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THREE NEW ISOFLAVONOIDS FROM *ERYTHRINA VARIEGATA*

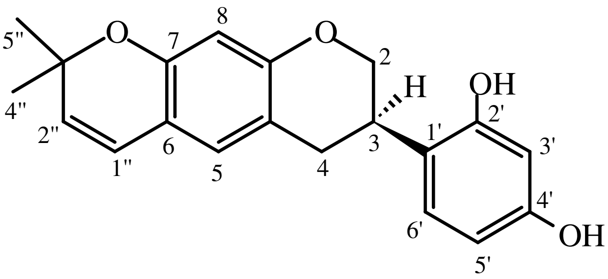
Hitoshi Tanaka,*[,a] Miyuki Hirata,[a] Hideo Etoh,[b] Naoharu Watanabe,[b] Hiroshi Shimizu,[c] Mansoor Ahmad,[d] Zahid Khan,[d] and Munir Anwar[d]

[a]Faculty of Pharmacy, Meijo University, Yagoto, Tempaku-ku, Nagoya, 468-8503, Japan, [b]Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan, [c]Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5 chome, Gifu 502-8585, Japan, [d]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan

Abstract—Three new isoflavonoids, eryvarins C-E, together with fourteen known compounds were isolated from the roots of *Erythrina variegata* (Leguminosae) and their structures were elucidated on the basis of spectroscopic evidence.

Erythrina variegata has been used as medicine in many parts of tropical and subtropical regions[1] and phytochemical studies on the non-alkaloidal secondary metabolites of the genus *Erythrina* showed the presence of isoflavones,[2] a cinnamylphenol,[3] and flavanones,[4] some of which have inhibitory activity against the Na^+/H^+ exchange system[5] and antimicrobial[3] and anti-inflammatory activities.[4] A recent investigation has revealed the isolation of two isoflavonoids (eryvarins A and B) from the roots of this plant.[6] Further investigation of the roots of this plant, cultured in Pakistan, has now led to the isolation of a new isoflavan, eryvarin C (**1**), and two new pterocarpenes, eryvarins D (**2**) and E (**3**), along with fourteen known compounds.

Silica gel chromatography from the $CHCl_3$ extract of the roots of this source gave three new isoflavonoids, eryvarins C-E (**1**-**3**), together with fourteen known compounds (**4**-**17**).



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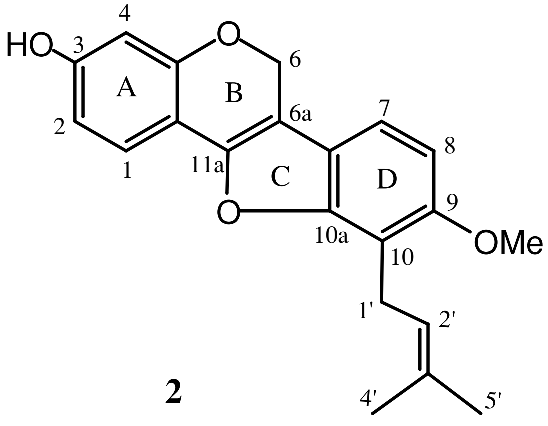

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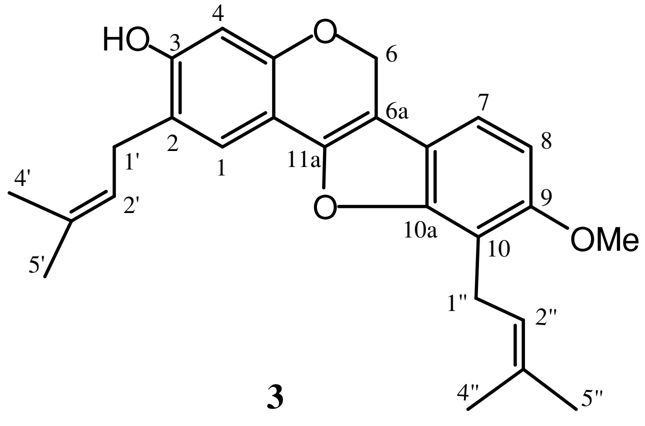

