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A NEW PTEROCARPAN FROM *ERYTHRINA FUSCA*

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Abstract - A new pterocarpan, 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan (**1**), together with seven known compounds, sandwicensin (**2**), erythrisenegalone (**3**), citflavanone (**4**), liquiritigenin (**5**), lonchocarpol A (**6**), lupinifolin (**7**) and 8-prenyldaidzein (**8**), were isolated from the stem bark of *Erythrina fusca*. The structure of **1** was determined on the basis of spectroscopic analyses. Among these compounds, lonchocarpol A (**6**) exhibited strongly antibacterial activities *in vitro* against *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*. Compounds (**2**, **3**, **6** and **7**) exhibited weakly antimycobacterial activity with minimum concentrations (MICs) of 100, 50, 50 and 25 μg/mL, respectively.

INTRODUCTION

The genus *Erythrina* (Papilionaceae) comprises over 100 species distributed in tropical and subtropical regions.[1] It is known to produce erythrinan alkaloids, flavonoids, isoflavonoids, and pterocarpans.[2-5] Some of these compounds have been found to display a wide varieties of biological activities, such as behavioral depression, muscle relaxant, antihypertensive and antimicrobial activities against Gram-positive bacteria.[6] The stem bark of *Erythrina fusca* (Thai name: Thong long) was collected from Pathumthani province, Thailand. This is the first reported on the presence of 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan (**1**) and antimicrobial activity study of four pure compounds (**1**, **3**, **4** and **7**).

RESULTS AND DISCUSSION

The hexane and EtOAc extracts from the stem bark of *E. fusca* have antimicrobial activity *in vitro* against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Enterococcus faecalis* ATCC 29212, but inactive with *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231. The compounds from hexane and EtOAc extracts of this plant were separated by silica gel column chromatography and reverse phase HPLC, yielding a new 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan (**1**), together with sandwicensin (**2**),[7] erythrisenegalone (**3**),[8] citflavanone (**4**),[9] liquiritigenin (**5**),[10] lonchocarpol A (**6**),[11] lupinifolin (**7**)[12] and 8-prenyldaidzein (**8**)[13] (**Figure 1**). Spectral data of compounds (**2-8**) are in good agreements with those published values.[7-13]

