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

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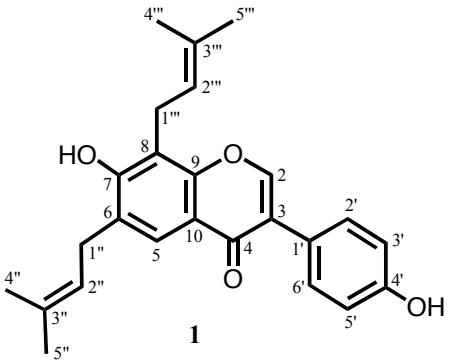
Abstract − Two new isoflavonoids, eryvarins S (**1**) and T (**2**), and a new 2-arylbenzofuran, eryvarin U (**3**), together with nine known compounds, were isolated from the roots of *Erythrina variegata*. Their structures were established on the basis of spectroscopic analysis. Eryvarin U is a rare naturally-occurring 2-arylbenzofuran. The antibacterial activity of these three new compounds against 13 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) was examined in which eryvarin U showed the highest antibacterial activity.

INTRODUCTION

Erythrina variegata (Leguminosae) has been used as folk medicine in southern parts of Japan and China,[1] and comprises bioactive secondary metabolites with antimicrobial activity.[2] We previously reported the isolation of anti-MRSA isoflavonoids (erycristagallin, eryvarin Q and orientanol B) from the roots of this plant collected in Pakistan and Indonesia.[3,4] In continuation of our screening of anti-MRSA compounds from *Erythrina* plants, we describe the isolation and structural elucidation of a new isoflavone, eryvarin S (**1**), a new isoflavan, eryvarin T (**2**), and a new 2-arylbenzofuran, eryvarin U (**3**), together with nine known isoflavonoids from the roots of Taiwanese *E. variegata*. We also report the antibacterial activities of the new compounds (**1**−**3**) against MRSA. The nine known compounds were



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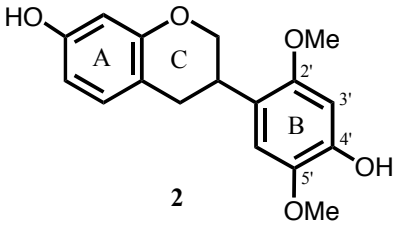

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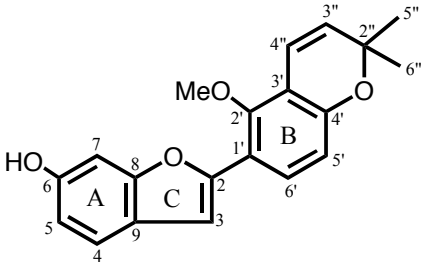

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identified as auriculatin,[5] bidwillols A[6] and B,[6] erystagallin A,[7] erysubin E,[8] erythrabyssin II (**8**),[9] eryvarin K,[10] phaseollidin[7] and phaseollin[11] by comparing spectroscopic data with those of authentic samples or reported values.

RESULTS AND DISCUSSION

Eryvarin S (**1**) was assigned a molecular formula of $C_{25}H_{26}O_4$ ($[M]^+$ *m/z* 390.1823) from the HREIMS spectrum. The IR spectrum showed the presence of conjugated carbonyl (1620 cm^{-1}) and hydroxyl (3380 cm^{-1}) groups. The UV spectrum and the typical singlet signal assigned to H-2 (δ 7.97) in the ^1H NMR spectrum revealed that compound (**1**) is an isoflavone derivative.[12,13] The ^1H NMR spectrum exhibited a singlet aromatic proton (δ 7.96) and two γ,γ-dimethylallyl (prenyl) groups (δ 1.80, 3.45 and 5.33, and 1.77, 1.86, 3.61 and 5.27), as well as a set of AA'BB'-type aromatic protons (δ 6.84 and 7.37) on a 4-hydroxyphenyl group. The placement of one of the prenyl groups at the C-6 position was assigned by NOESY experiment which revealed NOE interactions between H-1"/H-5 and H-2"/H-5 (H-5 was assigned with the HMBC spectrum; the cross-peak between H-5 and C-4). Further support for the assignment of the prenyl group at the C-6 position was obtained from the HMBC spectrum which indicated correlations between H-1"/C-5, H-1"/C-6, H-1"/C-7 and H-5/C-1". The other prenyl group was located at the C-8 position as showed from the HMBC spectrum, indicating correlations between H-1'"/C-7, H-1'"/C-8 and H-1'"/C-9. Therefore, the structure of eryvarin S is represented by **1**.