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Eryvarin C (**1**) was obtained as colorless oil and the molecular formula was confirmed to be $C_{20}H_{20}O_4$ by the HRMS (*m*/*z* 324.1356). The MS spectrum showed an intense and typical fragment at *m*/*z* 309 $[M-15]^+$, resulting by expulsion of methyl group of dimethylchromene ring.[7] The ^1H NMR spectrum displayed the presence of two singlet aromatic protons (δ 6.32 and 6.69), three aromatic protons in an ABX system [δ 6.31 (d, *J*=2.4 Hz, H-3'), 6.38 (dd, *J*=8.3, 2.4 Hz, H-5') and 6.95 (d, *J*=8.3 Hz, H-6')] and aliphatic protons of the C2-C4 portion of the isoflavan moiety (δ2.86, 2.95, 3.48, 4.02 and 4.31) as well as the characteristic signals of the dimethylpyran ring (δ 1.41, 5.49 and 6.25). The dimethylpyran ring fused to the C-6 and C-7 positions was assigned from the NOESY spectrum which exhibited NOE interaction between H-5 and H-1'' (Figure 1) and further the assignment was also observed in the HMBC spectrum (Figure 2), indicating correlations between H-8/C-6, H-1''/C-6, H-2''/C-6, H-5/C-7, H-8/C-7 and H-1''/C-7. The absolute stereochemistry at C-3 of the compound (**1**) was assigned to be *R* configuration from its positive Cotton effect at 280 nm[8] and, therefore, eryvarin C was represented by **1** (3*R*).

Eryvarin D (**2**) was obtained as colorless oil whose molecular formula was determined to be $C_{21}H_{20}O_4$ by the HRMS (*m*/*z* 336.1370). The UV spectrum and the characteristic singlet signal ascribed to H-6 (δ 5.54)

Table 1. ^{13}C NMR spectra data of **1-3** † in CDCl$_3$

C	1	2	3
1		121.3	121.4
2	69.9	108.4	119.8[a]
3	31.7	156.9	155.3
4	30.3	103.9	104.2
4a		155.2	153.6
5	126.9		
6	114.9	65.7	65.5
6a		105.8	105.9
6b		119.6	119.7[a]
7	152.3	115.3	115.2
8	104.2	107.9	107.8
9	154.9	154.9	154.8
10	114.4	114.4	114.4
10a		154.7	154.7
11a		147.3	147.4
11b		110.1	109.9
1'	119.9	22.9	29.1
2'	154.5	121.9	122.0[b]
3'	103.1	132.0	135.0
4'	155.3	17.8	17.8[c]
5'	107.9	25.8	25.8*
6'	128.4*		
1"	121.9		22.9
2"	128.4*		121.8[b]
3"	76.1		131.9
4"	27.9**		17.9[c]
5"	27.9**		25.8*
OMe		56.7	56.7

† Assignments are based on HSQC and HMBC spectra.

[a,b,c]Assignments may be interchanged in each column.

*,**Assignments are superimposed.

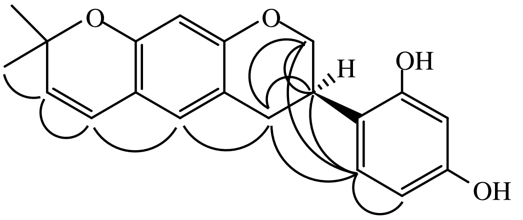

Figure 1.
NOESY interactions of compound (**1**).

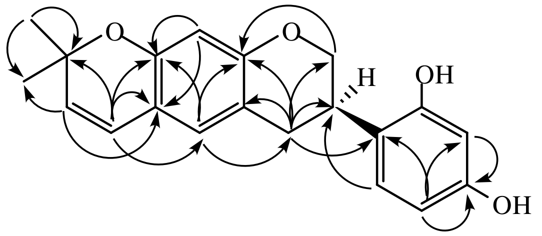

Figure 2.
HMBC correlations of compound (**1**).

