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Biological Activities of the Chemical Constituents of *Erythrina stricta* and *Erythrina subumbrans*

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Phytochemical investigation of the hexane and CH_2Cl_2 extracts of *Erythrina stricta* roots and *E. subumbrans* stems led to the isolation of six pterocarpans, one flavanone, one isoflavone, two alkaloids, five triterpenes, six steroids and alkyl *trans*-ferulates. The structures of all known compounds were determined on the basis of spectroscopic evidence. Sophoradiol (**15**), a mixture of stigmast-4-en-3-one (**19**) and stigmasta-4,22-dien-3-one (**20**), lupeol (**21**), cycloeucalenol (**22**), a mixture of 3β-hydroxystigmast-5-en-7-one (**23**) and 3β-hydroxystigmast-5,22-dien-7-one (**24**) and melilotigenin C (**25**) were first isolated from the genus *Erythrina*. The isolated compounds were evaluated for antiplasmodial activity, antimycobacterial activity and cytotoxicity. Among the tested compounds, 5-hydroxysophoranone (**8**) exhibited the highest antiplasmodial activity against *Plasmodium falciparum* (IC_{50} 2.5 μg/mL). Compound **8**, erystagallin A (**5**), erycristagallin (**7**) and erysubin F (**10**) showed the same level of antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 12.5 μg/mL). For cytotoxicity, erybraedin A (**2**) showed the highest activity against the NCI-H187 and BC cells (IC_{50} 2.1 and 2.9 μg/mL, respectively), whereas **10** exhibited the highest activity against the KB cells (IC_{50} 4.5 μg/mL).

Key words: *Erythrina stricta*, *Erythrina subumbrans*, Leguminosae, Antiplasmodial activity, Antimycobacterial activity, Cytotoxicity

INTRODUCTION

The genus *Erythrina* belongs to the family Leguminosae and comprises over 100 species, of which 6 are known to occur in Thailand. Previous investigations of *Erythrina species* have led to the isolation of several phenolic metabolites, such as pterocarpans, isoflavones, flavanones and chalcones, some of which displayed antiplasmodial activity (Yenesew *et al.*, 2003, 2004; Andayi *et al.*, 2006), antimycobacterial activity (Khaomek *et al.*, 2004) and cytotoxic activity against various cancer cell lines (Nkengfack *et al.*, 2001; El-Masry *et al.*, 2002).

Erythrina stricta Roxb. is used for various ailments (Chopra *et al.*, 1956), while the bark of *Erythrina subumbrans* Merr. is claimed to treat coughs, post partum vomiting and as poultices (Mitscher et al., 1987). *E. stricta* has been reported to contain alkaloids, erythraline, erysodine, erythrinine, erysopine, 11-hydroxyerysodine, 11-hydroxyerysovine, erysovine, hypaphorine and 11-acetylerysotrine, 7-methoxy-8-(15-hydroxypentadecyl)-coumarin, alkyl ferulates and other known compounds (Games *et al.*, 1974; Singh *et al.*, 1981; Hussain, 2002). Alkaloids erythramine, hypaphorine, erysodine and erysopine have also been reported from *E. subumbrans* (Folkers and Koniuszy, 1939; Folkers *et al.*, 1941). As part of our ongoing project on bioactive compounds from Thai medicinal plants for the treatment of tropical diseases, we have investigated these plant species. In the earlier report, we described the isolation of seven pterocarpans, erybraedin B (**1**), erybraedin A (**2**), phaseollin (**3**), erythrabyssin II (**4**), erystagallin A (**5**), erythrabissin-1 (**6**) and erycristagallin (**7**), two flavanones, 5-hydroxysophoranone (**8**) and glabrol (**9**), and one isoflavone, erysubin F (**10**) from the stems of *E. subumbrans*, some of which showed very high antibacterial activity against MRSA and VRSA clinical strains (Rukachaisirikul *et al.*, 2007). In the present paper, we wish to report the isolation of chemical constituents from *E. stricta* roots, the re-isolation of chemical constituents from *E. subumbrans* stems as well as the results of the antiplasmodial, antimy-

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cobacterial and cytotoxic evaluations on some isolates.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined on an Electrothermal apparatus and are uncorrected. Optical rotations were obtained using a JASCO-1020 polarimeter. UV spectra were measured with a Perkin Elmer Lamda 20 spectrophotometer. IR spectra were obtained using a Perkin-Elmer FT-IR 2000 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer, operating at 400 and 100 MHz, respectively. Electrospray mass spectra were recorded with Finnigan LC-Q mass spectrometer. Column chromatography and TLC were carried out using Merck silica gel 60 (finer than 0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent, followed by heating.