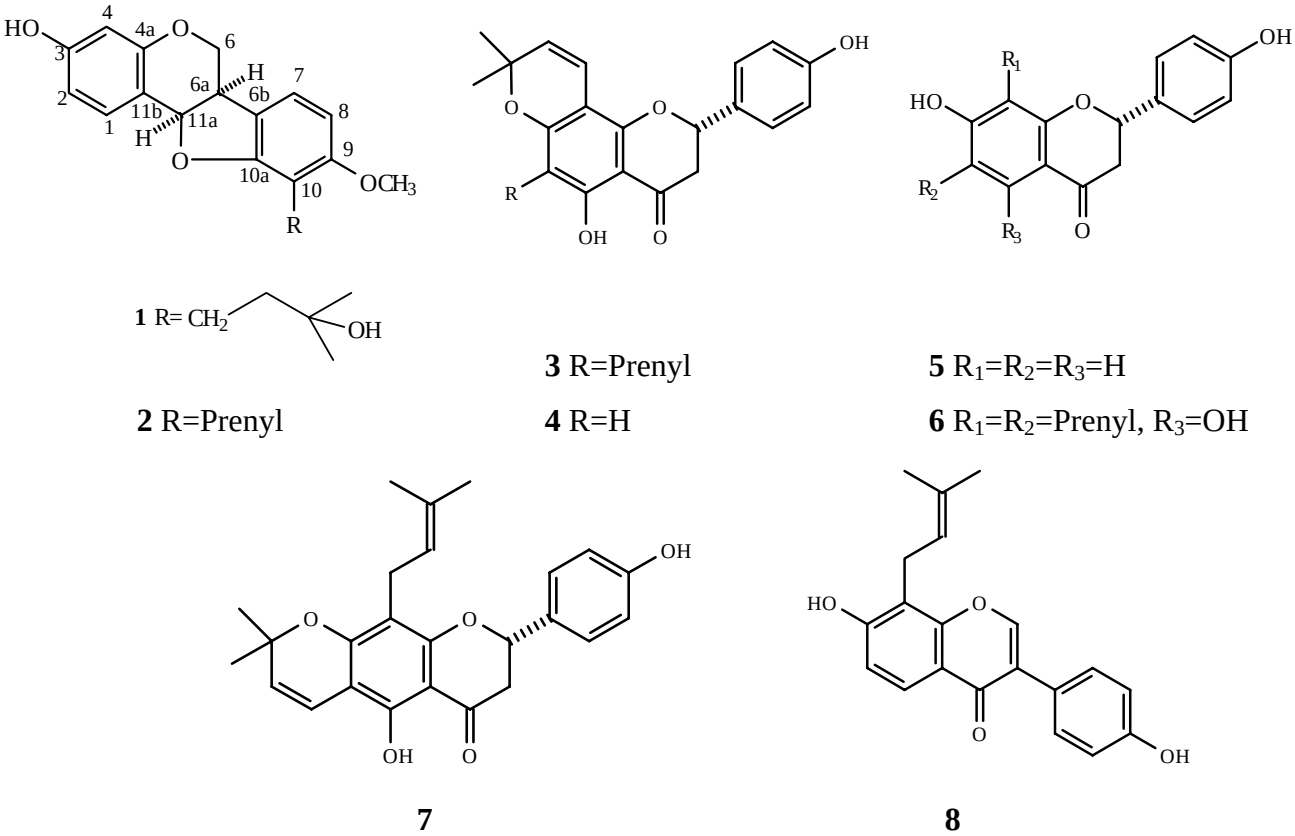

Figure 1. The structure of compounds (**1-8**)

Compound (**1**) was obtained as a brown oil and the molecular formula was confirmed to be $C_{21}H_{24}O_5$ by the ESITOFMS. The ^{1}H NMR spectra (δ 3.50, 3.60, 4.20 and 5.44) indicated it to be a pterocarpan derivative. The ^{1}H NMR spectrum of **1,** five aromatic protons (δ 6.36, 6.40, 6.50, 7.00 and 7.37) on rings A and D, and oxymethylene protons (δ 3.60 and 4.20), and two methine protons (δ 3.50 and 5.44) were assigned by comparison of the ^{1}H NMR and ^{13}C NMR spectra with those of sandwicensin.[7] The remaining signals were assignable to two methylene groups (δ 1.70 and 2.67), two methyl groups (δ 1.21 and 1.22) on a carbinol carbon. These partial structures were fully compatible with a 3-hydroxy-3-methylbutyl side chain as shown by

the ^{13}C NMR spectral assignments (δ 18.4, 29.0, 29.1, 42.4, 55.9 and 71.3) of **1** and comparison with the reported ^{1}H NMR spectra data. The assignment of the side chain moiety at the C-10 (δ 114.0) position was confirmed from the HMBC experiment (**Figure 2**), revealing that the aliphatic protons at C-1' (δ 2.67) correlated with carbons at C-9 (δ 158.5), C-10 (δ 114.0) and C-10a (δ 158.4). The remaining methoxyl group was located at the C-9 position as deduced from the HMBC spectrum (**Figure 2**), indicating correlation between C-9 (δ 158.5) and the methoxyl proton (δ 3.79). The unambiguous assignment of all the ^{1}H NMR and ^{13}C NMR signals of **1** was accomplished by analyses of its HMQC, HMBC and NOESY spectra, the details of NMR spectral assignment are shown in experimental section. The absolute stereochemistry at C-6a and C-11a was deduced as *R* from the negative optical rotation, and therefore 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan (**1**) (6a*R* and 11a*R*).[14]