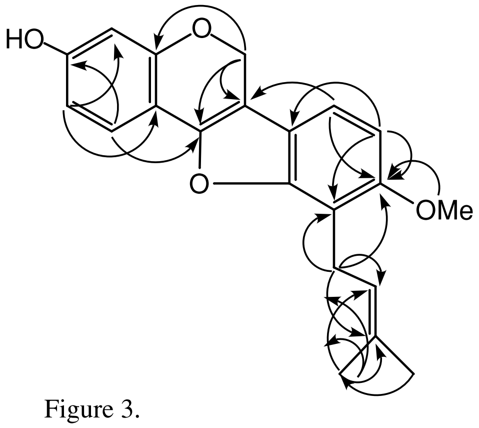

Figure 3.
HMBC correlations of compound (**2**).

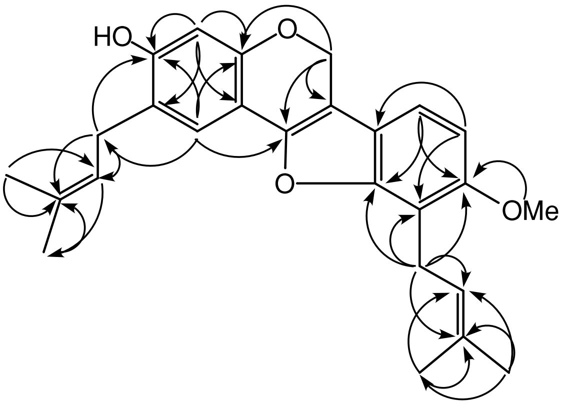

Figure 4.
HMBC correlations of compound (**3**).

in the ^{1}H NMR spectrum showed that the compound (**2**) has a pterocarpene skeleton.[9] The ^{1}H NMR spectrum displayed ABX type aromatic protons [δ6.43 (d, *J*=2.2 Hz, H-4), 6.45 (dd, *J*=8.1, 2.2 Hz, H-2) and 7.37 (d, *J*=8.1 Hz, H-1)], *ortho* coupled aromatic protons [δ6.85 (d, *J*=8.4 Hz, H-8) and 7.09 (d, *J*=8.4 Hz, H-7)], a methoxyl group (δ3.88) in addition to a prenyl group (δ1.69, 1.88, 3.62 and 5.35). The methoxyl group at C-9 and the prenyl group at C-10, respectively, were assigned by the NOESY experiment which exhibited NOE interactions between the methoxyl group and H-8, the methoxyl group and H-2'. Further support for the assignment of the prenyl group at C-10 was obtained by the HMBC experiment, indicating correlations between H-1'/C-9 and H-1'/C-10 (Figure 3). Therefore, the structure of eryvarin D was represented by **2**.

Eryvarin E (**3**) was isolated as colorless oil and the molecular formula of $C_{26}H_{28}O_4$ was established by the HRMS (*m*/*z* 404.1977). The UV spectrum and the typical singlet proton signal [H-6 (δ 5.51)] in the ^{1}H NMR spectrum also showed the compound (**3**) to be a pterocarpene derivative. Comparison of the ^{1}H NMR spectrum of **3** with that of **2** displayed the identical positions of *ortho* coupled aromatic protons [δ6.85 (d, *J*=8.3 Hz, H-8) and 7.09 (d, *J*=8.3 Hz, H-7)], a methoxyl group (δ3.89) and a prenyl group (δ1.69, 1.89, 3.63 and 5.353); these same partial structures were also exhibited by comparison of the ^{13}C NMR spectrum of **3** with that of **2** (Table 1). The remaining signals of two singlet aromatic protons [H-1 (δ 7.23) and H-4 (δ 6.42)] and an other prenyl group (δ1.80, 3.34 and 5.345) were observed and the location of the prenyl group was assigned from the NOSY spectrum which revealed NOE interaction between H-1 and H-1'. And further assignment of the prenyl group was decided by the HMBC experiment, indicating correlations H-1/C-1' and H-1'/C-3 (Figure 4). Therefore, the structure of eryvarin E was represented by **3**.