Plant material

E. stricta roots and *E. subumbrans* stems were collected from Maetaeng district, Chiang Mai province and Kangkajan national park, Kangkajan district, Phetchaburi province, Thailand, respectively. Voucher specimens of *E. stricta* (BKF 93574) and *E. subumbrans* (BKF 63781) have been deposited at the herbarium of the Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok.

Extraction and isolation

The air-dried, powdered *E. stricta* roots and *E. subumbrans* stems (1 kg each) were Soxhlet extracted successively with *n*-hexane and CH_2Cl_2. The hexane and CH_2Cl_2 extracts were filtered and concentrated until dryness under reduced pressure.

The hexane extract (5.9 g) of *E. stricta* roots was subjected to a quick CC (silica gel; hexane-EtOAc gradient) to give 20 fractions (H1-H20). Fr. H12 (1.5 g) was further fractionated by CC (silica gel; EtOAc-hexane, 15:85) to furnish 7 fractions (H21-H27). Fr. H22 (556 mg) was purified by repeated CC (silica gel; acetone-hexane, 10:90) to afford **11** (7 mg) whereas fr. H23 yielded a mixture of **12** and **13** (84 mg). Fr. H14 (255 mg) was separated by repeated CC (silica gel; EtOAc-hexane, 20:80) to give 7 fractions (H28-H34). Compounds **5** (30 mg) and **6** (5 mg) were obtained from frs. H29 and H31, respectively.

The CH_2Cl_2 extract (27.1 g) of *E. stricta* roots was subjected to a quick CC (silica gel; hexane-EtOAc gradient) to afford 20 fractions (C1-C20). Fr. C6 (1.0 g) was further purified by repeated CC (silica gel, EtOAc-hexane, 15:85) to give 5 fractions (C21-C25). Compounds **8** (10 mg) and **14** (29 mg) were obtained from frs. C22 and C24, respectively. Fr. C8 (780 mg) was separated by CC (silica gel; CH_2Cl_2-EtOAc gradient) to yield 15 fractions (C26-C40). Fr. C30 (115 mg) was further fractionated by CC (silica gel; acetone-hexane, 20:80) to furnish 12 fractions (C41-C52). Frs. C43 and C51 yielded **15** (9 mg) and **4** (21 mg), respectively. Fr. C11 (1.9 g) was subjected to CC (silica gel; hexane-acetone gradient) to give 12 fractions (C53-C64). Fr. C55 (75 mg) was further purified by repeated CC (silica gel; EtOAc-hexane, 30:70) to furnish **16** (19 mg). Fr. C17 (2.2 g) was purified by repeated CC (silica gel; MeOH-CH_2Cl_2, 3.5:96.5) to afford **17** (1 mg). Fr. C18 (560 mg) was subjected to repeated CC (silica gel; MeOH-CH_2Cl_2, 5:95) to yield **18** (7 mg).

The hexane extract (9.2 g) of *E. subumbrans* stems was subjected to a quick CC (silica gel; hexane-$CHCl_3$-MeOH gradient) to give 11 fractions (H1-H11). Fr. H6 (646 mg) was purified by CC (silica gel; $CHCl_3$-hexane, 7:93) to yield **11** (25 mg). Fr. H7 (2.0 g) was filtered to furnish a mixture of **12** and **13** (450 mg). Filtrate of fr. H7 (1.4 g) was further fractionated by CC (silica gel; EtOAc-hexane, gradient) to give 15 fractions (H12-H26). Fr. H14 (561 mg) was subjected to repeated CC (silica gel; EtOAc-hexane, 5:95) to afford 7 fractions (H27-H33). A mixture of **19** and **20** (10 mg) was obtained from fr. H29. Fr. H32 (162 mg) was separated by CC (silica gel; acetone-hexane, 7:93) to yield 5 fractions (H34-H38). Fr. H36 (33 mg) was further purified by CC (silica gel, EtOAc-hexane, 5:95) to afford **21** (5 mg) and **22** (9 mg). Fr. H15 (234 mg) was purified by CC (silica gel; MeOH-$CHCl_3$, 0.2:99.8) to furnish **2** (5 mg). Fr. H10 (3.8 g) was subjected to a quick CC (silica gel; hexane-acetone-MeOH gradient) to give 19 fractions (H39-H57). Fr. H43 (370 mg) was purified by repeated CC (silica gel; acetone-hexane, 15:85) to afford a mixture of **23** and **24** (7 mg). Fr. H44 (512 mg) was fractionated by repeated CC (silica gel; EtOAc-hexane, 20:80) to give 11 fractions (H58-H68). Frs. H59 (40 mg) was purified by CC (silica gel; EtOAc-hexane, 10:90) to yield **8** (7 mg). Fr. H45 (791 mg) was subjected to CC (silica gel; MeOH-$CHCl_3$, 20:80) to afford 14 fractions (H69-H82). Compound **4** (5 mg) was obtained from fr. H74. Fr. H79 (138 mg) was purified by CC (silica gel; EtOAc-hexane, 30:70) to yield **5** (10 mg). Fr. H47 (263 mg) was purified by CC (silica gel; MeOH-$CHCl_3$, 2:98) to furnish **6** (12 mg).

