

FOUR NEW ISOFLAVONOIDS AND A NEW 2-ARYLBENZOFURAN FROM THE ROOTS OF *ERYTHRINA VARIEGATA*

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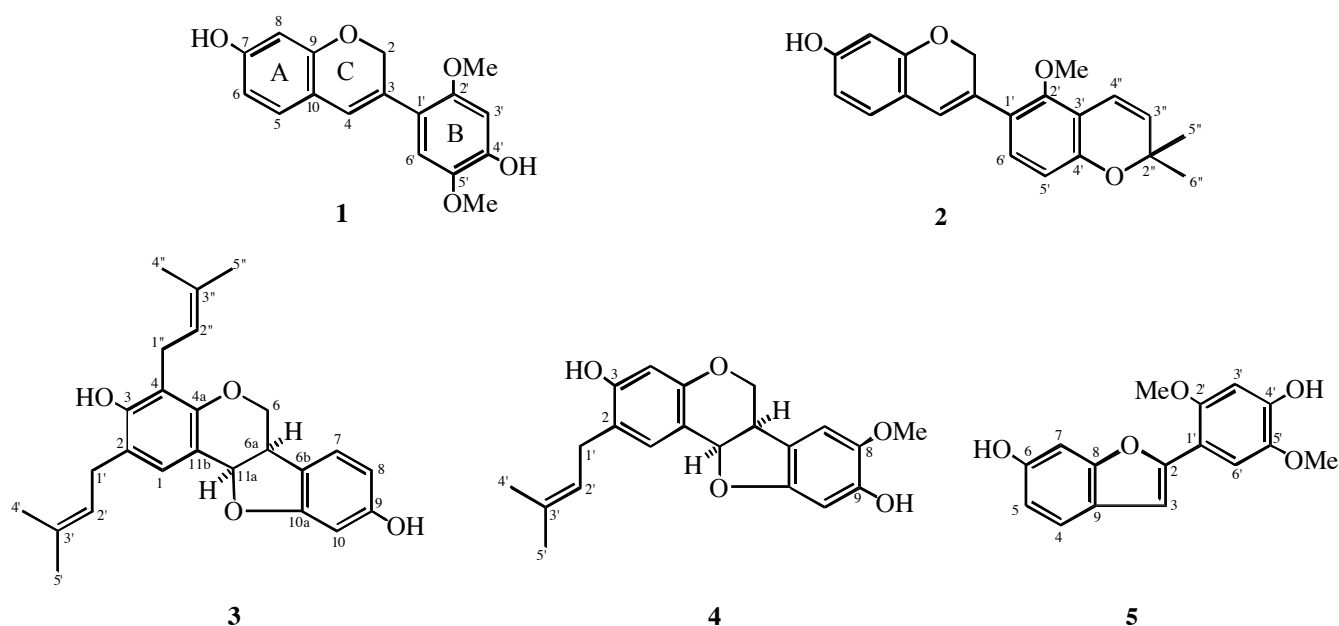
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Abstract—Four new isoflavonoids, eryvarins H-K, and a new 2-arylbenzofuran, eryvarin L, together with five known compounds, were isolated from the roots of *Erythrina variegata*. Their structures were established on the basis of spectroscopic analysis. Eryvarins H and I are rare naturally-occurring isoflav-3-enes. The antibacterial activity of these five new compounds against 13 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) was examined.

INTRODUCTION

Erythrina variegata is a member of the Leguminosae family with known antibacterial property.¹ Phytochemical studies on the non-alkaloidal secondary metabolites of this plant resulted in the isolation of a cinnamylphenol,² flavanones³ and isoflavonoids.^{4,5} In continuation of our phytochemical investigation of the genus *Erythrina*, we describe in this paper the isolation and structural elucidation of

two new isoflav-3-enes, eryvarins H (**1**) and I (**2**), two new pterocarpan, eryvarins J (**3**) and K (**4**), and a new 2-arylbenzofuran, eryvarin L (**5**), along with five known isoflavonoids (**6**–**10**). Compounds were isolated from the roots of *E. variegata* obtained from Indonesia. We also report on the antibacterial activities of the new compounds (**1**–**5**) against methicillin-resistant *Staphylococcus aureus* (MRSA). The five known compounds were identified as bidwillon C (**6**),⁶ erysubin C (**7**),⁷ erythrabyssin II (**8**),⁸ orientanol B (**9**)⁹ and phaseollin (**10**),¹⁰ by comparing spectroscopic data with those of authentic samples or reported values.