The known fourteen compounds were identified as orientanols C (**4**)[10] and F (**10**),[11] folitenol (**5**),[10] bidwillol A (**6**),[10] auriculatin (**7**),[12] erystagallin A (**8**),[13] phaseollidin (**9**),[13] cristacarpin (**11**),[13] sigmoidin K (**12**),[14] bidwillons A (**13**)[11] and B (**14**),[11] erysubin F (**15**),[15] erycristagallin (**16**)[10] and 2-(γ,γ-dimethylallyl)-6a-hydroxyphaseollidin (**17**),[13] respectively, by comparison of spectroscopic and physical data with those of authentic samples or the reported values.

EXPERIMENTAL

General. IR spectra were recorded on a JASCO IR-810 spectrophotometer and UV spectra were recorded on a Beckman DU-530 spectrophotometer. Optical rotations were taken on a JASCO DIP-370 digital polarimeter and CD spectra were measured on a JASCO J-725 spectropolarimeter. The ^{1}H NMR spectra were obtained on JEOL JNM-A 600 and 400 MHz spectrometers, while ^{13}C NMR spectra were recorded at 150.8 and 100.4 MHz on the same instruments. MS spectra were performed on a JEOL JMS-D 300 spectrometer. Column chromatography was performed using silica gel (230-400 mesh).

Plant material. The roots of *E. variegata* were collected at Karachi, Pakistan, in April 2000. A voucher specimen is deposited at the Department of Natural Product Chemistry in the Faculty of Pharmacy, Meijo University.

Extraction and isolation. The finely powdered roots (0.47 kg) were macerated with acetone (6 L) at 23°C for 72 h (2 times) and the solvent was removed to give a dark green residue (28.8 g). The residue was divided into *n*-hexane-, $CHCl_3$-, and EtOAc-soluble fractions. The $CHCl_3$-soluble fraction (19.26 g) was applied to silica gel column which was eluted with solvents of varying polarity of CH_2Cl_2, CH_2Cl_2-acetone (10:1→1:1) and CH_2Cl_2-MeOH (10:1→1:1) (each fraction; 200 mL, Column A) to afford 42 fractions. The fraction A2 (308 mg) was chromatographed on silica gel eluting with *n*-hexane-$CHCl_3$ (3:1) to yield orientanol C (**4**) (28 mg) and folitenol (**5**) (4 mg). The fractions A16-18 (2.23 g) were submitted to column chromatography on silica gel using $CHCl_3$-acetone (40:1→4:1) (each fraction; 20 mL, Column B) to furnish 40 fractions. The fractions B20-24 (556 mg) were separated by silica gel using benzene-EtOAc (10:1→5:1) to provide eryvarin E (**3**) (8 mg), bidwillol A (**6**) (194 mg), auriculatin (**7**) (7 mg), erystagallin A (**8**) (212 mg) and phaseollidin (**9**) (5 mg). The fraction A20 (4.81 g) was chromatographed on silica gel using $CHCl_3$-acetone (10:1.5→1:1) (each fraction; 25 mL, Column C) to give 40 fractions. The fractions C15-19 (456 mg) were separated by column chromatography on silica gel using *n*-hexane-acetone (2:1) to afford eryvarin D (**2**) (19 mg), orientanol F (**10**) (16 mg) and cristacarpin (**11**) (101 mg). The fraction A21 (5.41 g) was applied to silica gel column eluting with $CHCl_3$-acetone (10:1.5→4:1→1:1) (each fraction; 40 mL, Column D) to yield 58 fractions. The fractions D6-10 (137 mg) were purified by column chromatography on silica gel using benzene-EtOAc (4:1) to furnish sigmoidin K (**12**) (10 mg) and the fractions D21-24 (124 mg) were separated by column chromatography on silica gel using benzene-EtOAc (3:1→1:1) to provide bidwillon A (**13**) (9 mg), bidwillon B (**14**) (5 mg) and erysubin F (**15**) (11 mg). The fractions D33-40 (262 mg) were purified by column chromatography on silica gel successively using benzene-EtOAc (5:1→2:1) and *n*-hexane-acetone (2:1) to give eryvarin C (**1**) (7 mg). The fractions D46-54 (688 mg) were subjected to silica gel chromatography successively using benzene-EtOAc (3:1→1:1) and *n*-hexane-acetone (1.75:1) to afford bidwillon B (**14**) (8 mg), erycristagallin (**16**) (10 mg), and 2-(γ,γ-dimethylallyl)-6a-hydroxyphaseollidin (**17**) (130 mg).

