

**TWO NEW ISOFLAVONES FROM *ERYTHRINA SUBEROSA* VAR. *GLABRESCENCES***

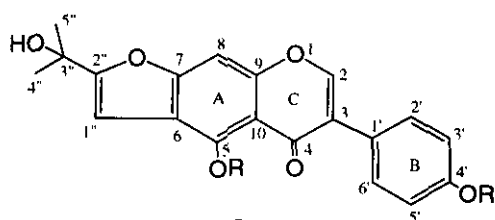
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**Abstract** - Two new isoflavones, erysubins A and B, were isolated from the wood of *Erythrina suberosa* var. *glabrescences* (Leguminosae) and their structures were elucidated on the basis of spectroscopic evidence.

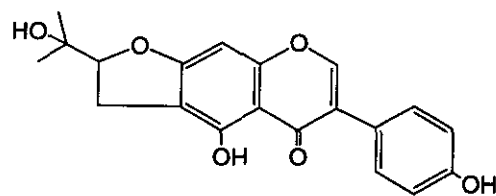
In a continuation of our study on genus *Erythrina*,<sup>1</sup> we have investigated the non-alkaloidal secondary metabolites of *Erythrina suberosa* var. *glabrescences* which has been used in Pakistan as an ornament and folk medicine. Phytochemical studies of this genus have led to the isolation of sterols,<sup>2</sup> a flavanone<sup>3</sup> and a number of alkaloids.<sup>4</sup> We now describe the isolation and structural elucidation of two new isoflavones, named erysubin A (**1**) and erysubin B (**4**), along with five known isoflavones (erythrinin C (**3**),<sup>5,6</sup> alpinumisoflavone (**5**),<sup>7</sup> a wighteone metabolite (**6**),<sup>8</sup> wighteone (**7**)<sup>9</sup> and laburnetin (**8**)),<sup>10,11</sup> and a known pterocarpan, cristacarpin (**9**),<sup>12</sup> from the methylene chloride extract of the wood of this plant.

Erysubin A (**1**) was obtained as pale yellow needles and the molecular formula was confirmed to be C<sub>20</sub>H<sub>16</sub>O<sub>6</sub> by the HRMS (*m/z* 352.0943). The UV spectrum showed absorption maxima at 203, 212 (sh), 266 and 354 nm and the IR spectrum exhibited the presence of a conjugated carbonyl (1660 cm<sup>-1</sup>) and hydroxyl (3400 cm<sup>-1</sup>) groups. Acetylation of **1** with acetic anhydride and pyridine provided a diacetate

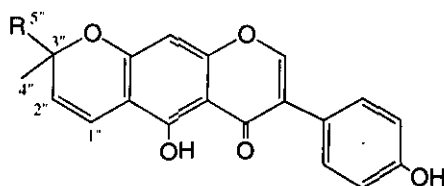


1 R = H

2 R = Ac

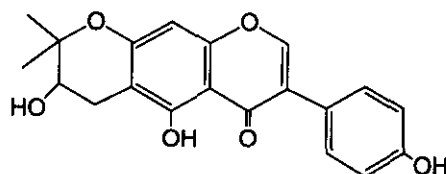


3



4 R = CH<sub>2</sub>OH

5 R = Me



6

derivative (**2**) whose <sup>1</sup>H NMR spectrum revealed signals of two acetyl groups [ $\delta$  2.32, 2.50 (each, 3H, s)] and one hydroxyl group [ $\delta$  3.54 (1H, s)]. Comparison of the <sup>1</sup>H NMR spectrum of **1** with that of **3**<sup>5</sup> displayed the same substituent patterns of B and C rings and the <sup>13</sup>C NMR assignment (Table 1) also suggested the presence of the structural moieties. In the <sup>1</sup>H NMR spectrum, a singlet aromatic proton at  $\delta$  7.33 was located at C-8 position by the HMBC spectrum (Figure 1) which showed correlations between H-8 and C-9 ( $\delta$  153.4), C-6 ( $\delta$  113.1), C-10 ( $\delta$  105.9). The remaining signals [ an other singlet olefinic proton at  $\delta$  6.77, geminal methyl groups [ $\delta$  1.55 (6H, s)] on a carbon carrying oxygen and a hydroxyl group [ $\delta$  5.49 (1H, s)] were ascribed to a 2-hydroxy-2-isopropylfuran moiety (C<sub>7</sub>H<sub>8</sub>O<sub>2</sub>) from the HMBC spectrum as follows: the signal of the geminal methyl groups correlated to a carbinol carbon signal ( $\delta$  67.4) which indicated the presence of a hydroxydimethyl group. The methyl groups signal also correlated to a oxygenated *sp*<sup>2</sup> quaternary carbon signal at C-2'' ( $\delta$  165.0) and thus the hydroxydimethyl group was linked to C-2'' position. In addition, the carbon signal of C-2'' showed cross peak with the aromatic proton at H-1'' ( $\delta$  6.77) which correlated to a *sp*<sup>2</sup> quaternary carbon signal at C-6 ( $\delta$  113.1) and, on the other hand, a chelated hydroxyl proton ( $\delta$  13.71) exhibited correlation with a *sp*<sup>2</sup> quaternary carbon signal at C-7 ( $\delta$  154.2) attached to oxygen of the furan ring. From these results, the hydroxyisopropylfuran moiety was deduced to be fused at the 6 and 7 positions which was further confirmed by the NOE interaction between

