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

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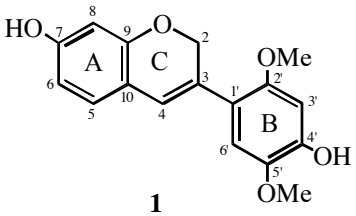
**Abstract**－Four new isoflavonoids, eryvarins H-K, and a new 2-arylbenzofuran, eryvarin L, together with five known compounds, were isolated from the roots of *Erythrina variegata*. Their structures were established on the basis of spectroscopic analysis. Eryvarins H and I are rare naturally-occurring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

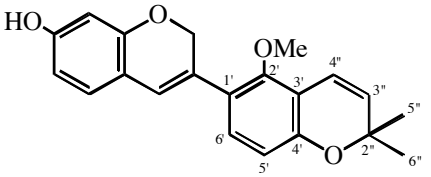
## INTRODUCTION

*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] flavanones[3] 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

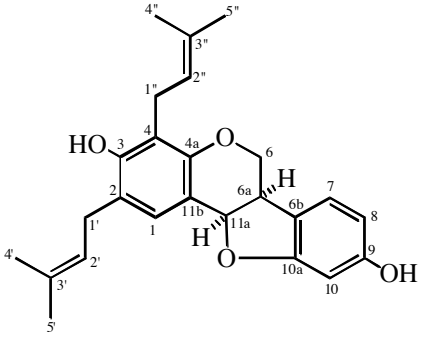
two new isoflav-3-enes, eryvarins H (**1**) and I (**2**), two new pterocarpans, eryvarins J (**3**) and K (**4**), and a new 2-arylbenzofuran, eryvarin L (**5**), along with five known isoflavonoids (**6**−**10**). Compounds were isolated from the roots of *E. variegata* obtained from Indonesia. We 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.



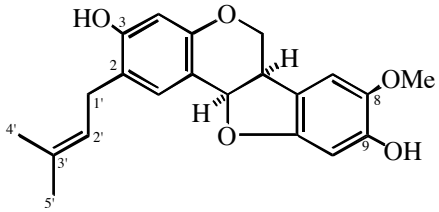

**1**



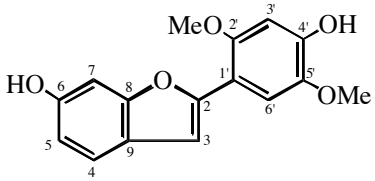

**2**




**3**




**4**




**5**

## 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 $^1$H 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 $^1$H 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. $^{13}$C 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 |
| 4a | | | 152.4 | 155.0 | |
| 5 | 128.3 | 128.6 | | | 111.6 |
| 6 | 109.4 | 109.5 | 66.8 | 66.5 | 153.1 |
| 6a | | | 39.5 | 40.3 | |
| 6b | | | 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 | |
| 10a | | | 160.8 | 153.9 | |
| 11a | | | 79.5 | 78.2 | |
| 11b | | | 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: $CDCl_3$.

*, **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 $C_{21}H_{20}O_4$ using HREIMS ($[M]^+$ *m/z* 336.1371). A comparison of the $^1$H and $^{13}$C NMR spectra of **2** with those of **1** showed the same substitution pattern on the A ring [$\delta_H$ 6.98 (H-5), 6.43 (H-6) and 6.35 (H-8); $\delta_C$ 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 [$\delta_H$ 4.95 (2H, s, H-2) and 6.64 (1H, s, H-4); $\delta_C$ 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 $^1$H 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 $[M\text{-}CH_3]^+$.[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 $C_{25}H_{28}O_4$ using HREIMS ($[M]^+$ *m/z* 392.1981). The UV spectral data, and a set of four aliphatic protons in the $^1$H NMR spectrum (δ 3.49, 3.56, 4.25 and 5.50), showed it to be a pterocarpan. The $^1$H 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 $C_{21}H_{22}O_5$ using HREIMS ($[M]^+$ *m/z* 354.1462). The UV spectral data, and the characteristic aliphatic proton signals in the $^1$H NMR spectrum (δ 3.48, 3.61, 4.22 and 5.43), showed that compound (**4**) also had a pterocarpan skeleton. The $^1$H 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 $C_{16}H_{14}O_5$ using HREIMS ($[M]^+$ *m/z* 286.0833). The UV spectral data and characteristic aromatic proton signal in the $^1$H NMR spectrum (δ 7.14) showed that compound (**5**) was a 2-arylbenzofuran derivative.[16] The $^1$H 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 μg $mL^{-1}$). This compound also inhibited the growth of 5 strains of vancomycin-resistant enterococci at 50 μg $mL^{-1}$.

## 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 → 10 : 1.5 → 1 : 1) and $CHCl_3$-MeOH (10 : 1 → 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→ 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) $\nu_{max}$ $cm^{-1}$: 3420, 1620; UV $\lambda_{max}$ nm (log ε): 206 (4.41), 235 (sh, 4.07), 288 (3.88), 326 (3.96); EIMS *m/z* (rel. int.): 300 ($[M]^+$, 100), 286 (38), 271 (19), 269 (23), 243 (8), 241 (8); HREIMS *m/z*: 300.0991 ($M^+$, Calcd for $C_{17}H_{16}O_5$, 300.0997); $^1$H 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) $\nu_{max}$ $cm^{-1}$: 3400, 1620; UV $\lambda_{max}$ nm (log ε): 205 (4.33), 241 (4.42), 284 (4.14), 322 (4.30); EIMS *m/z* (rel. int.): 336 ($[M]^+$, 81), 321 (100), 306 (19); HREIMS *m/z*: 336.1371 ($M^+$, Calcd for $C_{21}H_{20}O_4$, 336.1360); $^1$H 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; $[\alpha]_D^{23}$ –137° (*c* 0.1, MeOH); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3440; UV $\lambda_{max}$ nm (log ε): 209 (4.70), 235 (sh, 4.02), 287 (3.82); EIMS *m/z* (rel. int.): 392 ($[M]^+$, 100), 375 (7), 336 (10), 321 (30), 293 (10), 281 (22); HREIMS *m/z*: 392.1981 ($M^+$, Calcd for $C_{25}H_{28}O_4$, 392.1986); $^1$H 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; $[\alpha]_D^{23}$ –183° (*c* 0.1, MeOH); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3400; UV $\lambda_{max}$ nm (log ε): 208 (4.67), 235 (sh, 4.04), 291 (3.92); EIMS *m/z* (rel. int.): 354 ($[M]^+$, 100), 299 (29), 232 (6), 194 (15); HREIMS *m/z*: 354.1462 ($M^+$, Calcd for $C_{21}H_{22}O_5$, 354.1466); $^1$H 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) $\nu_{max}$ $cm^{-1}$: 3400, 1620; UV $\lambda_{max}$ 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 ($[M]^+$, 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 $C_{16}H_{14}O_5$, 286.0840); $^1$H 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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