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CONSTITUENTS OF STEM BARK OF *ERYTHRINA ARBORESCENS*

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From the methanolic extracts of stem bark of *Erythrina arborescens* Roxb. (Leguminosae) eight components were obtained. The structure of these compounds were elucidated as squalene (1), phytosterols (2), oleanolic acid (3),alpinumisoflavone (4), erythrivarone C (5), warangalone (6), erythrinin C (7) and a mixture of phytosteroid glycosides (8) by the usual spectroscopic methods and 2D NMR techniques.

Key words: *Erythrina arborescens* Roxb., Leguminosae, Prenylated isoflavonoids.

INTRODUCTION

Erythrina arborescens Roxb. (Leguminosae) is a large deciduous tree which is widely distributed in India and Southern China.[1] This plant was introduced as an ornamental plant in Taiwan[1,2] and is used for relieving fever and inflammation, promotion of gastrointestinal absorption and for treating rheumatism in folk medicine.[3] Previously,[4] we described the isolation of five known triterpenoids and one protobeberine alkaloid from the leaves of *Erythrina arborescens* Roxb. (Leguminosae). Continuation of our studies on the stem bark of this plant has yielded four prenylatedisoflavonoids (**4** - **7**) together with known compounds (**1 – 3, 8**). In this paper, we reported the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

The eight constituents were obtained as described in the experimental section. Components **2** and **8** are stigmastane derivatives and were identified as campesterol (**2a**, 14.96%), stigmasterol (**2b**, 57.70%), *β*-sitosterol (**2c**, 27.34%), campesteryl-3-*O*-*β*-*D*-glucopyranoside (**8a**, 4%), stigmasteryl-3-*O*-*β*-*D*-glucopyranoside (**8b**, 74%) and

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β- sitosteryl-3-*O*-*β*-*D*-glucopyranoside (**8c**, 22%) by the spectroscopic methods[5] and GC analysis. In addition, squalene(**1**),[6] oleanolic acid (**3**),[5] alpinumisoflavone (**4**),[7] erythrivarone C (**5**),[8] and warangalone (**6**),[9] were also identified by comparing their spectral data with literature values. Compound **7**, $C_{20}H_{18}O_6$ ([M$^+$] at *m/z* 354), obtained as yellow needles, gave a positive ferric chloride test, but negative Mg-HCl test. The ^{1}H NMR spectrum showed an one-proton singlet at δ 8.10 assignable to the H-2 of an isoflavone skeleton[10] and characteristic signals for a hydroxyisopropyldihydrofuran [an AMX system at δ 3.29 (J = 15.6, 9.5 Hz), 3.56 (J = 15.6, 7.6 Hz) and δ 4.95 (J = 9.5, 7.6 Hz), two three-proton singlets at δ 1.40 and 1.51 and mass spectrum showed the base peak at *m/z* 295 (M$^+$-59)]].[11] The presence of an A_2X_2 system at δ 7.26 and 7.70 (each J = 9.0 Hz) of the 4'-oxygenated B ring indicated that the hydroxyisopropyldihydrofuran ring was fused on the A ring.[10] The chelated hydroxyl group at C-5 (δ 13.74) and a one-proton singlet at δ 6.49 indicated a hydroxyisopropyldihydrofuran ring fused with the A ring in the linear form but not in the angular form.[10] Furthermore, the orientation of the furan ring was confirmed by ^{1}H-^{1}H NOESY, HMQC and HMBC NMR techniques. The HMBC spectrum showed correlations between H-2 (δ 8.10) and C-3 (δ 123.78), H-2 and C-4 (δ 181.43), H-2 and C-9 (δ 158.48), H-8 (δ 6.49) and C-6 (δ 110.10), H-8 and C-7 (δ 167.22), H-8 and C-9 (δ 158.48), H-8 and C-10 (δ 106.79), H-2" (δ 4.95) and C-7 (δ 167.22), H-2" and C-3" (δ 27.71), H-3"a (δ 3.29) and C-6 (δ 110.10), H-3"a and C-7 (δ 167.22), H-3"a and C-3" (δ 27.71), H-3"a and C-4" (δ 70.92), H-3"b (δ 3.56) and C-6 (δ 110.10), H-3"b and C-7 (δ 167.22), H-3"a and C-4" (δ 70.92), C-5-OH (δ 13.74) and C-5 (δ 157.62), C-5-OH and C-6 (δ 110.10), and C-5-OH and C-10 (δ 106.79) (Table 1). These correlations clearly confirmed that the hydroxyisopropyldihydrofuran ring was fused in a linear form. Consequently, **7** was deduced to be erythrinin C (5,4'-dihydroxy-5"-(hydroxyisopropyl) dihydrofuran [3",2":6,7] isoflavone) (**7**)[12]. Its ^{13}C NMR and 2D NMR spectral data are reported here for the first time. The known triterpenoids, squalene (**1**) and oleanolic acid (**3**) together with pre-nylated flavonoids, alpinumisoflavone (**4**), erythrivarone C (**5**), warangalone (**6**) anderythrinin C (**7**), have not previously been reported from this plant.

