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Eryvarins F and G, two 3-phenoxychromones from the roots of *Erythrina variegata*

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Abstract

Two 3-phenoxychromones, eryvarins F and G, were isolated from the roots of *Erythrina variegata*. Their structures were established to be 3-(2,4-dihydroxyphenoxy)-7-hydroxy-6,8-di(3,3-dimethylallyl)chromen-4-one and 3-(2,4-dihydroxyphenoxy)-8-(3,3-dimethylallyl)-2,2-dimethylpyrano[5,6:6,7]chromen-4-one on the basis of spectroscopic and chemical evidence. Eryvarins F and G are unusual 3-phenoxychromone derivatives with two isoprenoid groups.

Keywords: *Erythrina variegata*; Leguminosae; Chromones; 3-Phenoxychromones; Eryvarins F and G

1. Introduction

Erythrina variegata L. (Leguminosae) is found in many tropical and subtropical regions and is used medicinally as an antibacterial, anti-inflammatory, antipyretic, and antiseptic agent and as a collyrium in China. Phytochemical investigation of the non-alkaloidal secondary metabolites of the genus *Erythrina* revealed the presence of one cinnamylphenol (Telikepalli et al., 1990) and several isoflavonoids (Deshpande et al., 1977; Hegde et al., 1977; Kobayashi et al., 1997), some of which exhibit antibacterial (Telikepalli et al., 1990) and anti-inflammatory activities (Hegde et al., 1977) and inhibit the Na^+/H^+ exchange system (Kobayashi et al., 1997). Phytochemical analysis of the genus *Erythrina* allowed us to isolate five isoflavonoids (eryvarins A–E) from the wood and the roots of *E. variegata* (Tanaka et al., 2000, 2001). This study reports the isolation and structural elucidation of two novel 3-phenoxychromones, eryvarins F (**1**) and G (**2**), and four known isoflavonoids (**3**–**6**) from the roots of *E. variegata* that was obtained from Indonesia.

2. Results and discussion

Silica gel chromatography of the CH_2Cl_2 soluble portion of the acetone extract of the roots of the plant yielded new 3-phenoxychromones, eryvarins F (**1**) and G (**2**), and four known compounds (**3**–**6**). The four known compounds were identified as auriculatin (**3**) (Shabbir et al., 1968), bidwillol A (**4**) (Iinuma et al., 1994), cristacarpin (**5**) (Tanaka et al., 1996) and erystagallin A (**6**) (Tanaka et al., 1997) by comparison of the spectroscopic data with those of authentic samples or reported values.

The molecular formula of eryvarin F (**1**) was determined as $C_{25}H_{26}O_6$ using HREIMS ($[M]^+$ m/z 422.1725). Compound **1** was subsequently treated with

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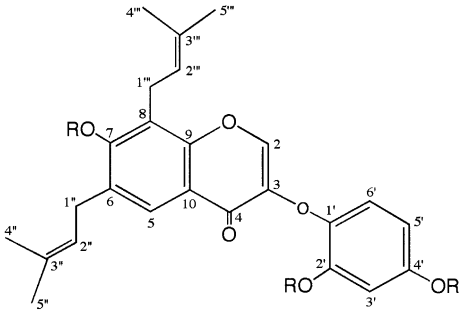
trimethylsilyldiazomethane to yield trimethyl ether **1a**. The UV spectral data of **1** demonstrated that **1** exhibits a chromen-4-one skeleton (Komiya et al., 1976). The characteristic signals assignable to the C-2 (δ_H 8.27 and δ_C 148.5) and C-3 positions (δ_C 144.0) [^{1}H and ^{13}C NMR spectra (Table 1)] suggested that **1** is a 3-oxygenated chromen-4-one derivative (Zeng et al., 1993). The ^{1}H NMR spectrum revealed the presence of another singlet aromatic proton (δ 7.90 at C-5), AMX-type aromatic protons assigned to a 2,4-dihydroxyphenoxy group (δ 6.28, 6.51 and 6.96), and protons of two prenyl groups (δ 1.77, 1.79, 3.41 and 5.29; δ 1.75, 1.85, 3.59 and 5.21). The ^{13}C NMR spectrum also revealed the presence of these structures. The placement of the prenyl group at the C-6 position was decided using the HMBC spectrum that revealed cross-peaks between H-1″/C-5, H-1″/C-6 and H-1″/C-7. The location of the other prenyl group at the C-8 position was confirmed using the HMBC spectrum that revealed correlations between H-1‴/C-7, H-1‴/C-8 and H-1‴/C-9. The presence of the 2,4-dihydroxyphenoxy moiety was identified using the HMBC spectrum which revealed 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-4′, H-6′/C-1′, H-6′/C-2′ and H-6′/C-4′. The placement of the 2,4-dihydroxyphenoxy substituent at the C-3 position was determined using the NOESY spectrum of the trimethyl ether **1a** that revealed NOE interactions between H-2/H-6′, H-5′/H-6′ and H-5′/OMe-4′ (Fig. 1). The structure of eryvarin F was subsequently identified as 3-(2,4-dihydroxyphenoxy)-7-hydroxy-6,8-di(3,3-dimethylallyl)chromen-4-one (**1**).