RESULTS AND DISCUSSION

The molecular formula of eryvarin H (**1**) was determined as $C_{17}H_{16}O_5$ using HREIMS ($[M]^+$ m/z 300.0991). The UV spectral data and characteristic signals in the 1H NMR spectrum [oxymethylene protons at C-2 (δ 4.92) and an isolated olefinic proton at C-4 (δ 6.56)] showed that compound (**1**) was an isoflav-3-ene.¹¹ The 1H NMR spectrum indicated three aromatic protons of an AMX system (δ 6.33, 6.41 and 6.94) on an A ring, as well as two singlet aromatic protons (δ 6.57 and 6.95) and two methoxyl groups (δ 3.77 and 3.84) on a 4-hydroxy-2,5-dimethoxyphenyl group. The placement of the methoxyl group at C-2' was determined from the HMBC spectrum, which revealed cross-peaks between 2'-OMe/C-2', H-3'/C-2' and H-6'/C-2'. The methoxyl group at C-5' was also confirmed from the HMBC spectrum, showing correlations between 5'-OMe/C-5', H-3'/C-5' and H-6'/C-5'. The assignment of these methoxyl groups at C-2' and C-5' were further clarified by DIFNOE analysis, revealing NOE interactions between 2'-OMe/H-2, 2'-OMe/H-3', 5'-OMe/H-6' and H-6'/H-4. Thus, eryvarin H was char-

Table 1. ¹³C NMR spectral data of compounds (1-5)

position	1 ^a	2 ^a	3 ^b	4 ^b	5 ^b
1			128.9	131.9	
2	68.9	68.6	121.2	121.0	151.7*
3	129.7	128.6	154.0	155.7	104.3
4	121.5	122.4	115.3	104.0	120.9
4 a			152.4	155.0	
5	128.3	128.6			111.6
6	109.4	109.5	66.8	66.5	153.1
6 a			39.5	40.3	
6 b			119.7	117.0	
7	159.0	159.3	124.9	107.7	97.9
8	103.4	103.5	107.4	141.1	154.4
9	155.7	155.8	156.8	146.6	123.7
10	117.1	116.9	98.3	98.1	
10 a			160.8	153.9	
11 a			79.5	78.2	
11 b			111.7	112.3	
1'	119.3	125.2	29.2	29.3	111.1
2'	153.1	154.7*	122.2	121.8	151.4*
3'	101.2	115.8	134.0*	135.0	99.5
4'	148.3	154.9*	17.8**	17.9	146.2
5'	142.3	113.3	25.8	25.8	140.5
6'	113.3	129.5			109.2
1''			22.5		
2''		76.7	122.0		
3''		131.7	134.2*		
4''		117.5	17.9**		
5''		28.0	25.8		
6''		28.0			
8-OMe				56.9	
2'-OMe	56.3	61.9			56.0
5'-OMe	57.1				56.7

a: acetone-*d*₆. b: CDCl₃.

*·**Assignments in same vertical column may be interchanged.

acterized as 7,4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene (1).¹²

The molecular formula of eryvarin I (2) was determined as C₂₁H₂₀O₄ using HREIMS ([M]⁺ *m/z* 336.1371).

A comparison of the ¹H and ¹³C NMR spectra of 2 with those of 1 showed the same substitution pattern on the A ring [δ_{H} 6.98 (H-5), 6.43 (H-6) and 6.35 (H-8); δ_{C} 128.6 (C-5), 109.5 (C-6), 159.3 (C-7), 103.5 (C-8), 155.8 (C-9) and 116.9 (C-10)] and the C ring [δ_{H} 4.95 (2H, s, H-2) and 6.64 (1H, s, H-4); δ_{C} 68.6 (C-2), 128.6 (C-3) and 122.4 (C-4)]; the comparison also revealed that compound (2) was an isoflav-3-ene.

