

## MINOR PHENOLIC CONSTITUENTS OF *CRINUM AUGUSTUM*

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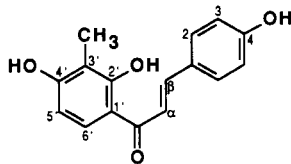
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**ABSTRACT.**—Three minor phenolic compounds were isolated from the bulbs of *Crinum augustum*. Two known compounds were identified as 2',4,4'-trihydroxychalcone and (–)-4'-hydroxy-7-methoxy-8-methylflavan. The structure of a new compound, 2',4,4'-trihydroxy-3'-methylchalcone [**1**], has been established from spectral and chemical evidence.

The genus *Crinum* (Amaryllidaceae) is well known for its alkaloids (1,2). The alkaloidal content of *Crinum augustum* Roxb. from Egypt has been established (2–6) as well as its mucilage and fatty acid combination (7). Because the neutral biologically active compounds of *C. augustum* have been only partly studied, we have undertaken a systematic chemical examination of the flavonoid constituents occurring in this species. In our previous paper, we reported the isolation of 4'-hydroxy-7-methoxyflavan (6). Here, we report the isolation and structure elucidation of a new, naturally occurring chalcone, 2',4,4'-trihydroxy-3'-methylchalcone [**1**], as well as the known 2',4,4'-trihydroxychalcone and (–)-4'-hydroxy-7-methoxy-8-methylflavan. The known compounds have not been previously isolated from the family Amaryllidaceae. We also report here the <sup>13</sup>C-nmr spectral data for the latter known compound.



**1**

## RESULTS AND DISCUSSION

Extraction of dried bulbs of *C. augustum* with both petroleum ether and EtOAc followed by Si gel chromatography afforded (–)-4'-hydroxy-7-methoxy-8-methylflavan, 2',4,4'-trihydroxychalcone, and the new compound, 2',4,4'-trihydroxy-3'-methylchalcone [**1**].

Compound **1**, C<sub>16</sub>H<sub>14</sub>O<sub>4</sub> ([M]<sup>+</sup> 270.0882, calcd 270.0892), separated from MeOH as yellow needles, mp 196°, and produced a brown color with FeCl<sub>3</sub> that indicated its phenolic nature. The color reactions (positive test with FeCl<sub>3</sub> and negative Shinoda) and uv absorption maxima [(258 (sh), 300 (sh), and 370 nm)] suggested the presence of a chalcone system (8). The uv data, on the other hand, resembled closely those of 2',4,4'-trihydroxychalcone (9–11). The displacement of the 258 nm band by 22 nm upon addition of NaOAc indicated the presence of a hydroxyl group at the 4'-position. The bathochromic shift and the increase of the intensity [370 nm (log 4.22) → 435 (log 4.29)] in methanolic NaOMe indicated the presence of the 4-hydroxyl group in the B ring (9–12). In AlCl<sub>3</sub> alcoholic solution the uv absorption maxima suggested the presence of a phenolic hydroxyl near the carbonyl group (9–13).

The ir spectrum of **1** exhibited absorptions at 3410 (free OH), 3230 (chelated OH), and 1630 cm<sup>-1</sup> (conjugated carbonyl). This skeleton was also supported by the <sup>13</sup>C-nmr spectrum (Table

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1) which showed peaks for C- $\alpha$ , C- $\beta$ , and a carbonyl carbon at  $\delta$  119.3, 146.6, and 194.5, respectively. The 200 MHz nmr spectrum (Table 1) showed two trans-olefinic protons at  $\delta$  7.62 (d,  $J = 16$  Hz) and  $\delta$  7.80 (d,  $J = 16$  Hz), providing evidence for the chalcone system (14,15). Six aromatic protons, four distinguished by an  $A_2B_2$  quartet centered at  $\delta$  6.82 and 7.66, supported the presence of a 4-hydroxyl group in the B ring. The other two aromatic hydrogen atoms were adjacent and centered at  $\delta$  8.0 and 6.5 (each 1H, d,  $J = 8.5$  Hz); this established the presence of two aromatic protons with ortho coupling in ring A. Irradiation of each of the doublets collapsed the other to a singlet. Also, a three-proton singlet appeared at  $\delta$  2.01 due to the 3'-methyl substituent in ring A.

Confirmation of this structure was obtained by the eims of **1** in which the molecular ion was detected at  $m/z$  270. In addition, a number of prominent peaks at  $m/z$  137 ( $C_7H_5O_3$ , 100%) and  $m/z$  120 ( $C_8H_8O$ , 94%) arising from rings A and B, respectively, confirmed

this hypothesis. The latter peak ( $m/z$  120) corresponded to a hydroxystyrene molecule. Other characteristic fragment ions were at  $m/z$  256 [ $M - CH_2$ ] $^+$  (78), 255 [ $M - Me$ ] $^+$  (40), 239 (10), 177 (11), 163 (31), 151 (51), 107 (32), and 91 (28), indicating the presence of two hydroxyl groups as well as one methyl group in ring A and one hydroxyl group in ring B.  $^{13}C$ -nmr spectral data ( $CD_3OD$ ) (Table 1) exhibited a total of 14 signals, two of which ( $\delta$  132.7 and 119.3) are of high intensity and closely resembled those of 2',4,4'-trihydroxychalcone (isoliquiritigenin) (16). In addition, one new signal appeared at  $\delta$  31.7 due to the aromatic methyl group. On the basis of the above physical, chemical, and spectroscopic studies, the structure **1** was concluded to be 2',4,4'-trihydroxy-3'-methylchalcone. No report on the isolation of this simple, biogenetically important chalcone from a plant source has been made previously.

