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NEW CONSTITUENTS FROM THE ROOTS OF *ERYTHRINA* X *BIDWILLII*

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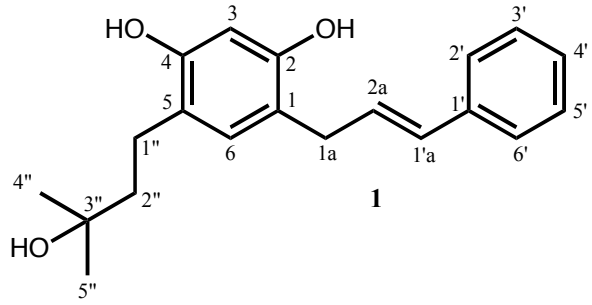
Abstract − Three new compounds, erythbidins C-E (**1**–**3**), together with five known compounds **4**–**8** were isolated from the roots of *Erythrina* x *bidwillii*. Their structures were established on the basis of spectroscopic evidence. Among the isolated compounds, erythbidin E (**3**) showed a potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

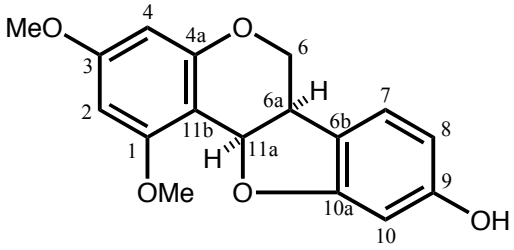
INTRODUCTION

The genus *Erythrina* (Leguminosae) is distributed in the tropical and subtropical regions of the world, and has been used as a folklore medicinal treatment of microbial infections. *Erythrina* x *bidwillii* has been grown as an ornamental plant, a shrub with scarlet flowers, in the southern area of Japan. There have been many secondary metabolites extracted from the roots, wood and flowers of *E.* x *bidwillii*,[1-3] among which the non-alkaloidal component, erycristagallin, showed a potent antibacterial activity against *Streptococcus mutanns*.[1] In continuation of our screening of antibacterial compounds against MRSA from *Erythrina* plants, we describe the isolation and structural elucidation of a new cinnamylphenol, erythbidin C (**1**), a new pterocarpan, erythbidin D (**2**), and a new 2-arylbenzofuran, erythbidin E (**3**), along with the known 2-arylbenzofuran (**4**) and four known isoflavonoids (**5**-**8**) from the roots of this plant, and report their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).

The five known compounds were identified as 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran (**4**),[4,5] erythbidin A (**5**),[2] hydroxycristacarpone (**6**),[6] 3,9-dihydroxypterocarp-6a-ene (**7**)[7] and 8-prenyldaidzein (**8**)[8] by comparing their spectroscopic data with those of authentic samples or reported values.



1



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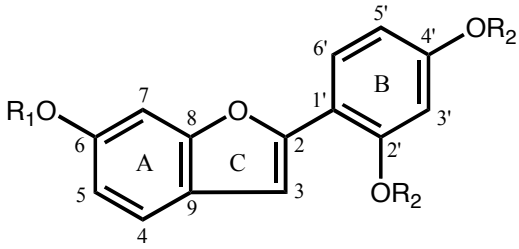

3 R_1 = Me, R_2 = H
3a R_1 = Me, R_2 = Ac
4 R_1 = R_2 = H

RESULTS AND DISCUSSION

Silica gel chromatography of the CH_2Cl_2-soluble portion of the acetone extract of the roots of *E.* x *bidwillii* gave three new compounds **1**–**3**, together with five known compounds **5**–**8**.