1 R = H

1a R = Me

The molecular formula of eryvarin G (**2**) was determined as $C_{25}H_{24}O_6$ by HREIMS ([M]$^+$ m/z 420.1567). Its UV spectrum revealed that **2** is also a chromen-4-one derivative. Comparison of the ^{1}H NMR spectrum of **2** (Table 2) with that of **1** demonstrated that a singlet aromatic proton (δ 7.73), a singlet olefinic proton (δ 8.26) and protons of a prenyl group (δ 1.68, 1.82, 3.49 and 5.15) and AMX-type aromatic protons on the 2,4-dihydroxyphenoxy moiety (δ 6.29, 6.50 and 6.97) are located in identical positions. This result was confirmed by comparison of the ^{13}C NMR spectrum of **2** with that of **1**. The remaining signals (δ 1.47, 5.77 and 6.41) were attributable to those of the 2,2-dimethylpyran ring. This was supported by analysis of the EIMS spectrum, revealing the fragment ion typical of a 2,2-dimethylpyran ring ([M–CH_3]$^+$ m/z 405) (Takayama et al., 1992). The attachment of the 2,2-dimethylpyran moiety to the C-6 and C-7 positions was assigned from the HMBC spectrum that revealed correlations between H-5/C-7, H-5/C-1″, H-1″/C-7 and H-2″/C-6. The structure of eryvarin G was subsequently identified as 3-(2,4-dihydroxyphenoxy)-8-(3,3-dimethylallyl)-2,2-dimethylpyrano[5,6:6,7]chromen-4-one (**2**).

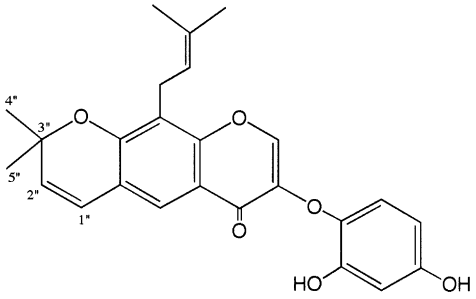


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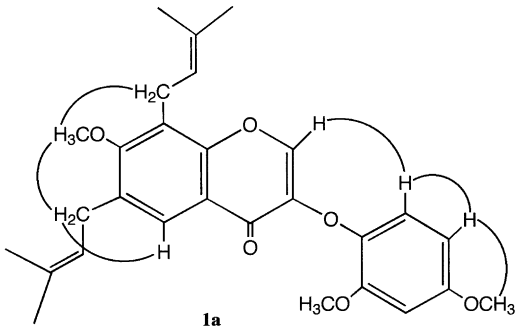
Table 1
NMR spectral data for eryvarin F (**1**) in $CDCl_3$

Position	^{1}H NMR	NOESY	^{13}C NMR	HMBC
2	8.27 *s*	6′	148.5	4, 9
3			144.0	
4			175.4	
5	7.90 *s*	1″	123.9	4, 7, 9, 1″
6			127.2	
7			158.5	
8			114.8	
9			154.2	
10			117.3	
1′			140.2	
2′			150.3	
3′	6.51 *d* (2.9)		105.5	1′, 2′, 4′, 5′
4′			154.0	
5′	6.28 *dd* (8.8, 2.9)	6′	106.7	1′, 3′, 4′
6′	6.96 *d* (8.8)	2, 5′	121.7	1′, 2′, 4′
1″	3.41 *d* (7.3)	5, 2″, 4″	29.5	5, 6, 7, 2″, 3″
2″	5.29 *t* (7.3)	1″, 5″	120.2[a]	1″, 4″, 5″
3″			136.2	
4″	1.77 *s*	1″	17.9[b]	5″
5″	1.79 *s*	2″	25.8	2″, 4″
1‴	3.59 *d* (7.3)	2‴, 4‴	22.3	7, 8, 9, 2‴, 3‴
2‴	5.21 *t* (7.3)	1‴, 5‴	120.4[a]	1‴, 4‴, 5‴
3‴			135.6	
4‴	1.85 *s*	1‴	18.0[b]	2‴, 5‴
5‴	1.75 *s*	2‴	25.8	2‴, 4‴
OH	5.08 *br s*			
OH	6.29 *br s*			
OH	9.48 *br s*			