2',4,4'-Trihydroxychalcone (isoliquiritigenin) was obtained as yellow needles from MeOH. Its molecular formula  $C_{15}H_{12}O_4$  was assigned from the hrms,

TABLE 1.  $^1H$ -nmr Data and  $^{13}C$ -nmr Assignments for 2',4,4'-Trihydroxy-3'-methylchalcone [**1**] and  $^1H$ -nmr Data of 2',4,4'-Trihydroxychalcone.

Atom	Compound				
	<b>1</b>			2',4,4'-Trihydroxychalcone	
	$^1H$	$J$ (Hz)	$^{13}C$	$^1H$	$J$ (Hz)
C- $\beta$	7.80, d	16.0	146.6	7.82, d	16.0
C- $\alpha$	7.62, d	16.0	119.3	7.65, d	16.0
C=O	—	—	194.5	—	—
C-2'	—	—	194.5	—	—
C-3'	—	—	167.5	—	—
C-4'	—	—	104.9	6.47, d	2.5
C-5'	6.50, d	8.50	110.1	6.47, q	8.5, 2.5
C-6'	8.00, d	8.50	134.4	6.98, d	8.5
C-1'	—	—	115.6	—	—
C-1	—	—	128.9	—	—
C-2	6.82, q	—	132.0	6.60, q	—
C-3	7.66, q	—	117.0	7.60, q	—
C-4	—	—	162.6	—	—
C-5	7.66, q	—	117.0	7.60, q	—
C-6	6.82, q	—	132.0	6.60, q	—
C-Me	2.01, s	—	31.7	—	—

which showed a molecular ion at  $m/z$  256.0813. It was most readily characterized as 2',4,4'-trihydroxychalcone by comparison of its physical and spectroscopic properties (uv,  $^1\text{H}$  nmr, and  $^{13}\text{C}$  nmr) with those of the reported isoliquiritigenin (9–12, 16–18). Its 200 MHz spectrum in  $\text{CD}_3\text{OD}$  (Table 1) was very similar to that of **1** apart from changes associated with replacement of the Me group at position 3' by a proton.

The co-occurrence of these two chalcones in the same plant is of biogenetic significance. It has been pointed out by Shinoda and Sato (19) and Andityachaudhury *et al.* (20) that chalcones containing the 2,4,6-trihydroxybenzoyl nucleus isomerize readily to the corresponding flavanones, while those containing the 2,4-dihydroxybenzoyl group are relatively more stable in the chalcone form. The facile isolation of these stable chalcones from *C. augustum* bulbs provides circumstantial evidence for the correctness of the above view.

(-)-4'-Hydroxy-7-methoxy-8-methylflavan was isolated as colorless crystals. Its physical properties, uv, ir, and  $^1\text{H}$ -nmr spectra are in full agreement with those reported for (-)-4'-hydroxy-7-methoxy-8-methylflavan (21,22).  $^{13}\text{C}$ -nmr spectral data are reported here for the first time. Flavans unsubstituted in the pyran ring are not common natural products (21,23). Most of the known flavans have oxygen at the 3 or 4 or the 3,4 positions (24). This compound is one of the few flavans to be obtained from a natural source not containing an oxygen attached to the heterocyclic ring.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Mp's are uncorrected; uv spectra were measured with a Beckman Du-65 spectrophotometer, using MeOH for the compound **1** and the other chalcone and in  $\text{CHCl}_3$  for the flavan; ir spectra were recorded with a Beckman 4230 spectrophotometer in KBr.  $^1\text{H}$ -nmr spectra were run in  $\text{CDCl}_3$  or  $\text{CD}_3\text{OD}$  and were referenced to internal TMS at  $\delta$  0.00 for  $^1\text{H}$ -nmr spectra and to deuterated solvents for  $^{13}\text{C}$ -nmr spectra. Eims spectra were recorded at 70 eV using a direct inlet

system with high resolution. Cc was performed on Si gel (E. Merck, 0.2–0.5 mm). Tlc was performed on a pre-coated Si gel layers.

**PLANT MATERIAL.**—Whole plants of *C. augustum*, cultivated in the campus of Assiut University, were collected in May 1987. The plants were previously propagated by offsets from plants identified by the late Prof. Vivi Tackholm of the Cairo University Herbarium, Giza, Cairo.