The ¹H NMR spectrum indicated a set of *ortho*-coupled aromatic protons (δ 6.57 and 7.13) and a methoxyl group (δ 3.70), as well as two methyl groups (δ 1.42) and two olefinic protons (δ 5.79 and 6.63) on a 2,2-dimethylpyran ring. The presence of the 2,2-dimethylpyran moiety was evidenced from the EI

mass spectrum that displayed a peculiar intense fragment ion at *m/z* 321 [M-CH₃]⁺.¹³ The placement of the 2,2-dimethylpyran moiety fused to C-3' and C-4' was determined from the HMBC spectrum, which exhibited correlations between H-3''/C-3' and H-4''/C-4'. The methoxyl group was assigned to C-2' based on the NOESY spectrum, indicating NOE interactions between OMe/H-2 and OMe/H-4''. Thus, eryvarin I was characterized as 7-hydroxy-2'-methoxy-2,2-dimethylpyrano[5'',6'':3',4']isoflav-3-ene (2). Eryvarins H (1) and I (2) are rare isoflav-3-enes from natural sources, because of their high reactivity (low stability).¹⁴

The molecular formula of eryvarin J (**3**) was determined as C₂₅H₂₈O₄ using HREIMS ([M]⁺ *m/z* 392.1981). The UV spectral data, and a set of four aliphatic protons in the ¹H NMR spectrum (δ 3.49, 3.56, 4.25 and 5.50), showed it to be a pterocarpan. The ¹H NMR spectrum indicated three aromatic protons of an ABX system (δ 6.35, 6.36 and 7.07), a singlet aromatic proton (δ 7.13) and two 3,3-dimethylallyl (prenyl) groups (δ 1.77, 3.32 and 5.32; δ 1.71, 1.79, 3.38 and 5.20). The aromatic proton of C-7 in the ABX type was clarified using the NOESY spectrum, revealing NOE interaction between the aromatic proton at C-7 (δ 7.07) and the aliphatic proton at C-6a (δ 3.49). The placement of prenyl group at C-2 was confirmed from the HMBC spectrum, which indicated cross peaks between H-1/C-1', H-1'/C-1 and H-1'/C-3. The prenyl group at C-4 was also confirmed from the HMBC spectrum, exhibiting correlations between H-1"/C-3, H-1"/C-4 and H-1"/C-4a. The absolute stereochemistry at C-6a and C-11a was assigned as 6a *R*:11a *R* from its negative optical rotation value.¹⁵ Thus, eryvarin J was characterized as (6a *R*, 11a *R*)-3,9-dihydroxy-2,4-di(3,3-dimethylallyl)pterocarpan (**3**).

The molecular formula of eryvarin K (**4**) was determined as C₂₁H₂₂O₅ using HREIMS ([M]⁺ *m/z* 354.1462). The UV spectral data, and the characteristic aliphatic proton signals in the ¹H NMR spectrum (δ 3.48, 3.61, 4.22 and 5.43), showed that compound (**4**) also had a pterocarpan skeleton. The ¹H NMR spectrum indicated four singlet aromatic protons (δ 6.41, 6.52, 6.79 and 7.24), a methoxyl group (δ 3.87) and a prenyl group (δ 1.78, 1.79, 3.33 and 5.32). The assignment of the methoxyl group at C-8 was obtained from the NOESY spectrum, in which NOE interactions between OMe/H-7, H-7/H-6 and H-7/H-6a were observed. The prenyl group at C-2 was confirmed from the HMBC spectrum, revealing cross-peaks between H-1/C-1', H-1'/C-1, H-1'/C-2 and H-1'/C-3. The absolute stereochemistry at C-6a and C-11a was also 6a *R*: 11a *R* (negative optical rotation value). Thus, eryvarin K was characterized as (6a *R*, 11a *R*)-3,9-dihydroxy-8-methoxy-2-(3,3-dimethylallyl)pterocarpan (**4**).