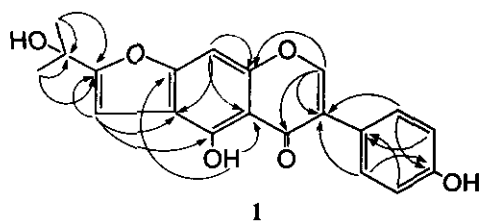


Figure 1. Long-range Correlations in the HMBC Spectrum of Erysubin A (1)

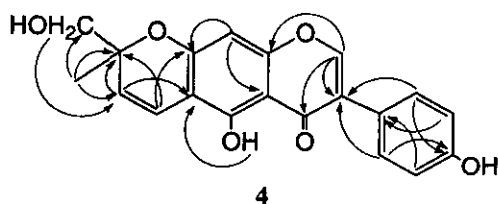


Figure 2. Long-range Correlations in the HMBC Spectrum of Erysubin B (4)

Table 1.  $^{13}\text{C}$  NMR spectral data of 1, 4 and 6

C	1	4	6
2	154.9	154.4	154.3
3	121.5	124.0	123.6
4	182.0	181.8	181.7
5	158.0	157.6	160.8
6	113.1	106.0	105.0
7	154.2	160.5	160.5
8	90.6	95.3	95.0
9	153.4	158.1	156.7
10	105.9	106.6	105.6
1'	121.0	122.9	123.1
2'	130.2	131.1	131.1
3'	115.1	115.9	115.9
4'	157.4	158.4	158.3
5'	115.1	115.9	115.9
6'	130.2	131.1	131.1
1''	97.1	117.4	26.0
2''	165.0	126.3	68.7
3''	67.4	82.0	79.7
4''	28.7	23.6	25.7
5''	28.7	68.7	21.2

Spectrum of 1 was recorded in  $\text{DMSO}-d_6$  and spectra of 4 and 6 were recorded in  $\text{acetone}-d_6$ .

H-1'' and the chelated hydroxyl proton. Therefore, the structure of erysubin A was represented as formula (1). This is the first naturally occurring isoflavone possessing the hydroxyisopropylfuran substituent.

Erysubin B (4) was obtained as pale yellow needles and IR spectrum showed also the presence of a conjugated carbonyl and hydroxyl groups. The MS spectrum exhibited the parent ion ( $C_{20}H_{16}O_6$ ) at  $m/z$  352 and the intense mass fragment at  $m/z$  321  $[M-CH_2OH]^+$ .<sup>13</sup> Comparison of the  $^1H$  NMR spectrum of 4 with that of 5<sup>7</sup> revealed that 4 have the same partial structures except for an oxymethylene group [ $\delta$  3.61, 3.67 (each 1H, d,  $J=11.7$  Hz)]. Thus, two compounds differed only by a hydroxyl substituted on one of the geminal methyl groups of the dimethylpyran portion: signals of hydroxymethyl group in the  $^1H$  NMR spectrum were observed at  $\delta$  3.61, 3.67 and  $\delta$  4.20 (1H, br s, OH, disappeared after addition of  $D_2O$ ). Next, the location of the 6, 7-fused pyran ring (linear isomer) was confirmed from the HMBC spectrum (Figure 2) which indicated correlations between H-2'' ( $\delta$  5.75) and C-6 ( $\delta$  106.0), H-1'' ( $\delta$  6.77) and C-7 ( $\delta$  160.5). The configuration of C-3'', however, was remained to be determined. Therefore, the structure of Erysubin B was represented as formula (4).

