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

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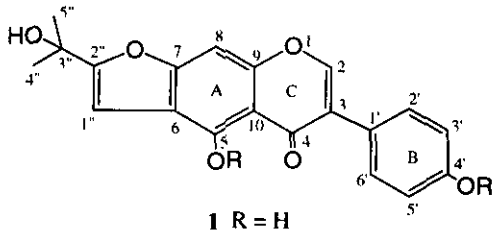
Abstract - Two new isoflavones, erysubins A and B, were isolated from the wood of *Erythrina suberosa* var. *glabrescences* (Leguminosae) and their structures were elucidated on the basis of spectroscopic evidence.

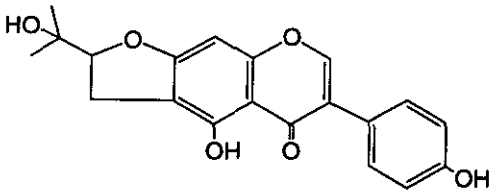
In a continuation of our study on genus *Erythrina*,[1] we have investigated the non-alkaloidal secondary metabolites of *Erythrina suberosa* var. *glabrescences* which has been used in Pakistan as an ornament and folk medicine. Phytochemical studies of this genus have led to the isolation of sterols,[2] a flavanone[3] and a number of alkaloids.[4] 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**),[5,6] alpinumisoflavone (**5**),[7] a wighteone metabolite (**6**),[8] wighteone (**7**)[9] and laburnetin (**8**)),[10,11] and a known pterocarpan, cristacarpin (**9**),[12] 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 $C_{20}H_{16}O_6$ 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^{-1}) and hydroxyl (3400 cm^{-1}) groups. Acetylation of **1** with acetic anhydride and pyridine provided a diacetate



1 R = H

2 R = Ac



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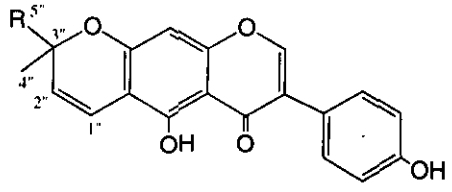
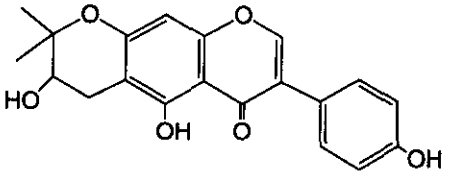


4 R = CH_2OH

5 R = Me



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derivative (**2**) whose ^{1}H NMR spectrum revealed signals of two acetyl groups [δ 2.32, 2.50 (each, 3H, s)] and one hydroxyl group [δ 3.54 (1H, s)]. Comparison of the ^{1}H NMR spectrum of **1** with that of **3**[5] displayed the same substituent patterns of B and C rings and the ^{13}C NMR assignment (Table 1) also suggested the presence of the structural moieties. In the ^{1}H NMR spectrum, a singlet aromatic proton at δ 7.33 was located at C-8 position by the HMBC spectrum (Figure 1) which showed correlations between H-8 and C-9 (δ 153.4), C-6 (δ 113.1), C-10 (δ 105.9). The remaining signals [an other singlet olefinic proton at δ 6.77, geminal methyl groups [δ 1.55 (6H, s)] on a carbon carrying oxygen and a hydroxyl group [δ 5.49 (1H, s)] were ascribed to a 2-hydroxy-2-isopropylfuran moiety ($C_7H_8O_2$) from the HMBC spectrum as follows: the signal of the geminal methyl groups correlated to a carbinol carbon signal (δ 67.4) which indicated the presence of a hydroxydimethyl group. The methyl groups signal also correlated to a oxygenated sp^2 quarternary carbon signal at C-2" (δ 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" (δ 6.77) which correlated to a sp^2 quarternary carbon signal at C-6 (δ 113.1) and, on the other hand, a chelated hydroxyl proton (δ 13.71) exhibited correlation with a sp^2 quarternary carbon signal at C-7 (δ 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

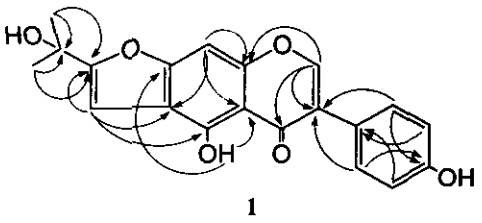

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Figure 1. Long-range Correlations in the HMBC Spectrum of Erysubin A (**1**)

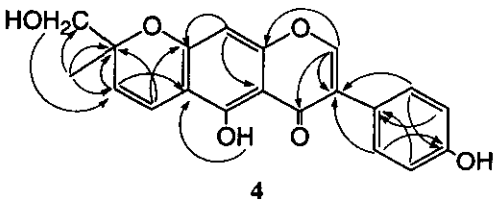

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Figure 2. Long-range Correlations in the HMBC Spectrum of Erysubin B (**4**)

Table 1. ^{13}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 DMSO-d_6 and spectra of **4** and **6** were recorded in 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 $[M\text{-}CH_2OH]^+$.[13] Comparison of the ^1H NMR spectrum of **4** with that of **5**[7] revealed that **4** have the same partial structures except for an oxymethylene group [δ 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 δ 3.61, 3.67 and δ 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" (δ 5.75) and C-6 (δ 106.0), H-1" (δ 6.77) and C-7 (δ 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)[8] 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 $[M\text{-}71]^+$.[14] ^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_2Cl_2-, and EtOAc-soluble fractions. The CH_2Cl_2-soluble fraction (50 g) was chromatographed on silica gel and eluted with $CHCl_3$ (fractions A1-10), $CHCl_3$-acetone (10:1)(frs. A11-32) and $CHCl_3$-acetone (1:1) (frs. A33-69) and $CHCl_3$-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.[5-12]

Erysubin A (1). Pale yellow needles from EtOH. mp 231-233°C. IR (KBr) ν_{max} cm^{-1}: 3400, 1660, 1620. UV λ_{max} (MeOH) nm (log ε): 203 (4.53), 212 (sh 4.45), 266 (4.68), 354 (3.51). MS *m/z*: 352 $[M]^+$, 337, 334 (base peak), 309, 305, 295, 283, 216, 167, 160. HRMS *m/z* 352.0943 (M^+, calcd for $C_{20}H_{16}O_6$: 352.0946). ^1H NMR (DMSO-d_6): δ 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). ^{13}C NMR: see Table 1.

Acetylation of 1. A mixture of **1** (9 mg), Ac_2O (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 ($CHCl_3$) ν_{max} cm^{-1}: 3600, 1750, 1710, 1630, 1600. UV λ_{max} (MeOH) nm (log ε): 202 (4.48), 253 (4.62), 331 (3.75). MS *m/z*: 436 $[M]^+$, 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). ^{1}H NMR ($CDCl_3$): δ 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° (*c* = 0.1, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 1660, 1620. UV λ_{max} (MeOH) nm (log ε): 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). ^{1}H NMR (acetone-d_6): δ 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° (*c* = 0.1, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 1650, 1610. UV λ_{max} (MeOH) nm (log ε): 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). ^{1}H NMR (acetone-d_6): δ 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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