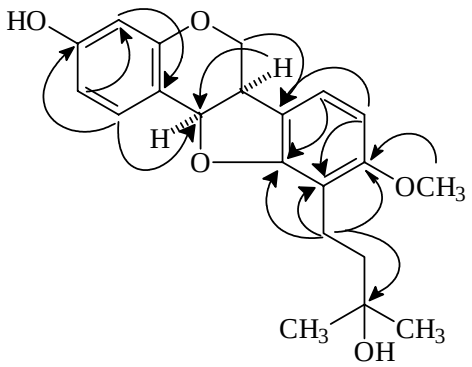

Figure 2. Selected HMBC correlations observed of **1**

Table 1. *In vitro* antimicrobial activity of pure compounds from *Erythrina fusca*

Compound	*Staphylococcus aureus*		*Bacillus subtilis*		*Enterococcus faecalis*	
	MIC[a]	MBC[b]	MIC	MBC	MIC	MBC
1	> 100	> 100	> 100	> 100	> 100	> 100
2	50	> 100	100	100	50	50
3	> 100	> 100	50	50	100	100
4	12.5	> 100	12.5	12.5	> 100	> 100
6	6.25	> 100	3.125	3.125	6.25	6.25
7	12.5	> 100	6.25	6.25	50	50
Tetracycline	< 0.25	< 0.25	16	16	0.125	0.125

[a] The minimum inhibitory concentration in μg/mL.

[b] The minimum bactericidal concentration in μg/mL.

Biological activities of 3-hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan (**1**), sandwicensin (**2**), erythrisenegalone (**3**), citflavanone (**4**), lonchocarpol A (**6**) and lupinifolin (**7**) were tested *in vitro* for their antimicrobial activities using an agar streak-dilution technique.[15] Lonchocarpol A (**6**) exhibited strongly antibacterial activities (**Table 1**) *in vitro* against *S. aureus*, *B. subtilis* and *E. faecalis*, and inactive with *E. coli* and *C. albicans*. Compounds (**2**, **3, 6** and **7**) were tested *in vitro* for their antimycobacterial activities against *Mycobacterium tuberculosis* H37Ra. These compounds showed weakly antimycobacterial activities with MIC 100, 50, 50 and 25 μg/mL, respectively.

EXPERIMENTAL

General Experimental Procedures. The UV and IR spectra were recorded on a Milton Roy Spectronic 3000 Array spectrophotometer and Perkin-Elmer FT-IR 2000 spectrophotometer, respectively. The optical rotation was measured on a Perkin-Elmer Polarimeter 341. The ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC spectra were obtained from a Varian Unity 400 NMR spectrometer, operating at 400 MHz for proton and 100 MHz for carbon, or a JEOL JMN-A 500 NMR spectrometer, operating at 500 MHz for proton and 125 MHz for carbon. The EIMS spectra were obtained from a JEOL JMS-AX505HA spectrometer. The HR-EIMS, HR-FABMS spectra were performed on a JEOL JMS-700 Mstation spectrometer and the ESITOFMS spectra were obtained from a Micromass LCT mass spectrometer.

Plant Material. The stem bark of *Erythrina fusca* Lour. was collected from Muang District, Pathumthani Province, Thailand, in February 2002. Authentication was achieved by comparison with the herbarium specimen (BKF No. 112379) at the Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand. A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and Isolation. The pulverized dried stem bark of *E. fusca* (5.1 kg) were macerated 4 times for each solvent at room temperature with n-hexane (*ca.* 14 L for each), EtOAc (*ca.* 14 L for each) and 95% EtOH (*ca.* 14 L for each) for 3 days, respectively. The concentrated hexane extract (15 g) was separated by flash column chromatography on silica gel with gradient solvent of hexane, $CHCl_3$ and MeOH to give 17 fractions (300 mL each). Fraction 11 was rechromatographed on silica gel column with $CHCl_3$, followed by semi-preparative HPLC (C_{18} reversed-phase column, eluted with MeCN : H_2O (85 : 15) and 0.05% TFA) and repeatedly purified by PTLC ($CHCl_3$: MeOH, 95 : 5) to give **1** (2.7 mg) and **2** (159 mg). The EtOAc extract (150 g) was separated by vacuum liquid column chromatography on silica gel with gradient solvent of n-hexane, $CHCl_3$ and MeOH to give 22 fractions (500 mL each). Fraction 9 (10 g) was purified by flash column chromatography on silica gel with gradient solvent of hexane, $CHCl_3$ and MeOH to give 13 fractions (300 mL each) and fraction 7 was rechromatographed on silica gel column with $CHCl_3$: MeOH (95 : 5), followed by semi-preparative HPLC (C_{18} reversed-phase column, eluted with MeCN : H_2O (85:15) and 0.05% TFA) and