Eryvarin T (**2**) was obtained in racemic form and its molecular formula was determined as $C_{17}H_{18}O_5$ ($[M]^+$ *m/z* 302.1161) from the HREIMS spectrum. This compound was found to be an isoflavan on the basis of its characteristic spectral data: λ_{max} 230 and 289 nm in the UV spectrum and a set of aliphatic proton signals (δ 2.77, 2.96, 3.47, 3.96 and 4.17) in the ^1H NMR spectrum.[14] The ^1H NMR spectrum showed three aromatic protons in an AMX system (δ 6.28, 6.36 and 6.89), and two singlet aromatic protons (δ 6.57 and 6.85) and two methoxyl groups (δ 3.77 and 3.78) on a 1,2,4,5-tetrasubstituted benzene moiety. The placement of the C-5 position in the AMX-type was confirmed from both the

Table 1. ^{13}C NMR spectral data for **1**−**3**

position	**1**[a]	**2**[b]	**3**[a]
2	152.3	70.6	151.7
3	124.5	32.5	103.5
4	176.8	31.3	120.9
5	124.4	131.0	111.7
6	126.3	108.7	153.3
7	157.7	157.4	98.0
8	114.6	103.6	154.7
9	154.2	156.1	123.5
10	118.0	114.3	
1'	124.0	120.6	116.7
2'	130.3	152.9	153.1
3'	115.7	101.0	115.4
4'	155.9	146.9	154.1
5'	115.7	142.2	112.9
6'	130.3	113.0	127.1
1"	29.8		
2"	121.0		76.3
3"	135.8		130.8
4"	18.0		116.8
5"	25.9		27.9
6"			27.9
1'"	22.3		
2'"	120.7		
3'"	135.1		
4'"	18.0		
5'"	25.8		
2'-OMe		56.4	61.1
5'-OMe		57.3	

a: $CDCl_3$. b: acetone-d_6.

NOESY data (NOE interaction: H-5/H-4b) and the HMBC spectrum (correlations: H-5/C-4, H-5/C-9 and H-5/C-7). The positions of the methoxyl groups at the C-2' and C-5' were assigned from the NOESY spectrum which displayed NOE interactions between OMe-2'/H-3' and OMe-5'/H-6'. The further assignments of the methoxyl groups were obtained by the HMBC experiment, revealing correlations between OMe-2'/C-2' and OMe-5'/C-5'. The attachment of the B-ring to the isoflavan moiety at the C-3 position was established from both the NOESY data (NOE interactions: H-6'/H-2a and H-6'/H-4a) and the HMBC experiment (correlation: H-6'/C-3). Therefore, the structure of eryvarin T is represented by **2**.

Eryvarin U (**3**) was assigned a molecular formula of $C_{20}H_{18}O_4$ ($[M]^+$ *m/z* 322.1214) from the HREIMS spectrum. This compound was found to be a 2-arylbenzofuran derivative on the basis of the UV spectral data and the characteristic olefinic proton signal (δ 7.10) in the ^{1}H NMR spectrum.[15,16] The ^{1}H NMR spectrum showed three aromatic protons in an AMX system (δ 6.76, 6.99 and 7.40), a set of *ortho*-coupled aromatic protons (δ 6.69 and 7.71) and a methoxyl group (δ 3.78), as well as two methyl groups (δ 1.46) and two olefinic protons (δ 5.70 and 6.68) on a 2,2-dimethylpyran ring. The location of the C-4 position in the AMX-type was determined from the HMBC spectrum which displayed correlations between H-4/C-3 and H-4/C-8. The assignment of the methoxyl group at the C-2' position was confirmed from both the NOESY spectrum (NOE interactions: MeO-2'/H-4" and MeO-2'/H-3) and the HMBC spectrum (correlation: MeO-2'/C-2'). The presence of the 2,2-dimethylpyran moiety was evidenced from the EIMS spectrum that revealed the characteristic fragment ion at *m/z* 307 $[M-CH_3]^+$.[17] The placement of the 2,2-dimethylpyran moiety fused to the C-3' and C-4' positions was decided from the HMBC spectrum, indicating correlations between H-3"/C-3' and H-5'/C-4'. Therefore, the structure of eryvarin U is represented by **3**.