The molecular formula of eryvarin L (**5**) was determined as C₁₆H₁₄O₅ using HREIMS ([M]⁺ *m/z* 286.0833). The UV spectral data and characteristic aromatic proton signal in the ¹H NMR spectrum (δ 7.14) showed that compound (**5**) was a 2-arylbenzofuran derivative.¹⁶ The ¹H NMR spectrum indicated three AMX aromatic protons (δ 6.75, 7.00 and 7.37), and two singlet aromatic protons (δ 6.66 and 7.50) and two methoxyl groups (δ 3.91 and 3.96) on a 4-hydroxy-2,5-dimethoxyphenyl group. The methoxyl groups were placed at C-2' and C-5' based on the NOESY spectrum, which exhibited NOE interactions between 2'-OMe/H-3, 2'-OMe/H-3' and 5'-OMe/H-6'. The methoxyl group assignment was further confirmed by the HMBC experiment, revealing cross-peaks between 2'-OMe/C-2', H-3'/C-2', H-6'/C-2', 5'-OMe/C-5', H-3'/C-5' and H-6'/C-5'. Thus, eryvarin L was characterized as 6-hydroxy-2-(4'-hydroxy-2',5'-dimethoxyphenyl)benzofuran (**5**).

The antibacterial activity of the five new compounds (**1-5**) against 13 strains of MRSA was investigated

using the previous procedure.¹⁷ Only eryvarin L (**5**) exhibited weak anti-MRSA activity (each minimum inhibitory concentration is 25 $\mu\text{g mL}^{-1}$). This compound also inhibited the growth of 5 strains of vancomycin-resistant enterococci at 50 $\mu\text{g mL}^{-1}$.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO IR-810 spectrophotometer and UV spectra were obtained in MeOH using a Beckman DU-530 spectrophotometer. Mass spectra were obtained using a JEOL JMS-D 300 spectrometer. ^1H and ^{13}C NMR spectra were measured on a JEOL ALPHA-600 spectrometer. The ^1H and ^{13}C NMR (Table 1) signals of **1–5** were assigned based on the ^1H - ^1H COSY, NOESY, HSQC, and HMBC spectra. Column chromatography was performed using Merck silica gel (230–400 mesh). The procedure for the MIC measurement has been described in a previous publication.¹⁷

Plant material. Plant materials were as reported in our previous paper.⁵

Extraction and isolation. The finely powdered roots (1.53 kg) were macerated with acetone (18 L) at 23 °C for 72 h (2 times) and the solvent was removed to yield a residue that was divided into *n*-hexane-, CH_2Cl_2 -, and EtOAc-soluble fractions. The CH_2Cl_2 -soluble fraction (65.4 g) was applied onto a silica gel column first eluted with CHCl_3 -acetone (40 : 1 \rightarrow 10 : 1.5 \rightarrow 1 : 1) and CHCl_3 -MeOH (10 : 1 \rightarrow 1 : 1) (each volume; 400 mL) to afford 33 fractions (frs. 1–33). The above procedure was reported in our previous paper.⁵ Fractions 10–12 (739 mg) were separated by silica gel column chromatography using *n*-hexane-acetone (3 : 1) to provide erysubin C (**7**) (4.3 mg), eryvarin J (**3**) (1.7 mg) and orientanol B (**9**) (59 mg). Fraction 13 (5.38 g) was separated by silica gel column chromatography using benzene-EtOAc (5 : 1) (each volume; 100 mL) to afford 15 fractions (frs. 34–48). Fractions 36 and 37 (488 mg) were separated by silica gel column chromatography using *n*-hexane-acetone (4 : 1) to give eryvarin I (**2**) (7.6 mg) and phaseollin (**10**) (44 mg). Fractions 38 and 39 (1.98 g) were purified by repeated silica gel column chromatography using *n*-hexane-acetone (3 : 1) to yield erythrabyssin II (**8**) (321 mg). Fraction 14 (3.33 g) was subjected to silica gel column chromatography successively using benzene-EtOAc (10 : 1) and CHCl_3 -acetone (20 : 1) to provide eryvarin K (**4**) (4.6 mg). Fractions 16 and 17 (3.10 g) were applied onto a silica gel column using benzene-EtOAc (10 : 1 \rightarrow 5 : 1) (each volume; 80 mL) to afford 15 fractions (frs. 49–63). Fractions 56–58 (1.29 g) were subjected to repeated silica gel column chromatography using *n*-hexane-acetone (2 : 1) to give eryvarin H (**1**) (3 mg) and eryvarin L (**5**) (16 mg). Fractions 59–63 (630 mg) were purified by repeated silica gel column chromatography using *n*-hexane-acetone (2 : 1) to yield bidwillon C (**6**) (11 mg).