Erythbidin C (**1**) was assigned the molecular formula of $C_{20}H_{24}O_3$ ($[M]^+$ *m/z* 312.1734) based on the HREIMS spectrum. This compound was found to have an *E*-cinnamylphenol skeleton from the characteristic UV spectrum (λ_{max} 203, 250, 285 and 293) and a typical ABX_2 system associated with a *E*-CH=CH–CH_2- structure in the ^{1}H NMR spectrum (δ 3.45, 6.36 and 6.46).[9,10] The ^{1}H NMR spectrum showed two singlet aromatic protons (δ 6.37 and 6.82) and a 3-hydroxy-3-methylbutyl moiety (δ 1.27, 1.76 and 2.63), along with an unsubstituted phenyl group (δ 7.19, 7.28 and 7.34). The placement of the 3-hydroxy-3-methylbutyl group at the C-5 position was determined from the HMBC spectrum, which revealed correlations between H-6/C-1", H-1"/C-4, H-1"/C-5, H-1"/C-6 and H-2"/C-5. The attachment of a 2,4-dihydroxy-5-(3-hydroxy-3-methylbutyl)benzene ring to the C-1a position was established from both the NOESY data (NOE interaction: H-6/H-1a) and the HMBC spectrum (correlations: H-6/C-1a, H-1a/C-1, H-1a/C-2 and H-1a/C-6). The assignment of the unsubstituted phenyl moiety at the C-1'a position was obtained from both the NOESY data (NOE interactions: H-2'/H-2a and H-6'/H-1'a) and the HMBC spectrum (correlations: H-2'(6')/C-1'a, H-3'(5')/C-1', H-1'a/C-1', H-1'a/C-2'(6') and H-2a/C-1'). Thus, erythbidin C was characterized as *E*-1-[2,4-dihydroxy-5-(3- hydroxy-3-methylbutyl)benzyl]-2-phenylethylene (**1**).

This is the first report of the naturally-occurring cinnamylphenol derivative that possesses a 3-hydroxy-3-methylbutyl group.

Erythbidin D (**2**), $C_{17}H_{16}O_5$ ($[M]^+$ *m/z* 300.0998), was revealed to have a pterocarpan structure on the basis of

Table 1. ^{13}C NMR spectral data for **1**−**4**

position	**1**[a]	**2**[b]	**3**[b]	**4**[b]
1	117.3	161.9		
2	153.2	92.7	153.4	153.0
3	104.0	162.7	103.9	104.0
4	153.4	94.2	121.4	121.4
4a		158.4		
5	121.2		112.2	112.6
6	131.4	67.1	158.6	156.0
6a		39.5		
6b		119.1		
7		125.7	96.2	98.2
8		108.0	155.3	155.5
9		159.5	124.1	123.5
10		98.5		
10a		161.9		
11a		75.9		
11b		103.0		
1'	137.3		110.9	111.1
2'	126.2		156.3	156.3
3'	128.5		103.8	104.0
4'	127.2		159.3	159.2
5'	128.5		108.3	108.3
6'	126.2		128.0	128.0
1"	24.0			
2"	43.4			
3"	71.9			
4"	29.5			
5"	29.5			
6"				
1a	33.4			
2a	128.8			
1'a	130.9			
1-OMe		56.0		
3-OMe		55.6		
6-OMe			55.9	

a: $CDCl_3$.

b: acetone-d_6.

its UV spectrum data and a set of four aliphatic proton signals (δ 3.41, 3.54, 4.21 and 5.55) in the ^{1}H NMR spectrum.[11] The ^{1}H NMR spectrum displayed *metha*-coupled aromatic protons (δ 6.07 and 6.22) and three aromatic protons in an AMX system (δ 6.29, 6.36 and 7.13), as well as two methoxyl groups (δ 3.78 and 3.87). The locations of the AMX-type aromatic protons at the C-7, C-8 and C-10 positions were based on both the COSY spectrum (correlation: H-7/H-8) and the NOESY data (NOE interaction: H-6a/H-7). The further assignment of the three aromatic protons was obtained by the HMBC technique (correlations: H-7/C-9, H-7/C-10a, H-8/C-6b, H-8/C-9, H-8/C-10, H-10/C-6b, H-10/C-8, H-10/C-9 and H-10/C-10a). The locations of the two methoxyl groups at the C-1 and C-3 positions were achieved from both the NOESY data (NOE interactions: OMe-1/H-2 and OMe-1/H-11a for the methoxyl group at C-1; OMe-3/H-2 and OMe-3/H-4 for the methoxyl group at C-3) and the HMBC spectrum (correlations: OMe-1/C-1 for OMe-C(1); OMe-3/C-3 for OMe-C(3)). The absolute stereochemistry at C-6a and C-11a was assigned as 6a *R*: 11a *R* from the negative optical rotation value.[12] Thus, erythbidin D was characterized as (6a*R*,11a*R*)-9-hydroxy-1,3-dimethoxypterocarpan (**2**)

