187 (22), 185 (12), 153 (12).

CROTARIN TRIACETATE [**2**].—A mixture of **1** (20 mg), $Ac_2O$ (0.5 ml), and pyridine (0.5 ml) was kept at ambient temperature for 15 h. The resulting mixture was poured over crushed ice. The precipitate was washed with $H_2O$ to neutral pH to give the triacetate **2** (18 mg), mp 196° (MeOH); ir ν max (KBr) 1760, 1620, 1590, 1270, 1200, 1160, 1135, 1045 $cm^{-1}$; $^1$H nmr δ ($CDCl_3$) 1.72 (s, 3H, C-5″-$CH_3$), 2.08, 2.24, 2.3 (s each, 3H each, 3×OA*c*), 2.7-3.4 (m, 2H, C-1″-$H_2$), 4.82 (bs, 1H, C-4″-*H*), 5.0 (bs, 1H, C-4″-*H*), 5.15 (t, *J*=7 Hz, C-2″-*H*), 6.62 (d, *J*=9 Hz, 1H, C-6′-*H*), 6.78 (d, *J*=2 Hz, 1H, C-6-*H*), 6.94 (d, *J*=9 Hz, 1H, C-5′-*H*), 7.14 (d, *J*=2 Hz, 1H, C-8-*H*), 7.68 (1H, s, C-2-*H*); eims

*m/z* (rel. int.) 478 ($M^+$, 39), 436 ($M^+$-42, 40), 394 (436-42, 70), 352 (394-42, 19), 337 (352-15, 75).

CROTALARIN [**3**].—The product from the fractions 46 to 60 of silica column eluted with $C_6H_6$-MeOH (95:5) was subjected to preparative tlc using $C_6H_6$-MeOH (94:6) as eluent followed by crystallization, ($CHCl_3$/MeOH) yielded crotalarin [**3**] (60 mg) mp 281-285°; yield 0.0012%; uv λ max (MeOH) 260 (log ε, 5.6152), 320 (sh) (log ε, 4.8386); λ max (MeOH) ($AlCl_3$) 260 and 360 nm; ir ν max (KBr) 3200, 1620, 1560, 1450, 1305, 1260, 1220, 1160, 1120, 1080, 1060, 1015, 870, 840 $cm^{-1}$; $^1H$ nmr δ ($CDCl_3$+DMSO-$d_6$) 1.32 (s, 6H, C-4″, 5″ C-$(CH_3)_2$, 1.74 (t, *J*=7 Hz, 2H, C-2″-$H_2$), 2.6 (t, *J*=7 Hz, 2H, C-3″, $CH_2$), 6.36 (s, 1H, C-6, CH), 6.75 (d, *J*=9 Hz, 2H, C-3′, 5′-*H*) 7.32 (d, *J*=9 Hz, 2H, C-2′, 6′-*H*), 7.62 (s, 1H, C-2-*H*); $^{13}C$ nmr refer to Table 1; eims *m/z* (rel. int.) 338 ($M^+$, 100), 323 ($M^+$-$CH_3$, 17), 295 ($M^+$-43, 51), 282 ($M^+$-56, 28), 283 ($M^+$-55, 100), 176 (25), 118 (21).

CROTALARIN DIACETATE [**4**].—A mixture of crotalarin **3** (50 mg), $Ac_2O$ (0.5 ml), and pyridine (0.5 ml) was kept at ambient temperature for 20 h. The resulting mixture was worked up as for **2** to give the diacetate **4**, mp 179-180° (MeOH); ir ν max (KBr) 1760, 1375, 1240, 1200, 1125, 1080, 1058, 920, 905, 875, 840 $cm^{-1}$; $^1H$ nmr δ ($CDCl_3$) 1.38 (s, 6H, C-4″, 5″-$C(CH_3)_2$, 1.78 (t, *J*=7 Hz, 2H, C-2″-$H_2$), 2.22 (s, 3H, OAc), 2.3 (s, 3H, OAc), 2.58 (t, *J*=7 Hz, 2H, C-3″-$H_2$), 6.66 (s, 1H, C-6-H), 7.05 (d, *J*=9 Hz, 2H, C-3′, 5′-*H*), 7.45 (d, *J*=9 Hz, 2H, C-2′, 6′-*H*), 7.7 (s, 1H, $C_2$-*H*); eims *m/z* (rel. int.) 422 ($M^+$, 16.2), 380 ($M^+$-42, 29), 338 (380-42, 11), 295 (12), 283 (36).

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