Eryvarin H (1). Amorphous powder; IR (KBr) ν_{\max} cm^{-1} : 3420, 1620; UV λ_{\max} nm (log ϵ): 206 (4.41), 235 (sh, 4.07), 288 (3.88), 326 (3.96); EIMS m/z (rel. int.): 300 ($[\text{M}]^+$, 100), 286 (38), 271 (19), 269 (23), 243 (8), 241 (8); HREIMS m/z : 300.0991 (M^+ , Calcd for $\text{C}_{17}\text{H}_{16}\text{O}_5$, 300.0997); ^1H NMR (acetone- d_6): δ 3.77 (3H, s, OMe-2'), 3.84 (3H, s, OMe-5'), 4.92 (2H, s, H-2), 6.33 (1H, d, $J = 2.2$ Hz, H-8), 6.41 (1H, dd, $J = 8.1, 2.2$ Hz, H-6), 6.56 (1H, s, H-4), 6.57 (1H, s, H-3'), 6.94 (1H, d, $J = 8.1$ Hz, H-5), 6.95 (1H, s, H-6'), 7.81 (1H, br s, OH), 8.47 (1H, br s, OH); ^{13}C NMR: see Table 1.

Eryvarin I (2). Amorphous powder; IR (Film) ν_{\max} cm^{-1} : 3400, 1620; UV λ_{\max} nm (log ϵ): 205 (4.33), 241 (4.42), 284 (4.14), 322 (4.30); EIMS m/z (rel. int.): 336 ($[\text{M}]^+$, 81), 321 (100), 306 (19); HREIMS m/z : 336.1371 (M^+ , Calcd for $\text{C}_{21}\text{H}_{20}\text{O}_4$, 336.1360); ^1H NMR (acetone- d_6): δ 1.42 (6H, s, H-5'' and H-6''), 3.70 (3H, s, OMe), 4.95 (2H, s, H-2), 5.79 (1H, d, $J = 10.3$ Hz, H-3''), 6.35 (1H, d, $J = 2.2$ Hz, H-8), 6.43 (1H, dd, $J = 8.1, 2.2$ Hz, H-6), 6.57 (1H, d, $J = 8.8$ Hz, H-5'), 6.63 (1H, d, $J = 10.3$ Hz, H-4''), 6.64 (1H, s, H-4), 6.98 (1H, d, $J = 8.1$ Hz, H-5), 7.13 (1H, d, $J = 8.8$ Hz, H-6'), 8.48 (1H, br s, OH); ^{13}C NMR: see Table 1.