Table 1. HMBC Correlations and NOESY of Erythrinin C (7)

H	Carbon correlated	NOESY
H-2	3, 4, 9	H-2',6'
H-8	6, 7, 9, 10	
H-2',6'	3, 4'	H-2, 3', 5'
H-3',5'	1', 4'	H-3', 6'
H-2"	7, 3"	H-3"a, 5", 6"
H-3"a	6, 7, 4"	H-2", 3"b
H-3"b	6, 7, 2", 4"	H-2", 3"b
H-5"	2", 4", 6"	H-3"a, 5", 6"
H-6"	2", 4", 5"	H-2", 3"b
5-OH	5, 6, 10	

4'-OH	3', 5'

EXPERIMENTAL

General Method

Melting points were measured on a Yanaco micro melting-point apparatus and were uncorrected. IR spectra were recorded on Jasco A-302 or Hitachi I-2001 infrared spectrometers. EIMS spectra were recorded on a Jeol JMX-SX/SX 102A mass spec-trometer. ^{1}H NMR and ^{13}C NMR, COSY, HMQC, HMBC and DEPT spectra were recorded on Varian VXR-300, 600 or Bruker TMX 400 spectrometers with TMS as an internal standard. UV spectra were recorded on a Shimadzu UV 260 ultraviolet spectrometer. GC spectra were obtained on a Hewlett Packard 5890 series II gas chromatograph. Optical rotations were measured on a Perkin Elmer 241 MC digital polarimeter.

Plant Material

The stem bark of *Erythrina arborescens* Roxb. (Leguminosae) was collected at Taichung, Taiwan, in June, 1996.

The plant was identified by Mr. Nien-Yung Chiu (Institute of Chinese Pharmaceutical Sciences, China Medical College).

Extraction and Separation

The air-dried stem bark of *Erythrina arborescens* Roxb. (Leguminosae) (4.40 Kg) was extracted with boiling methanol. The extracts were combined and concentrated *in vacuo*. The residue (207.1 g) was suspended in methanol-water (1 L, 1:9, v/v) solution and then extracted with chloroform to give chloroform solution and aqueous solution. The concentrated chloroform extract (111.7 g) was subjected to silica gel column chromatography using a mixture of $CHCl_3$-MeOH of increasing solvent polarity as eluent to give 10 fractions. Fractions 1-3 were combined and purified by repeated column chromatography on silica gel with *n*-hexane-ethyl acetate (19:1) to afford squlene (**1**, 20 mg), phytosterols (**2**, 136 mg) and oleanolic acid (**3**, 18 mg), respectively. Fractions 4-7 were rechromatographed on a silica gel column with *n*-hex-ane and increasing concentrations of ethyl acetate to yield alpinumisoflavone (**4**, 779 mg), erythrivarone C (**5**, 23 mg), warangalone (**6**, 28 mg) and erythrinin C (**7**, 482 mg), respectively. Fractions 9 were rechromatographed on silica gel with $CHCl_3$-MeOH of increasing polarity to give phytosteryl glucosides (**8**, 483 mg).

Squalene (1)

Colorless oil, IR (KBr) cm^{-1}: 2870, 1440, 1375, 840; ^{1}H-NMR (300 MHz, $CDCl_3$):δ 1.60 (12 H, *s*, Me x 4), 1.67 (12 H, *s*, Me x 4), 2.00 (20H, *m*, CH_2 x 10), 5.11 (6H, *t, J* = 6.1 Hz, C=C-H x 6); EIMS *m/z* (rel. int): 410 [M^+] (10), 341 (16), 273 (8), 205 (8), 137 (76), 84 (100), 69 (64).

phytosterols (2)