Compound (6) was the known metabolite (M-Wi-2)<sup>8</sup> which occurred as a metabolite of 7 in cultures of *Aspergillus flavus* and *Botrytis cinerea* and was isolated in racemic form. The MS spectrum, as that of the reported metabolite, showed the parent ion ( $C_{20}H_{18}O_6$ ) at  $m/z$  354 and the characteristic fragment of a hydroxylated dimethyldihydropyran substituent at  $m/z$  283  $[M-71]^+$ .<sup>14</sup>  $^1H$  NMR and  $^{13}C$  NMR spectral data of 6 are reported here. Assignment of all the  $^1H$  NMR and  $^{13}C$  NMR signals of 1, 4 and 6 was accomplished by analyses of the  $^1H$ - $^1H$  COSY, HSQC and HMBC spectra.

## EXPERIMENTAL

**General.** The instruments used for this study were as follows: a JASCO DIP-370 digital polarimeter (for specific rotation, measured at 23°C); a JASCO IR-810 spectrophotometer (for IR spectra); a Shimadzu UV-2100 spectrophotometer (for UV spectra); a JEOL JMS-D 300 spectrometer (for MS and HRMS spectra); a JEOL JNM-A 600 spectrometer (for NMR spectra using tetramethylsilane as an internal standard). Column chromatography was carried out with silica gel 60 (230-400 mesh; MERCK).

**Plant material.** The dried wood of *E. suberosa* var. *glabrescences* was collected at Karachi, Pakistan, in July, 1997. A voucher specimen was deposited at Department of Natural Product Chemistry in Faculty of Pharmacy, University of Meijo.

**Extraction and isolation.** The wood (2.55 kg) was extracted with acetone (36 L) at 23° C for 72 h and evaporated to give a dark green residue (133.7 g). The residue was divided into *n*-hexane-, CH<sub>2</sub>Cl<sub>2</sub>-, and EtOAc-soluble fractions. The CH<sub>2</sub>Cl<sub>2</sub>-soluble fraction (50 g) was chromatographed on silica gel and eluted with CHCl<sub>3</sub> (fractions A1-10), CHCl<sub>3</sub>-acetone (10:1)(frs. A11-32) and CHCl<sub>3</sub>-acetone (1:1) (frs. A33-69) and CHCl<sub>3</sub>-MeOH (10:1) (frs. A70-81)(each fraction; 200 mL, Column A). The fraction A45 was separated by silica gel column chromatography [benzene-EtOAc (10:1) (frs. B1-25), benzene-EtOAc (3:1) (frs. B26-41) and benzene-EtOAc (1:1) (frs. B42-60)(each fraction; 20 mL, Column B)] to give alpinumisoflavone (**5**) (73 mg)(frs. B10-15) and wighteone (**7**) (124 mg)(frs. B35-42). The fractions A52- 55 were separated by silica gel column chromatography [*n*-hexane-acetone (3:1)(frs. C1-40) and *n*-hexane-acetone (1:1)(frs. C41-50)(each fraction; 20 mL, Column C)] and subsequently the fraction C22-44 were rechromatographed by silica gel column [benzene-EtOAc (5:1)(frs. D1-60) and benzene-EtOAc (1:1)(frs. D61-91) (each fraction; 10 mL, Column D)] to afford laburnetin (**8**) (31 mg) (frs. D70-81) and cristacarpin (**9**) (20 mg) (frs. D3-4). The fractions A69-70 were separated by repeated silica gel column chromatography [benzene-EtOAc (5:1)(Frs. E1-60) and benzene-EtOAc (1:1) (Frs. E61-131) (each fraction; 20 mL, Column E)] to yield erysubin A (**1**)(37 mg) (fr. E92), erythrinin C (**3**)(167 mg) (frs. E94-96), erysubin B (**4**)(13 mg) (fr. E99) and **6** (65 mg)(fr. E85).

The identification of **3** and **5-9** was made by comparison of the physical and spectral data with those published in the literature.<sup>5-12</sup>

**Erysubin A (1).** Pale yellow needles from EtOH. mp 231-233°C. IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3400, 1660, 1620. UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 203 (4.53), 212 (sh 4.45), 266 (4.68), 354 (3.51). MS  $m/z$ : 352 [M]<sup>+</sup>, 337, 334 (base peak), 309, 305, 295, 283, 216, 167, 160. HRMS  $m/z$  352.0943 (M<sup>+</sup>, calcd for C<sub>20</sub>H<sub>16</sub>O<sub>6</sub>: 352.0946). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  1.55 (6H, *s*, 4''- and 5''-Me), 5.49 (1H, *s*, 3''-OH), 6.77 (1H, *s*, H-1''), 6.85 (2H, *d*, *J*=8.8 Hz, H-3' and H-5'), 7.33 (1H, *s*, H-8), 7.42 (2H, *d*, *J*=8.8 Hz, H-2' and H-6'), 8.46 (1H, *s*, H-2), 9.59 (1H, *s*, 4'-OH), 13.71 (1H, *s*, 5-OH). <sup>13</sup>C NMR: see Table 1.