Eryvarin C (1). Colorless oil. $[\alpha]_D$ +35° (*c* 0.1, MeOH). CD (MeOH; *c* 3.13 x 10^{-5}): Δε+2.11 (310), +1.56 (302), +2.50 (280), +1.23 (247), +4.04 (232), ±0 (218). IR (KBr) ν_{max} cm^{-1}: 3400, 1620. UV λ_{max} nm (MeOH) (log ε): 204 (4.58), 222 (4.61), 277 (4.06), 311 (3.93). MS *m/z* (rel. int.): 324 ($[M]^+$, 28), 309 (100), 189 (7), 187 (13), 173 (18). HRMS *m/z:* 324.1356 (M^+, Calcd for $C_{20}H_{20}O_4$: 324.1360). ^{1}H NMR ($CDCl_3$): δ 1.41 (6H, s, H-4'' and -5''), 2.86 (1H, ddd, *J*=15.6, 5.4, 1.5 Hz, H-4), 2.95 (1H, dd, *J*=15.6, 10.7 Hz, H-4), 3.48 (1H, m, H-3), 4.02 (1H, t-like, *J*=10.3 Hz, H-2), 4.31 (1H, ddd, *J*=10.3, 3.4, 2.0 Hz, H-2), 4.73, 4.90 (2H, 2 x s, 2 x OH), 5.49 (1H, d, *J*=9.8 Hz, H-2''), 6.25 (1H, d, *J*=9.8 Hz, H-1''), 6.31 (1H, d, *J*=2.4 Hz, H-3'), 6.32 (1H, s, H-8), 6.38 (1H, dd, *J*=8.3, 2.4 Hz, H-5'), 6.69 (1H, s, H-5), 6.95 (1H, d, *J*=8.3 Hz, H-6'). ^{13}C NMR: see Table 1.

Eryvarin D (2). Colorless oil. IR (KBr) ν_{max} cm^{-1}: 3420, 1620. UV λ_{max} nm (MeOH) (log ε): 211 (4.42), 233 (sh, 4.32), 286 (3.83), 334 (3.92), 353 (sh, 3.76). MS *m/z* (rel. int.): 336 ($[M]^+$, 100), 321 (4), 305 (4), 278 (15), 265 (4). HRMS *m/z:* 336.1370 (M^+, Calcd for $C_{21}H_{20}O_4$: 336.1360). ^{1}H NMR ($CDCl_3$): δ 1.69 (3H, s, H-5'), 1.88 (3H, s, H-4'), 3.62 (2H, d, *J*=7.3 Hz, H-1'), 3.88 (3H, s, OMe), 5.15 (1H, br s, OH), 5.35 (H, t, *J*=7.3 Hz, H-2'), 5.54 (2H, s, H-6), 6.43 (1H, d, *J*=2.2 Hz, H-4), 6.45 (1H, dd, *J*=8.1, 2.2 Hz, H-2), 6.85 (1H, d, *J*=8.4 Hz, H-8), 7.09 (1H, d, *J*=8.4 Hz, H-7), 7.37 (1H, d, *J*=8.1 Hz, H-1). ^{13}C NMR: see Table 1.