Colorless needles from $CHCl_3$/MeOH; mp: 149-151℃; Liebermann-Burchard (L.B.) test: positive; IR (KBr) cm^{-1}: 3426 (OH), 1598 (C = C); ^{1}H NMR (300 MHz, $CDCl_3$):δ 0.70 (*s*, H-18), 1.01 (*s*, H-19), 3.53 (*m*, H-3α), 5.01 (*dd*, *J* = 15.4, 8.4 Hz, H-23), 5.15 (*dd*, *J* = 15.4, 8.4 Hz, H-22), 5.35 (*d, J* = 5.4 Hz , H-6); EIMS *m/z* (rel. int): 414 $[M]^+$ (100), 412 $[M]^+$ (18), 400 $[M]^+$ (53); The GC was run under the following conditions : HP-5 capillary column (30 m × 0.25 μm × 0.32 mm), carrier gas N_2 19.1 mL/min, column temperature 290℃, injection and FID temperature 300℃. Mixture **1** was identified as campestesterol (**2a**, t_R = 10.92 min, 14.96%); stigmasterol (**2b**, t_R = 11.31 min, 57.70%) and *β*-sitosterol (**2c**, t_R = 12.12 min, 27.34%) by coinjection with an authentic sample.

Oleanolic acid (3)

Colorless needles from $CHCl_3$/MeOH; mp >300℃; $[\alpha]_D$ +58.18^o ($CHCl_3$, c = 0.17); L.B. test: positive; IR (KBr) cm^{-1}: 3615 (OH), 2945, 1682 (COOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75 (3H, s, H-24), 0.77 (3H, s, H-26), 0.90 (3H, *s*, H-23), 0.92 (3H, *s*, H-29), 0.95 (3H, *s*, H-30), 0.98 (3H, *s*, H-25), 1.13 (3H, *s*, H-27), 2.84 (1H, *dd, J*=13.7, 3.5 Hz, H-18), 3.22 (1H, *dd, J* = 10.2, 5.1, Hz, H-3 α), 5.28 (1H, *t, J* = 3.6 Hz, H-12); ^{13}C-NMR (75 MHz, $CDCl_3$-CD_3OD): δ 37.89 (*t*, C-1), 26.12 (*t*, C-2), 79.08 (*d*, C-3),39.82 (*s*, C-4), 55.39(*d*, C-5),18.44 (*t*, C-6), 32.99 (*t*, C-7), 39.42 (*s*, C-8), 46.73 (*d*, C-9), 34.17 (*s*, C-10), 23.09 (*t*, C-11), 122.80 (*d*, C-12),143.20 (*s*, C-13), 41.08 (*s*, C-14), 28.12 (*t*, C-15), 23.73 (*t*, C-16), 46.68 (*s*, C-17), 39.46 (*d*, C-18), 45.05 (*t*, C-19), 32.48 (*s*, C-20), 33.28 (*t*, C-21), 28.23 (*t*, C-22), 30.84 (*q*, C-23), 15.50 (*q*, C-24), 15.63 (*q*, C-25), 17.16 (*q*, C-26), 26.99 (*q*, C-27),182.23 (*s*, C-28), 33.05 (*q*, C-29), 23.87 (*q*, C-30); EI MS *m/z* (rel. int): 456 [M^+] (4), 248 (100), 203 (58), 189 (11), 133 (10).

Alpinumisoflavone (4)

Yellow needles from chloroform-acetone, mp: 213-214℃, IR (KBr) cm^{-1} : 3415 (OH), 2935, 1710, 1468, 1385, 1048 cm^{-1} ; UV (MeOH) λ_{max} nm (log ε) : 228 (4.16), 283 (4.56), 340 (3.26); ^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD): δ 1.47 (6H, *s*, H-5'', 6''), 5.63 (1H, *d, J* = 10.2 Hz, H-3''), 5.80 (1H, *s*, 4'-OH), 6.34 (1H, *s*, H-8), 6.73 (1H, *d, J* = 10.2 Hz, H-4''), 6.89 (2H, *d, J* = 8.7 Hz, H-3', 5'), 7.39 (2H, *d, J* = 8.7 Hz, H-2', 6'), 7.81 (1H, *s*, H-2), 13.08 (1H, *s*, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD): δ 152.71 (C-2), 122.92 (C-3), 181.22 (C-4), 157.03 (C-5), 106.75 (C-6), 157.50 (C-7), 95.04 (C-8), 156.16 (C-9), 105.73 (C-10), 123.86 (C-1'), 130.41 (C-2', 6'), 115.88 (C-3', 5'), 159.70 (C-4'), 78.24 (C-2''), 128.28 (C-3''), 115.54 (C-4''), 28.29 (C-5'', 6''); EIMS *m/z* (rel. int) : 336 $[M]^+$ (30), 322 (22), 321 (100), 203 (6), 149 (35), 57 (70).