**Acetylation of 1.** A mixture of **1** (9 mg), Ac<sub>2</sub>O (0.5 mL) and pyridine (0.5 mL) was stirred overnight at rt. After work-up in the usual way, the residue was purified by silica gel column chromatography [benzene-EtOAc (3:1)] to yield a diacetate derivative (**2**) (8 mg) as an amorphous powder. IR (CHCl<sub>3</sub>)  $\nu_{\max}$  cm<sup>-1</sup>: 3600, 1750, 1710, 1630, 1600. UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 202 (4.48), 253 (4.62), 331 (3.75). MS  $m/z$ : 436 [M]<sup>+</sup>, 418, 394 (base peak), 379, 376, 352, 337, 334, 309, 295, 236. HRMS  $m/z$ : 436.1148

( $M^+$ , calcd for  $C_{24}H_{20}O_8$ : 436.1157).  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  1.70 (6H, s, 4''- and 5''-Me), 2.32 (3H, s, 4'-Ac), 2.50 (3H, s, 5-Ac), 3.54 (1H, br s, OH), 6.68 (1H, s, H-1''), 7.16 (2H, d,  $J=8.8$  Hz, H-3' and H-5'), 7.45 (1H, s, H-8), 7.53 (2H, d,  $J=8.8$  Hz, H-2' and H-6'), 7.92 (1H, s, H-2). These signals were assigned by the NOESY spectrum.

**Erysubin B (4)**. Pale yellow needles from EtOH. mp 247-249°C.  $[\alpha]_D - 16^\circ$  ( $c = 0.1$ , MeOH). IR (KBr)  $\nu_{max}$   $cm^{-1}$ : 3400, 1660, 1620. UV  $\lambda_{max}$  (MeOH) nm (log  $\epsilon$ ): 203 (4.46), 226 (4.33), 283 (4.57). MS  $m/z$ : 352 [ $M^+$ ], 321 (base peak), 295, 283, 270, 203, 161. HRMS  $m/z$  352.0953 ( $M^+$ , calcd for  $C_{20}H_{16}O_6$ : 352.0946).  $^1H$  NMR (acetone- $d_6$ ):  $\delta$  1.43 (3H, s, 4''-Me), 3.61 (1H, d,  $J=11.7$  Hz, H-5''), 3.67 (1H, d,  $J=11.7$  Hz, H-5''), 4.20 (1H, br s, 5''-OH), 5.75 (1H, d,  $J=10.3$  Hz, H-2''), 6.36 (1H, s, H-8), 6.77 (1H, d,  $J=10.3$  Hz, H-1''), 6.91 (2H, d,  $J=8.8$  Hz, H-3' and H-5'), 7.46 (2H, d,  $J=8.8$  Hz, H-2' and H-6'), 8.19 (1H, s, H-2), 8.60 (1H, br s, 4'-OH), 13.42 (1H, s, 5-OH).  $^{13}C$  NMR: see Table 1.

**Compound (6)**. Colorless needles from benzene. mp 264-266°C.  $[\alpha]_D 0^\circ$  ( $c = 0.1$ , MeOH). IR (KBr)  $\nu_{max}$   $cm^{-1}$ : 3400, 1650, 1610. UV  $\lambda_{max}$  (MeOH) nm (log  $\epsilon$ ): 203 (4.44), 213 (4.38), 265 (4.49). MS  $m/z$ : 354 [ $M^+$ ], 337, 321, 295, 284, 283 (base peak), 282, 268, 254, 165, 118. HRMS  $m/z$  354.1098 ( $M^+$ , calcd for  $C_{20}H_{18}O_6$ : 354.1102).  $^1H$  NMR (acetone- $d_6$ ):  $\delta$  1.36 (3H, s, 5''-Me), 1.39 (3H, s, 4''-Me), 2.62 (1H, dd,  $J=16.9, 7.3$  Hz, H-1''), 2.95 (1H, dd,  $J=16.9, 5.1$  Hz, H-1''), 3.88 (1H, dd,  $J=7.3, 5.1$  Hz, H-2''), 4.42 (1H, br s, 2''-OH), 6.35 (1H, s, H-8), 6.91 (2H, d,  $J=8.8$  Hz, H-3' and H-5'), 7.46 (2H, d,  $J=8.8$  Hz, H-2' and H-6'), 8.15 (1H, s, H-2), 8.55 (1H, br s, 4'-OH), 13.38 (1H, s, 5-OH).  $^{13}C$  NMR: see Table 1.

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