Erythbidin E (**3**), $C_{15}H_{12}O_4$ ($[M]^+$ *m/z* 256.0726), was found to be a 2-arylbenzofuran derivative based on the UV spectral data and the characteristic olefinic proton signal (δ 7.17) in the ^{1}H NMR spectrum.[13,14] The acetylation of **3** with acetic anhydride and pyridine produced a

diacetate (**3a**), indicating the presence of two hydroxyl groups. The ^{1}H NMR spectrum revealed three aromatic protons in an AMX-system (δ 6.82, 7.10 and 7.43) and methoxyl group (δ 3.84) on an A-ring, along with other AMX-type aromatic protons (δ 6.51, 6.57 and 7.73) on a B-ring. The location of the methoxyl group at the C-6 position was determined from both the NOESY data (NOE interactions: OMe-6/H-5 and OMe-6/H-7) and the HMBC experiment (correlation: OMe-6/C-6). The assignment of the AMX-type aromatic protons at the C-3', C-5' and C-6' positions on the B-ring was obtained from the HMBC spectrum which indicated correlations between H-3'/C-1', H-3'/C-2', H-3'/C-4', H-3'/C-5', H-5'/C-1', H-5'/C-3', H-5'/C-4', H-6'/C-2' and H-6'/C-4'. The attachment of the B-ring to the arylbenzofuran moiety at the C-2 position was established by identification of the cross-peak between the aromatic proton at H-6' (δ 7.73) and an sp^2 quaternary carbon at C-2 (δ 153.4) in the HMBC spectrum. Thus, erythbidin E was characterized as 2-(2,4-dihydroxyphenyl)-6-methoxybenzofuran (**3**).

To the best of our knowledge, erythbidin E (**3**) is the first naturally-occurring product, which had been previously prepared by the hydrogenolysis of 2-benzyloxy-4-methoxybenzyl-4-benzyloxy-2-hydroxyphenylketone.[15] The ^{1}H and ^{13}C NMR spectra of **3** are reported here for the first time.

Compound **4**, 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran, has been isolated from the heartwood of *Lespedeza cyrtobotrya* and its acetylated compound was identified with that in the literature.[4] However, in the reported ^{13}C NMR spectral data of **4** several erroneous assignments have been found.[5] The revised assignments of the ^{13}C NMR spectrum are demonstrated in this paper.

The assignment of all the ^{1}H and ^{13}C NMR signals of **1**–**4** was accomplished by analyses of the ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC spectra.

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against MRSA of the isolated compounds **1**–**8**, vancomycin and methicillin, are summarized in Table 2. Among the isolated compounds, erythbidin E (**3**) showed the highest antibacterial potency against the MRSA strains, followed by erythbidin A (**5**). These compounds also exhibited MBC values comparable to or lower than those of vancomycin, indicating their potent ability to kill MRSA cells.

EXPERIMENTAL

General Experimental Procedures. The Optical rotation was measured using a JASCO DIP-370 digital polarimeter. The IR spectra were recorded by a JASCO IR-810 spectrophotometer, and the UV spectra were obtained in MeOH using a Beckman DU-530 spectrophotometer. The MS spectra were obtained using a JEOL JMS-SX102A spectrometer. The ^{1}H and ^{13}C NMR spectra were measured by a JEOL ALPHA-600 MHz spectrometer. Column chromatography was performed using Merck silica gel (230–400 mesh). The MICs and MBCs of the isolated compounds against 13 MRSA strains were determined as previously reported.[16]

Table 2. Minimum inhibitory and minimum bactericidal concentrations (MICs and MBCs) of compounds **1**–**8** against 13 MRSA strains

	MICs (μg/mL)			MBCs (μg/mL)		
Compounds	MIC range	MIC_{50}[a]	MIC_{90}[a]	MBC range	MBC_{50}[b]	MBC_{90}[b]
1	> 50	> 50	> 50	–[c]	–[c]	–[c]
2	25–50	50	50	50–> 50	> 50	> 50
3	3.13–6.25	6.25	6.25	12.5–25	12.5	25
4	6.25–25	12.5	25	25–> 50	50	50
5	6.25–12.5	12.5	12.5	12.5–25	12.5	12.5
6	> 50	> 50	> 50	–[c]	–[c]	–[c]
7	12.5–25	25	25	25－50	50	50
8	> 50	> 50	> 50	–[c]	–[c]	–[c]
Vancomycin	0.78–1.56	1.56	3.13	3.13–25	6.25	25
Methicillin	12.5–> 50	> 50	> 50	> 50	> 50	> 50

a: Minimum concentration needed to inhibit growth of 50 and 90% of strains tested.

b: Minimum concentration needed to inhibit bacterial recovery of 50 and 90% of strains tested.

c: Not tested.