Erythrivarone C (5)

Yellow needles from $CHCl_3$-MeOH, mp 202-203℃, $[\alpha]_D$ +11.2° (MeOH; c = 0.08); IR (KBr) cm^{-1}: 3489 (OH), 2977, 1640 (C = O), 1606, 1517, 1433, 1223, 1026, 833; UV (MeOH) λ_{max} nm (log ε) : 227 (4.04), 287 (3.86), 340 (3.33); ^{1}H NMR (600 MHz, acetone-d_6): δ 1.51 (6H, *s*, H-5", 6"), 1.86 (3H, *s*, H-5'''), 2.95 (1H, *dd, J* = 13.8, 7.8 Hz, H-1'''a), 2.99 (1H, *dd, J* = 13.8, 6.0 Hz, H-1'''b), 4.37 (1H, *dd, J* = 7.8, 6.0 Hz, H-2'''), 4.69 (1H, *br s*, H-4'''a), 4.77 (1H, *br s*, H-4'''b), 5.78 (1H, *J* = 10.2 Hz, H-3"), 6.70 (1H, *d, J* = 10.2 Hz, H-4"), 6.91 (2H, *d, J* = 8.4 Hz, H-3', 5'), 7.48 (2H, *d, J* = 8.4 Hz, H-2', 6'), 8.27 (1H, s, H-2), 13.46 (1H, *s*, 5-OH); ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD): δ 151.84 (*d*, C-2), 120.88 (*s*, C-3), 180.65 (*s*, C-4), 154.34 (*s*, C-5), 104.89 (*s*, C-6), 156.35 (*s*, C-7), 103.60 (*s*, C-8), 154.70 (*s*, C-9), 104.23 (*s*, C-10), 122.68 (*s*, C-1'), 129.33 (*d*, C-2', 6'), 114.62 (*d*, C-3', C-5'), 156.17 (*s*, C-4'), 77.45 (*s*, C-2"), 127.14 (*d*, C-3"), 114.78 (*d*, C-4"), 27.36 (*q*, C-5", 6"), 27.85 (*t*, C-1'''), 74.52 (*d*, C-2'''), 146.04 (*s*, C-3'''), 110.25 (*t*, C-4'''), 16.48 (*q*, C-5'''); EIMS *m/z* (rel. int):420 $[M]^+$(20), 349 (100), 331 (7), 295 (4), 231 (10), 118 (20).

Warangalone (6)

Yellow needles from methanol-acetone; mp : 161-162℃; IR (KBr) cm^{-1} : 3430 (OH), 3074, 1652 (C = O), 1572; UV (MeOH) λ_{max} nm (log ε) : 226 (4.22), 287 (4.54) ; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.48 (3H, *s*, H-5"), 1.56 (3H, *s*, H-6"), 1.68 (3H, *d, J* = 1.2 Hz, H-4'''), 1.81 (3H, *s*, H-5'''), 3.38 (2H, *d, J* = 7.5 Hz, H-1'''), 5.18 (1H, *tq*, *J* = 7.5, 1.2 Hz, H-2'''), 5.60 (1H, *d, J* = 10.2 Hz, H-3"), 6.72 (1H, *d, J* = 10.2 Hz, H-4"), 6.90 (2H, *d*, *J* = 8.4 Hz, H-3', 5'), 7.40 (2H, *d, J* = 8.4 Hz, H-2', 6'), 7.88 (1H, *s*, H-2), 8.20 (1H, *s*, 4'-OH), 13.10 (1H, *s*, 5-OH); ^{13}C NMR (75 MHz, CD_3Cl_3): δ 155.40 (*d*, C-2), 123.78 (*s*, C-3), 182.27 (*s*, C-4), 155.81 (*s*, C-5), 105.92 (*s*, C-6), 157.47 (*s*, C-7), 108.32 (*s*, C-8), 155.52 (*s*, C-9), 105.86 (*s*, C-10), 123.04 (*s*, C-1'), 131.19 (*d*, C-2', 6'), 115.95 (*d*, C-3', 5'), 158.54 (*s*, C-4'), 78.73 (*s*, C-2"), 129.24 (*d*, C-3"), 116.22 (*d*, C-4"), 28.27 (*q*, C-5", 6"), 21.78 (*t*, C-1'''), 122.91 (*d*, C-2'''), 132.12 (*s*, C-3'''), 17.92 (*q*, C-4'''), 25.80 (*q*, C-5'''); EIMS *m/z* (rel. int.): 404 $[M]^+$ (48), 389 (100), 361 (25), 349 (38), 321 (16), 215 (9). 118 (22).