[a, b] Assignments may be interchanged.



1a

Fig 1. Selected NOSEY correlations of compound **1a**.

To the best of our knowledge, a natural 3-phenoxychromone derivative (glyasperin E) has only been previously isolated from the roots of *Glycyrrhiza aspera* (Leguminosae) (Zeng et al., 1993). Eryvarins F (**1**) and G (**2**) are rare 3-phenoxychromones, and this is the first isolation of 3-phenoxychromones from the genus *Erythrina*.

3. Experimental

3.1. General

UV spectra were obtained using a Beckman DU-530 spectrophotometer and IR spectra were recorded on a JASCO IR-810 spectrophotometer. Mass spectra were obtained using a Jeol JMS-D 300 spectrometer. The ^{1}H NMR spectra were measured using Jeol JNM-A 400 and 600 MHz spectrometers, while the ^{13}C NMR spectra were recorded at 100.4 and 150.8 MHz using the same instruments. Column chromatography (CC) was performed using Merck silica gel (230–400 mesh). TLC was performed using Merck precoated silica gel (60 F_{254}). UV light and iodine vapor were used for the detection of compounds.

3.2. Plant material

The roots of *E. variegata* were collected from Bogor Botanical Garden, Indonesia, in January 2001. The identification of the plant was confirmed by one of the authors (J. Murata). A voucher specimen (No. 010124) was deposited in the Department of Natural Product Chemistry in the Faculty of Pharmacy, Meijo University, Japan.

3.3. Extraction and isolation

The finely powdered roots (1.53 kg) were macerated with acetone (2×18 l) and the solvent was removed to yield a residue that was divided into hexane, CH_2Cl_2 and EtOAc soluble fractions. The CH_2Cl_2-soluble fraction (65.4 g) was applied to a silica gel column, and was eluted with solvents of varying polarity of $CHCl_3$–acetone (40:1→10:1.5→1:1) and $CHCl_3$–MeOH (10:1→1:1) to yield 33 fractions (each fraction; 400 ml, column A). Fractions A18-20 (2.53 g) were subjected to silica gel chromatography using C_6H_6–EtOAc (5:1) to yield 30 fractions (each fraction; 30 ml, column B). Fractions B6-8 (150 mg) were separated by CC on a silica gel using hexane–acetone (2:1) to yield auriculatin (**3**) (7 mg) and bidwillol A (**4**) (49 mg). Fractions B17-22 (351 mg) were purified by CC on a silica gel using $CHCl_3$–acetone (10:1) to yield cristacarpin (**5**) (175 mg). Fractions B9-16 (705 mg) were subjected to CC on a silica gel using hexane–acetone (2:1) to yield 20 fractions (each fraction; 10 ml, column C). Fractions C7 and 8 (61 mg) were subjected to CC on a silica gel using $CHCl_3$–acetone (20:1) and C_6H_6–EtOAc (8:1) to yield erystagallin A (**6**) (18 mg) and eryvarin G (**2**) (6 mg). Fractions C9-11 (120 mg) were purified by CC on a silica gel using $CHCl_3$–acetone (20:1) to yield eryvarin F (**1**) (19 mg).