Antibacterial activity of the three new compounds (**1**–**3**) against 13 strains of MRSA was evaluated by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC and MBC were determined by a broth dilution method as previously reported.[4] Eryvarin U inhibited the growth of MRSA strains with MIC values of 6.25–12.5 μg mL^{-1}, and both MIC_{50} and MIC_{90} (minimum concentration needed to inhibit the growth of 50% and 90% of the tested strains, respectively) were 6.25 μg mL^{-1}. Eryvarin S also showed growth inhibitory potency (MIC values of 6.25–25 μg mL^{-1}), but failed to inhibit 2 strains at 25 μg mL^{-1} (the maximum concentration in the present study). Eryvarin T did not show anti-MRSA activity at 25 μg mL^{-1}. The MIC values of mupirocin, an authentic antibiotic for MRSA, against tested strains ranged from 0.20 to 3.13 μg mL^{-1} (MIC_{50} and MIC_{90}: both 0.39 μg mL^{-1}). Although the growth inhibitory potency of eryvarin U was lower than that of mupirocin, it inhibited the recovery of MRSA cells at 6.25–12.5 μg mL^{-1} (MBC_{50}: 12.5 μg mL^{-1}). This value was lower than that of mupirocin (MBC_{50}: 25 μg mL^{-1}). Thus, eryvarin U was revealed to have strong bactericidal potency against MRSA. The high bactericidal activity of eryvarin U is expected to have a great advantage in treating MRSA infections, because it would reduce a risk of development of resistant mutants. The new arylbenzofuran could be a potent leading compound for the development of phytotherapeutic agents against MRSA infections.

EXPERIMENTAL

General Experimental Procedures. Optical rotation was measured using a JASCO DIP-370 digital polarimeter, and CD spectrum was recorded on a JASCO J-725 spectropolarimeter. IR spectra were recorded on a JASCO IR-810 spectrophotometer, and UV spectra were obtained in MeOH using a Beckman DU-530 spectrophotometer. MS spectra were obtained using a JEOL JMS-SX102A spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a JEOL ALPHA-600 MHz spectrometer. The ^{1}H and ^{13}C NMR (Table 1) signals of the compounds (**1**–**3**) were assigned based on the ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC spectra. Column chromatography was performed using Merck silica gel (230−400 mesh). The procedure for the MIC and MBC measurement has been described in a previous publication.[4]

Plant material. The roots of *E. variegata* were collected in Kaohsiung, Taiwan, R.O.C. in November 2001. A voucher specimen (No. 011130) was deposited at the Department of Natural Product Chemistry in the Faculty of Pharmacy, Meijo University.

Extraction and isolation. The finely powdered roots (3.19 kg) were macerated with acetone (73 L) at 23 °C for 48 h (2 times) and the solvent was removed to give a residue that was divided into *n*-

hexane-, CH_2Cl_2-, and EtOAc-soluble fractions. The CH_2Cl_2-soluble fraction (228.4 g) was applied to silica gel column first eluted with $CHCl_3$-acetone (40 : 1 → 10 : 1.5 → 3 : 1 → 1 : 1) and acetone (each volume; 3 L, Column A) to afford 7 fractions. Fraction A3 (27.7 g) was subjected to silica gel column chromatography using $CHCl_3$-acetone (40 : 1 → 20 : 1) (each volume; 200 mL, Column B) to yield 24 fractions. Fraction B7 (1.93 g) was separated by silica gel column chromatography successively using *n*-hexane-acetone (5 : 1) and benzene-EtOAc (80 : 1) to furnish eryvarin U (**3**) (2.2 mg) and phaseollin (4.1 mg). Fraction B14 (1.51 g) was subjected to silica gel column chromatography successively using benzene-EtOAc (10 : 1) and *n*-hexane-acetone (2 : 1) to provide bidwillol A (314 mg), erythrabyssin II (264 mg) and eryvarin K (22 mg). Fraction B20 (845 mg) was purified by repeated silica gel column chromatography using *n*-hexane-acetone (2.5 : 1) to give auriculatin (33 mg). Fractions B22–24 (1.62 g) were separated by silica gel column chromatography successively using *n*-hexane-acetone (3 : 1) and benzene-EtOAc (10 : 1) to afford bidwillol B (51 mg), eryvarin S (**1**) (7.2 mg) and phaseollidin (83 mg). Fraction A4 (70.4 g) was applied to silica gel column chromatography using $CHCl_3$-acetone (10 : 1.5 → 1 : 1) and acetone (each volume; 500 mL, Column C) to yield 21 fractions. Fraction C5 (1.28 g) was separated by silica gel column chromatography successively using *n*-hexane-acetone (2.5 : 1 → 1.5 : 1) and benzene-EtOAc (20 : 1 → 3 : 1) to furnish bidwillol A (24 mg) and bidwillol B (4.7 mg). Fractions C6 and C7 (8.72 g) were applied to silica gel column chromatography successively using benzene-EtOAc (5 : 1 → 3 : 1) (each volume; 40 mL, Column D) to provide 40 fractions. Fractions D5–10 (3.38 g) were separated by silica gel column chromatography successively using *n*-hexane-acetone (1.5 : 1) and benzene-EtOAc (10 : 1 → 5 : 1) to give auriculatin (34 mg), erystagallin A (41 mg) and erysubin E (24 mg). Fractions D11–18 (2.58 g) were purified by silica gel column chromatography successively using *n*-hexane-acetone (3 : 1 → 5 : 1) and benzene-EtOAc (10 : 1) to afford eryvarin T (**2**) (8.6 mg).