Eryvarin J (3). Amorphous powder; $[\alpha]_{\text{D}}^{23} -137^\circ$ (c 0.1, MeOH); IR (KBr) ν_{\max} cm^{-1} : 3440; UV λ_{\max} nm (log ϵ): 209 (4.70), 235 (sh, 4.02), 287 (3.82); EIMS m/z (rel. int.): 392 ($[\text{M}]^+$, 100), 375 (7), 336 (10), 321 (30), 293 (10), 281 (22); HREIMS m/z : 392.1981 (M^+ , Calcd for $\text{C}_{25}\text{H}_{28}\text{O}_4$, 392.1986); ^1H NMR (CDCl_3): δ 1.71 (3H, s, H-5'''), 1.77 (6H, s, H-4' and H-5'), 1.79 (3H, s, H-4''), 3.32 (2H, d, $J = 7.3$ Hz, H-1'), 3.38 (2H, d, $J = 7.3$ Hz, H-1''), 3.49 (1H, m, H-6a), 3.56 (1H, t-like, $J = 11.0$ Hz, H-6), 4.25 (1H, dd, $J = 11.0, 5.1$ Hz, H-6), 4.89 (1H, br s, OH), 5.20 (1H, t, $J = 7.3$ Hz, H-2''), 5.32 (1H, t, $J = 7.3$ Hz, H-2'), 5.50 (1H, d, $J = 7.3$ Hz, H-11a), 5.57 (1H, s, OH), 6.35 (1H, dd, $J = 8.1, 2.2$ Hz, H-8), 6.36 (1H, d, $J = 2.2$ Hz, H-10), 7.07 (1H, d, $J = 8.1$ Hz, H-7), 7.13 (1H, s, H-1); ^{13}C NMR: see Table 1.

Eryvarin K (4). Amorphous powder; $[\alpha]_{\text{D}}^{23} -183^\circ$ (c 0.1, MeOH); IR (KBr) ν_{\max} cm^{-1} : 3400; UV λ_{\max} nm (log ϵ): 208 (4.67), 235 (sh, 4.04), 291 (3.92); EIMS m/z (rel. int.): 354 ($[\text{M}]^+$, 100), 299 (29), 232 (6), 194 (15); HREIMS m/z : 354.1462 (M^+ , Calcd for $\text{C}_{21}\text{H}_{22}\text{O}_5$, 354.1466); ^1H NMR (CDCl_3): δ 1.78 (3H, s, H-5'), 1.79 (3H, s, H-4'), 3.33 (2H, d, $J = 7.3$ Hz, H-1'), 3.48 (1H, m, H-6a), 3.61 (1H, t-like, $J = 11.0$ Hz, H-6), 3.87 (3H, s, OMe), 4.22 (1H, dd, $J = 11.0, 5.1$ Hz, H-6), 5.27 (1H, br s, OH), 5.32 (1H, t, $J = 7.3$ Hz, H-2'), 5.43 (1H, d, $J = 6.6$ Hz, H-11a), 5.68 (1H, s, OH), 6.41 (1H, s, H-4), 6.52 (1H, s, H-10), 6.79 (1H, s, H-7), 7.24 (1H, s, H-1); ^{13}C NMR: see Table 1.

Eryvarin L (5). Amorphous powder; IR (KBr) ν_{\max} cm^{-1} : 3400, 1620; UV λ_{\max} nm (log ϵ): 212 (4.37), 251 (sh, 3.81), 274 (sh, 3.98), 281 (4.01), 329 (4.42), 340 (4.38); EIMS m/z (rel. int.): 286 ($[\text{M}]^+$, 100), 271 (49), 243 (19), 228 (14), 200 (4), 187 (5), 172 (6), 149 (7), 143 (10); HREIMS m/z : 286.0833 (M^+ , Calcd for $\text{C}_{16}\text{H}_{14}\text{O}_5$, 286.0840); ^1H NMR (CDCl_3): δ 3.91 (3H, s, OMe-2'), 3.96 (3H, s, OMe-5'), 5.10 (1H, br s, OH), 5.82 (1H, br s, OH), 6.66 (1H, s, H-3'), 6.75 (1H, dd, $J = 8.1, 2.2$ Hz, H-5), 7.00 (1H, d, J

= 2.2 Hz, H-7), 7.14 (1H, s, H-3), 7.37 (1H, d, $J = 8.1$ Hz, H-4), 7.50 (1H, s, H-6'); ^{13}C NMR: see Table 1.

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12. After submission of this article, we found that eryvarin H (**1**) was quite recently isolated from Kenyan *Erythrina abyssinica*: A. Yenesew, S. Derese, B. Irungu, J. O. Midiwo, N. C. Waters, P. Liyala, H. Akala, M. Heydenreich, and M. G. Peter, *Planta Med.*, 2003, **69**, 658. Spectroscopic data of **1** were identical with those of 7,4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene reported.
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