Plant material. The roots were collected in February 2005 from an *E.* x *bidwillii* planted in the Ogasawara Islands, Tokyo, Japan. A voucher specimen (No.050201) was deposited at the Department of Natural Product Chemistry, Faculty of Pharmacy, Meijo University.

Extraction and isolation. The fine powdered roots (2.34 kg) were macerated with acetone (48 L) at 23 ℃ for 48 h (2 times) and the solvent was removed to give a residue that was divided into the *n*-hexane-, CH_2Cl_2-, and EtOAc-soluble fractions. The CH_2Cl_2-soluble fraction (24.44 g) was first applied to a silica gel column eluted with $CHCl_3$-acetone (20 : 1 → 10 : 1 → 5 : 1 → 1 : 1) and acetone (each volume; 1 L, Column A) to afford 9 fractions. Fraction A4 (656 mg) was purified by repeated silica gel chromatography using *n*-hexane-acetone (3 : 1) to give **2** (15 mg). Fractions A5 and A6 (6.47 g) were subjected to silica gel column chromatography using $CHCl_3$-acetone (20 : 1 → 5 : 1) (each volume; 30 mL, Column B) to give 40 fractions. Fractions B20-40 (3.76 g) were separated by silica gel column chromatography using benzene-EtOAc (5 : 1) and *n*-hexane-acetone (3 : 1) to give **5** (200 mg) and **7** (3.7 mg). Fraction A7 (2.56 g) was purified by silica gel column chromatography using $CHCl_3$-acetone (10 : 1.5) and *n*-hexane-acetone (3 : 1 → 2 : 1) to give **3** (22 mg). Fraction A9 (5.82 g) was analyzed by silica gel chromatography using *n*-hexane-acetone (2 : 1 → 1 : 1) (each volume; 30 mL, Column C) to give 30 fractions. Fractions C10-14 (173 mg) were separated by silica gel column chromatography using benzene-acetone (5 : 1 → 1 : 1) to give **1** (6.8 mg), **6** (14 mg) and **8**

(4.4 mg). Fractions C18-28 (1.06 g) were separated by silica gel column chromatography using $CHCl_3$-acetone (10 : 1.5) and benzene-EtOAc (5 : 1) to give **4** (13 mg) and **6** (26 mg).

Erythbidin C (1). Amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3370, 1620; UV λ_{max} nm (log ε): 203 (4.27), 250 (3.79), 285 (3.54), 293 (sh, 3.49); EIMS *m/z* (rel. int.): 312 ($[M]^+$, 19), 294 (42), 239 (100), 225 (10), 147 (9), 117 (16), 91 (32); HREIMS *m/z* 312.1734 (M^+, Calcd for $C_{20}H_{24}O_3$, 312.1724); ^{1}H NMR ($CDCl_3$): δ 1.27 (6H, s, H-4" and H-5"), 1.76 (2H, t, *J* = 7.4 Hz, H-2"), 2.63 (2H, t, *J* = 7.4 Hz, H-1"), 3.45 (2H, d, *J* = 6.2 Hz, H-1a), 6.36 (1H, dt, *J* = 15.9, 6.2 Hz, H-2a), 6.37 (1H, s, H-3), 6.46 (1H, d, *J* = 15.9 Hz, H-1'a), 6.82 (1H, s, H-6), 7.19 (1H, t, *J* = 7.4 Hz, H-4'), 7.28 (2H, t, *J* =7.4 Hz, H-3' and H-5'), 7.34 (2H, d, *J* = 7.4 Hz, H-2' and H-6'); ^{13}C NMR: see Table 1.

Erythbidin D (2). Amorphous powder; $[\alpha]_D$ –385° (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3420; UV λ_{max} nm (log ε): 210 (4.96), 239 (sh, 4.22), 285 (3.92); EIMS *m/z* (rel. int.): 300 ($[M]^+$, 100), 285 (11), 178 (13), 167 (7), 150 (8); HREIMS *m/z* 300.0998 (M^+, Calcd for $C_{17}H_{16}O_5$, 300.0997); ^{1}H NMR (acetone-d_6): δ 3.41 (1H, m, H-6a), 3.54 (1H, t-like, *J* = 11.0 Hz, H-6α), 3.78 (3H, s, OMe-3), 3.87 (3H, s, OMe-1), 4.21 (1H, dd, *J* = 11.0, 4.4 Hz, H-6β), 5.55 (1H, d, *J* = 6.6 Hz, H-11a), 6.07 (1H, d, *J* = 2.2 Hz, H-4), 6.22 (1H, d, *J* = 2.2 Hz, H-2), 6.29 (1H, d, *J* = 2.2 Hz, H-10), 6.36 (1H, dd, *J* = 8.1, 2.2 Hz, H-8), 7.13 (1H, d, *J* = 8.1 Hz, H-7), 8.34 (1H, br s, OH); ^{13}C NMR: see Table 1.