Eryvarin S (1). Amorphous powder; IR (film) ν_{max} cm^{-1}: 3380, 1620; UV λ_{max} nm (log ε): 205 (4.62), 255 (4.46), 309 (3.97); EIMS *m/z* (rel. int.): 390 ($[M]^+$, 100), 373 (24), 347 (16), 335 (47), 319 (72), 291 (21), 279 (29); HREIMS *m/z*: 390.1823 (M^+, Calcd for $C_{25}H_{26}O_4$, 390.1830); ^1H NMR ($CDCl_3$): δ 1.77 (3H, s, H-5'''), 1.80 (6H, s, H-4'' and H-5''), 1.86 (3H, s, H-4'''), 3.45 (2H, d, *J* = 7.3 Hz, H-1''), 3.61 (2H, d, *J* = 7.3 Hz, H-1'''), 5.27 (1H, t, *J* = 7.3 Hz, H-2'''), 5.33 (1H, t, *J* = 7.3 Hz, H-2''), 5.97 (1H, br s, OH), 6.18 (1H, s, OH), 6.84 (2H, d, *J* = 8.8 Hz, H-3' and H-5'), 7.37 (2H, d, *J* = 8.8 Hz, H-2' and H-6'), 7.96 (1H, s, H-5), 7.97 (1H, s, H-2); ^{13}C NMR: see Table 1.

Eryvarin T (2). Amorphous powder; $[\alpha]_D$ ±0°; CD (MeOH; *c* 2.32 x 10^{-5}): no Cotton effect; IR (film) ν_{max} cm^{-1}: 3420; UV λ_{max} nm (log ε): 205 (4.66), 230 (sh, 4.11), 289 (3.87), 320 (3.43); EIMS

m/z (rel. int.): 302 ([M]$^+$, 68), 180 (100), 167 (46), 165 (46), 137 (17), 122 (6); HREIMS *m/z*: 302.1161 (M$^+$, Calcd for $C_{17}H_{18}O_5$, 302.1153); ^{1}H NMR (acetone-d_6): δ 2.77 (1H, ddd, *J* = 15.6, 5.4, 2.0 Hz, H-4b), 2.96 (1H, dd, *J* = 15.6, 11.2 Hz, H-4a), 3.47 (1H, m, H-3), 3.77 (3H, s, OMe-5'), 3.78 (3H, s, OMe-2'), 3.96 (1H, t-like, *J* = 10.3 Hz, H-2a), 4.17 (1H, ddd, *J* = 10.3, 3.4, 2.0 Hz, H-2b), 6.28 (1H, d, *J* = 2.4 Hz, H-8), 6.36 (1H, dd, *J* = 8.1, 2.4 Hz, H-6), 6.57 (1H, s, H-3'), 6.85 (1H, s, H-6'), 6.89 (1H, d, *J* = 8.1 Hz, H-5), 7.54 (1H, s, OH), 8.07 (1H, br s, OH); ^{13}C NMR: see Table 1.

Eryvarin U (3). Amorphous powder; IR (film) ν_{max} cm^{-1}: 3420, 1630; UV λ_{max} nm (log ε): 214 (sh, 4.02), 236 (4.17), 273 (4.15), 319 (4.31), 329 (sh, 4.27); EIMS *m/z* (rel. int.): 322 ([M]$^+$, 62), 307 (100), 292 (66); HREIMS *m/z*: 322.1214 (M$^+$, Calcd for $C_{20}H_{18}O_4$, 322.1204); ^{1}H NMR ($CDCl_3$): δ 1.46 (6H, s, H-5" and H-6"), 3.78 (3H, s, OMe-2'), 5.70 (1H, d, *J* = 10.3 Hz, H-3"), 6.68 (1H, d, *J* = 10.3 Hz, H-4"), 6.69 (1H, d, *J* = 8.8 Hz, H-5'), 6.76 (1H, dd, *J* = 8.1, 2.2 Hz, H-5), 6.99 (1H, d, *J* = 2.2 Hz, H-7), 7.10 (1H, s, H-3), 7.40 (1H, d, *J* = 8.1 Hz, H-4), 7.71 (1H, d, *J* = 8.8 Hz, H-6'); ^{13}C NMR: see Table 1.

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