repeatedly purified by PTLC (with $CHCl_3$: MeOH, 95 : 5) to give **3** (17 mg) , **4** (2 mg), **6** (319 mg) and **7** (200 mg). Fraction 15 (7 g) was purified by flash column chromatography on silica gel with gradient solvent of $CHCl_3$ and MeOH to give 10 fractions (300 mL each) and fraction 6 was rechromatographed on silica gel column with $CHCl_3$: MeOH (95 : 5), followed by semi-preparative HPLC (C_{18} reversed-phase column, eluted with MeCN : H_2O (85 : 15) and 0.05% TFA) and repeatedly purified by PTLC (with $CHCl_3$: MeOH, 95 : 5) to give **5** (1 mg) and **8** (1 mg).

3-Hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan (1): Brown oil; $[\alpha]_D^{20}$ – 317.8° (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 219(1.85), 285(1.41); IR (neat) ν_{max} cm^{-1} : 3400, 1621; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.37 (1H, d, *J*=8.0 Hz, H-1), 7.00 (1H, d, *J*=8.0 Hz, H-7), 6.50 (1H, dd, *J*=8.5, 2.5 Hz, H-2), 6.40 (1H, d, *J*=8.0 Hz, H-8), 6.36 (1H, d, *J*=2.5 Hz, H-4), 5.44 (1H, d, *J*=7.0 Hz, H-11a), 4.20 (1H, dd, *J*=11.0, 5.5 Hz, H-6), 3.79 (3H, s, OMe), 3.60 (1H, t, *J*=11.0 Hz, H-6), 3.50 (1H, ddd, *J*=11.0, 7.0, 5.5 Hz, H-6a), 2.67 (2H, t, *J*=8.0 Hz, H-1'), 1.70 (2H, m, H-2'), 1.22 (3H, s, H-5'), 1.21 (3H, s, H-4'). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 158.5 (C-9), 158.4 (C-10a), 157.1 (C-3), 156.6 (C-4a), 132.3 (C-1), 121.7 (C-7), 119.2 (C-6b), 114.0 (C-10), 112.7 (C-11b), 109.7 (C-2), 103.6 (C-4), 103.0 (C-8), 78.1 (C-11a), 71.3 (C-3'), 66.5 (C-6), 55.9 (OMe), 42.4 (C-2'), 39.9 (C-6a), 29.1 (C-4'), 29.0 (C-5'), 18.4 (C-1'). EIMS *m/z* (rel. int.): 356 ($[M]^+$, 43), 338 (100), 282 (77), 267 (22), 253 (20); ESITOF MS *m/z* 379.1523 $(M + Na)^+$, calcd for $(C_{21}H_{24}O_5 + Na)^+$ 379.1514.

Antimicrobial activity. Compounds (**1**, **2**, **3**, **4**, **6** and **7**) were tested against *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 by broth microdilution test.[15] The lowest concentration of the test compounds which inhibit growth and kill these microorganisms were defined as the MIC and MBC respectively. Tetracycline and nystatin were as positive control for antibacterial and anticandidal activities, respectively.

***In vitro* antimycobacterial activity.** Antimycobacterial activity of **2**, **3**, **6** and **7** was performed by a microplate alamar blue assay.[16] *M. tuberculosis* H37Ra was used as a tested microorganism. The minimum inhibitory concentrations (MICs) of the tested compounds were measured in μg/mL.

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