Erythbidin E (3). Amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3380, 1620; UV λ_{max} nm (log ε): 211 (4.46), 229 (sh, 4.14), 274 (sh, 4.16), 282 (4.19), 306 (sh, 4.30), 320 (4.55), 334 (4.53); EIMS *m/z* (rel. int.): 256 ($[M]^+$, 100), 241 (83), 149 (5), 128 (9), 101 (10); HREIMS *m/z* 256.0726 (M^+, Calcd for $C_{15}H_{14}O_4$, 256.0735); ^{1}H NMR (acetone-d_6): δ 3.84 (3H, s, OMe-6), 6.51 (1H, dd, *J* = 8.8, 2.2 Hz, H-5'), 6.57 (1H, d, *J* = 2.2 Hz, H-3'), 6.82 (1H, dd, *J* = 8.8, 2.2 Hz, H-5), 7.10 (1H, d, *J* = 2.2 Hz, H-7), 7.17 (1H, s, H-3), 7.43 (1H, d, *J* = 8.8 Hz, H-4), 7.73 (1H, d, *J* = 8.8 Hz, H-6'), 8.58 (1H, br s, OH-4'), 9.14 (1H, br s, OH-2'); ^{13}C NMR: see Table 1.

Acetylation of 3. A mixture of **3** (8.3 mg), Ac_2O (0.5 mL) and pyridine (0.5 mL) was stirred overnight at rt. The reaction mixture was worked up as usual and the resulting residue was purified by silica gel column chromatography [benzene-EtOAc (40 : 1)] to yield a diacetate derivative (**3a**) (10 mg) as an amorphous powder. IR (KBr) ν_{max} cm^{-1}: 1770, 1620, 1590; UV λ_{max} nm (log ε): 203 (4.41), 237 (sh, 3.94), 281 (sh, 3.94), 315 (4.44), 327 (sh, 4.37); EIMS *m/z* (rel. int.): 340 ($[M]^+$, 44), 298 (36), 256 (100), 241 (31), 212 (6), 128 (3); HREIMS *m/z* 340.0917 (M^+, Calcd for $C_{19}H_{16}O_6$, 340.0946); ^{1}H NMR (acetone-d_6): δ 2.29 (3H, s, OAc-4'), 2.44 (3H, s, OAc-2'), 3.87 (3H, s, OMe-6), 6.90 (1H, dd, *J* = 8.8, 2.0 Hz, H-5), 7.12 (1H, d, *J* = 2.0 Hz, H-3'), 7.17 (1H, d, *J* = 2.0 Hz, H-7), 7.19 (1H, s, H-3), 7.20 (1H, dd, *J* = 8.8, 2.0 Hz, H-5'), 7.54 (1H, d, *J* = 8.8 Hz, H-4), 8.00 (1H, d, *J* = 8.8 Hz, H-6').

2-(2,4-Dihydroxyphenyl)-6-hydroxybenzofuran (4). Amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3340, 1630; UV λ_{max} nm (log ε): 209 (4.55), 231 (sh, 4.17), 282 (4.11), 308 (sh, 4.19), 320 (4.39), 335 (4.38);

EIMS *m/z* (rel. int.): 242 ([M]$^+$, 100), 213 (22), 197 (8), 185 (14), 171 (7), 149 (17), 139 (15), 121 (28), 115 (26); HREIMS *m/z* 242.0574 (M^+, Calcd for $C_{14}H_{10}O_4$, 242.0578); ^{1}H NMR (acetone-d_6): δ 6.50 (1H, dd, *J* = 8.5, 2.3 Hz, H-5'), 6.56 (1H, d, *J* = 2.3 Hz, H-3'), 6.77 (1H, dd, *J* = 8.5, 2.3 Hz, H-5), 6.97 (1H, br s, H-7), 7.14 (1H, s, H-3), 7.36 (1H, d, *J* = 8.5 Hz, H-4), 7.72 (1H, d, *J* = 8.5 Hz, H-6'), 8.41 (1H, br s, OH), 9.10 (1H, br s, OH); ^{13}C NMR: see Table 1.

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