Eryvarin E (3). Colorless oil. IR (KBr) ν_{max} cm^{-1}: 3400, 1650, 1620. UV λ_{max} nm (MeOH) (log ε): 204 (4.45), 253 (sh, 4.08), 287 (3.85), 340 (4.17), 356 (4.09). MS *m/z* (rel. int.): 404 ($[M]^+$, 100), 388 (25), 373 (19), 349 (34), 333 (8), 317 (7), 291 (6). HRMS *m/z:* 404.1977 (M^+, Calcd for $C_{26}H_{28}O_4$: 404.1986). ^{1}H NMR ($CDCl_3$): δ 1.69 (3H, s, H-5''), 1.80 (6H, s, H-4' and -5'), 1.89 (3H, s, H-4''), 3.34 (2H, d, *J*=7.3

Hz, H-1'), 3.63 (2H, d, *J*=7.3 Hz, H-1"), 3.89 (3H, s, OMe), 5.345 (1H, t, *J*=7.3 Hz, H-2'), 5.353 (1H, t, *J*=7.3 Hz, H-2"), 5.51 (2H, s, H-6), 6.42 (1H, s, H-4), 6.85 (1H, d, *J*=8.3 Hz, H-8), 7.09 (1H, d, *J*=8.3 Hz, H-7), 7.23 (1H, s, H-1). ^{13}C NMR: see Table 1.

REFERENCES AND NOTES

1. Dictionary of Chinese Herbal Medicine, ed. by Jiangsu New Medical College, Shanghai People's Press, Shanghai, 1977, p. 1941.
2. V. H. Deshpande, A. D. Pendse, and R. Pendse, *Indian J. Chem.,* 1977, **15B**, 205.
3. H. Telikepalli, S. R. Gollapudi, A. K-Shokri, L. Velazquez, R. A. Sandmann, E. A. Veliz, K. V. J. Rao, A. S. Madhavi, and L. A. Mitscher, *Phytochemistry,* 1990, **29,** 2005.
4. V. R. Hegde, P. Dai, M. G. Patel, M. S. Puar, P. Das, J. Pai, R. Bryant, and P. A. Cox, *J. Nat. Prod.,* 1997, **60**, 537.
5. M. Kobayashi, T. Mahmud, N. Yoshioka, H, Shibuya, and I. Kitagawa, *Chem. Pharm, Bull.,* 1997, **45**, 1615.
6. H. Tanaka, H. Etoh, H. Shimizu, T. Makita, and Y. Tateishi, *Planta Med.,* 2000, **66**, 578.
7. M. Takayama, T. Fukai, Y. Hano, and T. Nomura, *Heterocycles*, 1992, **33**, 405.
8. E. Wong, in The Flavonoids, ed. by J. B. Harborne, T. J. Mabry, and H. Mabry, Chapman and Hall, London, 1975, p. 776.
9. A. V. K. Prasad, R. S. Kapil, and S. P. Popli, *Indian J. Chem.*, 1985, **24B**, 236.
10. H. Tanaka, T. Tanaka, and H. Etoh, *Phytochemistry,* 1998, **47,** 475.
11. H. Tanaka, T. Tanaka, A. Hosoya, Y. Kitada, and H. Etoh, *Phytochemistry*, 1998, **48**, 355.
12. M. Shabbir, A. Zaman, L. Crombie, B. Tuck, and D. A. Whiting, *J. Chem. Soc. (C),* 1968, 1899.
13. H. Tanaka, T. Tanaka, and H. Etoh, *Phytochemistry,* 1997, **45**, 835.
14. A. E. Nkengfack, T. W. Vouffo, J. C. Vardamides, Z. T. Fomum, O. Bergendorff, and O. Sterner, *J. Nat. Prod.*, 1994, **57**, 1172.
15. H. Tanaka, H. Etoh, N. Watanabe, H. Shimizu, M. Ahmad, and G. H. Rizwani, *Phytochemistry*, 2001, **56**, 769.