Erythrinin C (7)

Yellow needles from methanol-chloroform; mp:218-219℃; IR (KBr) cm^{-1}:3450 (OH), 1675 (C = O), 1618, 1565; UV (MeOH) λ_{max} (log ε):216 (3.85), 267 (3.92) nm; ^{1}H-NMR (400 MHz, pyridine–d_5): δ 1.40 (3H, *s*, H-5"), 1.51 (3H, *s*, H-6"), 3.29 (1H, *dd*, *J*=15.6, 9.5 Hz, H-3"a), 3.56 (1H, *dd*, *J* = 15.6, 7.6 Hz, H-3"b), 4.95 (1H, *dd*, *J* = 9.5, 7.6 Hz, H-2"), 6.49 (1H, *s,* H-8), 7.26 (2H, *d, J* = 9.0 Hz, H-3', 5'), 7.70 (2H, *d, J* = 9.0Hz, H-2', 6'), 8.10 (1H, *s*, H-2), 11.77 (1H, *s*, 4'-OH), 13.74 (1H, *s*, 5-OH); ^{13}C NMR (100 MHz, pyridine–d_5) : δ 25.83 (C-5"), 26.08 (C-6"), 27.71 (C-3"), 70.92 (C-4"), 89.04 (C-8), 92.93 (C-2"), 106.79 (C-10), 110.10 (C-6), 116.27 (C-3', 5'), 122.29 (C-1'), 123.77 (C-3), 131.12 (C-2', 6'), 153.34 (C-2), 157.62 (C-5), 158.48 (C-9), 159.38 (C-4'), 167.22 (C-7), 181.43 (C-4); EIMS *m/z* (rel. int): 354 $[M^+]$ (74), 334 (18), 321 (42), 295 (100). 118 (7).

Phytosteryl-3-*O*-*β*-*D*-glucosides (8)

Colorless granules from $CHCl_3$-MeOH, mp > 300℃, IR (KBr) cm^{-1}: 3410 (OH), 1570 (C = C); ^{1}H NMR (300 MHz, pyridine-d_5) :δ 0.63 (*s*, H-18), 1.02 (*s*, H-19), 5.02 (*d, J* = 7.8 Hz, H-1'), 5.37 (*d, J* = 3.1 Hz, H-6); EIMS *m/z* (rel. int): 396 (100); Positive ion FABMS (matrix NBA) *m/z* (rel. int.): 577 (8), 397 (26), 154 (NBA+1,100). Mixture **8** (10 mg) was hydrolyzed with 10 % HCl-EtOH (10 mL) for 6 h and worked up as usual. The residue was recrystallized from $CHCl_3$-MeOH to yield colorless needles and identified as campesterol (**8a**, t_R = 10.77 min, 3.52 %), stigmasterol (**8b**, t_R = 11.21 min, 74.40 %) and *β*-sitosterol (**8c**, t_R = 12.95 min, 22.08 %) by GLC analysis under the conditions described above. The aqueous layer was characterized as *D*-glucose by paper chromatography with authentic *D*-glucose. Thus, mixture **8** was identified as campesteryl-3-*O*-*β*-*D*-glucopyranoside (**8a**, 4 %); stigmasteryl-3-*O*-*β*-*D*-glucopyrano-side (**8b**, 74 %) and *β*-sitosteryl-3-*O*-*β*-*D*-glucopyranoside (**8c**, 22 %).

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大葉刺桐莖皮成分之研究

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本研究由大葉刺桐 (*Erythrina arborescens* Roxb.) 莖皮部分離得到八個成分，經光譜分析確定其結構分別為 squalene (**1**), phytosterols (**2**), oleanolic acid (**3**), alpinumisoflavone (**4**), erythrivarone C (**5**), warangalone (**6**), erythrinin C (**7**) 及 phytosteroid glycosides (**8**)。

關鍵字：大葉刺桐，豆科，異戊烯基異黃酮。

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