**EXTRACTION AND ISOLATION.**—Air-dried chipped bulbs (10 kg) were extracted with EtOH (95%) by maceration followed by percolation. The solvent was evaporated under reduced pressure. The residue was successively extracted with petroleum ether, EtOAc, and *n*-BuOH. Concentration of the extracts gave 750 g (7.5%) of petroleum ether extract, 250 g (2.50%) of EtOAc extract, and 600 g of *n*-BuOH extract. Only the petroleum ether extract and EtOAc extract were examined in this investigation. Part of the petroleum ether extract (60 g) was directly chromatographed over Si gel (1.20 kg) with solvents of increasing polarity using petroleum ether,  $\text{C}_6\text{H}_6$ ,  $\text{CHCl}_3$ , and MeOH. (-)-4'-Hydroxy-7-methoxy-8-methylflavan (12 mg) was eluted with  $\text{C}_6\text{H}_6$ - $\text{CHCl}_3$  (4:1) followed by a sterol fraction (150 mg). Direct chromatography of a portion of the EtOAc extract (80 g) over Si gel (1.60 kg) was performed in the same way as for the petroleum ether extract. The  $\text{C}_6\text{H}_6$ - $\text{CHCl}_3$  (1:4) eluate afforded a yellow solid (55 mg). Further cc on Si gel eluted with  $\text{C}_6\text{H}_6$ - $\text{CHCl}_3$  (1:4) yielded compound **1** (30 mg). The  $\text{CHCl}_3$ -MeOH (99:1) eluate gave 2',4,4'-trihydroxychalcone (10 mg).

**2',4,4'-TRIHYDROXY-3'-METHYLCHALCONE [1].**—Yellow crystals: mp 196°; ir  $\nu$  max (KBr) 3410, 3230, 1630, 1500, 1450, 1200, 1060, 820  $\text{cm}^{-1}$ ; uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 258 (4.06), 300 (4.13), 370 nm (4.22); (NaOAc) 280 (4.09), 379 (4.23); (NaOMe) 256 (4.06), 435 (4.29); ( $\text{AlCl}_3$ ) 258 sh (4.06), 315 (4.15), 382 sh (4.23), 419 (4.27);  $^1\text{H}$  nmr (200 MHz,  $\text{CD}_3\text{OD}$ ) see Table 1;  $^{13}\text{C}$  nmr ( $\text{CD}_3\text{OD}$ ) see Table 1; hrms calcd for  $\text{C}_{16}\text{H}_{14}\text{O}_4$   $m/z$   $[\text{M}]^+$  270.08921, found  $m/z$  270.08828.

**2',4,4'-TRIHYDROXYCHALCONE.**—Yellow needles: mp 200° [lit. (11) 199.5–200.5°]; ir  $\nu$  max (KBr) 3400, 3200, 1635, 1610, 1520, 1220, 1040, 990, 730  $\text{cm}^{-1}$ ; uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 260 (4.06), 300 (4.13), 373 (4.23); (NaOAc) 282 (4.09), 340 (4.03), 350 sh (4.05), 378 (4.23), (NaOEt) 258 (4.06), 438 (4.30); ( $\text{AlCl}_3$ ) 260 sh (4.06), 315 (4.15), 382 sh (4.23), 419 (4.27) (9–12);  $^1\text{H}$ -nmr (200 MHz,  $\text{CD}_3\text{OD}$ ) see Table 1.

**(-)-4'-HYDROXY-7-METHOXY-8-METHYLFLAVAN.**—Mp 126–127° [lit. (21) 126–127°];  $[\alpha]_D^{25}$  -22.2° ( $c$  = 0.8;  $\text{CHCl}_3$ ); uv  $\lambda$  max

(CHCl<sub>3</sub>) (log  $\epsilon$ ) 238 (4.12), 277 sh (3.56), 281 (3.54), 285 sh (3.51);  $\nu$  max (KBr) 3300, 1613, 1590 cm<sup>-1</sup>. <sup>1</sup>H-nmr (200 MHz, CDCl<sub>3</sub>)  $\delta$  7.32 and 6.94 (4H, A<sub>2</sub>B<sub>2</sub> quartet, ring B protons), 6.91 and 6.52 (2H, d,  $J$  = 8.5 Hz, ring A protons), 5.10 (1H, dd,  $J$  = 8.0, 4.0 Hz, H-2), 2.10 (2H, m, H-3), 2.80 (2H, m, H-4), 2.01 (3H, s, Me-8), 3.77 (3H, s, OMe); <sup>13</sup>C nmr (CDCl<sub>3</sub>)  $\delta$  159.9 (C-7), 155.8 (C-9), 155.2 (C-4'), 133.8 (C-5), 129.9 (C-1'), 127.6 (C-2', 6'), 115.3 (C-3', 5'), 113.9 (C-10), 107.3 (C-6), 101.5 (C-8), 77.6 (C-2), 55.3 (OMe), 29.3 (C-3, -4) and 21.1 (Me-8).

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