Table 2
NMR spectral data for eryvarin G (**2**) in $CDCl_3$

Position	^{1}H NMR	NOESY	^{13}C NMR	HMBC
2	8.26 *s*	6′	148.5	4, 9
3			144.0	
4			175.3	
5	7.73 *s*	1″	120.4	4, 7, 1″
6			120.0	
7			156.1	
8			117.2	
9			155.5	
10			117.6	
1′			140.3	
2′			150.4	
3′	6.50 *d* (2.9)		105.5	1′, 2′, 4′, 5′
4′			154.0	
5′	6.29 *dd* (8.8, 2.9)	6′	106.6	1′, 3′, 4′
6′	6.97 *d* (8.8)	2, 5′	121.8	1′, 2′, 4′
1″	6.41 *d* (9.5)	5, 2″	121.5	5, 7, 3″,
2″	5.77 *d* (9.5)	1″, 4″, 5″	132.2	6, 3″, 4″, 5″
3″			78.1	
4″	1.47 *s*	2″	28.4	2″, 3″, 5″
5″	1.47 *s*	2″	28.4	2″, 3″, 4″
1‴	3.49 *d* (7.3)	2‴, 4‴	21.9	7, 8, 2‴, 3‴
2‴	5.15 *t* (7.3)	1‴, 5‴	120.8	4‴, 5‴
3‴			132.6	
4‴	1.82 *s*	1‴	18.0	2‴, 3‴, 5‴
5‴	1.68 *s*	2‴	25.7	2‴, 3‴, 4‴
OH	4.99 *br s*			
OH	9.52 *br s*			

3.4. Eryvarin F (*1*)

Amorphous powder; UV (MeOH) λ_{max} nm (log ε): 203 (4.56), 246 *sh* (4.34), 288 *sh* (3.97), 309 (4.07); IR (KBr) ν_{max} cm^{-1}: 3400, 1630, 1600; ^{1}H and ^{13}C NMR (see Table 1); EIMS *m*/*z* (rel. int.): 422 ([M]$^+$, 100), 405

(9), 351 (8), 273 (15), 227 (9), 217 (13), 173 (10), 161 (18), 150 (11); HREIMS *m/z*: 422.1725 ($[M]^+$, calc. for $C_{25}H_{26}O_6$: 422.1728).

3.5. Methylation of *1*

A mixture of **1** (9.5 mg) and trimethylsilyldiazomethane (2.0 M solution in hexane) (0.5 ml) in MeOH (1 ml) was allowed to stand overnight at room temperature. After excess trimethylsilyldiazomethane was decomposed with a solution of CH_3COOH–MeOH (1:1), the solvent was removed. The resulting residue was purified using CC and C_6H_6–EtOAc (20:1) on a silica gel to yield trimethyl ether **1a** (3.9 mg, 37%) as an amorphous powder; UV (MeOH) λ_{max} nm (log ε): 203 (4.52), 238 (4.28), 280 (3.82), 312 (3.67); IR (KBr) ν_{max} cm^{-1}: 1650, 1600; ^1H NMR ($CDCl_3$): δ 1.69 (3H, *s*, H-5‴), 1.72 (3H, *s*, H-4″), 1.74 (3H, *s,* H-5″), 1.80 (3H, *s*, H-4‴), 3.42 (2H, *d*, *J* = 7.3 Hz, H-1″), 3.56 (2H, *d*, *J* = 6.6 Hz, H-1‴), 3.78 (3H, *s*, OMe-4′), 3.80 (3H, *s*, OMe-7), 3.86 (3H, *s*, OMe-2′), 5.19 (1H, *t*, *J* = 6.6 Hz, H-2‴), 5.30 (1H, *t*, *J* = 7.3 Hz, H-2″), 6.38 (1H, *dd*, *J* = 8.8, 2.9 Hz, H-5′), 6.56 (1H, *d,* *J* = 2.9 Hz, H-3′), 6.95 (1H, *d*, *J* = 8.8 Hz, H-6′), 7.83 (1H, *s*, H-2), 7.99 (1H, *s*, H-5); EIMS *m/z* (rel. int.): 464 ($[M]^+$, 100), 433 (60), 287 (48), 231 (37), 177 (20), 138 (12); HREIMS *m/z*: 464.2208 ($[M]^+$, calc. for $C_{28}H_{32}O_6$: 464.2197).

3.6. Eryvarin G (*2*)

Amorphous powder; UV (MeOH) λ_{max} nm (log ε): 204 (4.55), 268 (4.46), 331 (3.88), 346 *sh* (3.83); IR (film) ν_{max} cm^{-1}: 3500, 1630, 1600; ^1H and ^{13}C NMR (see Table 2); EIMS *m/z* (rel. int.): 420 ($[M]^+$, 81), 405 (100), 349 (12), 281 (15), 255 (20), 187 (10); HREIMS *m/z*: 420.1567 ($[M]^+$, calc. for $C_{25}H_{24}O_6$: 420.1571).

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