The CH_2Cl_2 extract (13.4 g) of *E. subumbrans* stems was subjected to a quick CC (silica gel; $CHCl_3$-MeOH gradient) to yield 8 fractions (C1-C8). Fr. C2 (12.2 g) was further fractionated by CC (silica gel; acetone-hexane gradient) to give 13 fractions (C9-C21). A mixture of **12** and **13** (28 mg) was obtained from fr. C14. Fr. C15 (354 mg) was purified by repeated CC (silica gel; acetone-hexane, 20:80) to afford **15** (11 mg). Fr. C16 (1.9 g) was separated by CC (silica gel; acetone-hexane, 22:78) to give 14 fractions (C22-C35). Fr. C27 (93 mg) was further purified by CC (silica gel; EtOAc-CH_2Cl_2, 20:80) to yield **25** (2 mg) and **16**

(13 mg). Fr. C19 (798 mg) was purified by repeated CC (silica gel; acetone-hexane, 30:70) to yield **7** (8 mg).

Antiplasmodial activity

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain) which was cultured continuously according to the method of Trager and Jensen (1976). Quantitative assessment of antiplasmodial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins *et al.* (1979). The inhibitory concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 1 ng/mL was observed for the standard compound, dihydroartemisinin, in the same test system.

Antimycobacterial activity

The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra strain using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC. The standard drugs rifampicin, isoniazid and kanamycin sulfate showed MIC of 0.004, 0.06 and 2.5 μg/mL, respectively.

Cytotoxicity activity

The cytotoxicity assays against oral human epidermal carcinoma (KB), human breast cancer (BC) and human small cell lung cancer (NCI-H187) cells were performed employing colorimetric method (Skehan *et al.*, 1990). The standard drug ellipticine exhibited IC_{50} values against these cell lines at 1.33, 1.46 and 0.39 μg/mL, respectively.

RESULTS AND DISCUSSION

Chromatographic separation of the hexane and CH_2Cl_2 extracts of the roots of *E. stricta* yielded four pterocarpans, one flavanone, two triterpenes, two alkaloids, esters and two steroids. These compounds were identified as erythrabyssin II (**4**) (Tanaka *et al.*, 1998), erystagallin A (**5**) (Tanaka *et al.*, 1997), erythrabissin-1 (**6**) (Fomum *et al.*, 1986), 5-hydroxysophoranone (**8**) (Baruah *et al.*, 1984; Matsuura *et al.*, 1994), sandwicensin (**14**) (Mitscher *et al.*, 1988a), sophoradiol (**15**) (Kinjo *et al.*, 1985; Mahato and Kundu, 1994), soyasapogenol B (**16**) (Mahato and Kundu, 1994; Kitagawa *et al.*, 1976; Maximo and Lourenco, 1998), 8-oxoerythrinine (**17**) (Dagne and Steglich, 1984), erythratine (**18**) (Barton *et al.*, 1968), alkyl *trans*-ferulates (**11**) (Rukachaisirikul *et al.*, 2000) and a mixture of β-sitosterol (**12**) and stigmasterol (**13**) (Pouchert and Behnke, 1993) by comparison of their spectral data with literature values. From the hexane and CH_2Cl_2 extracts of the stems of *E. subumbrans*, five triterpenes, six steroids and esters were isolated, in addition to five pterocarpans, one flavanone and one isoflavone, which have been reported previously (Rukachaisirikul *et al.*, 2007). These compounds were identified as erybraedin A (**2**) (Mitscher *et al.*, 1988b), erythrabyssin II (**4**), erystagallin A (**5**), erythrabissin-1 (**6**), erycristagallin (**7**) (Mitscher *et al.*, 1984), 5-hydroxysophoranone (**8**), erysubin F (**10**) (Tanaka *et al.*, 2001), sophoradiol (**15**), soyasapogenol B (**16**), lupeol (**21**) (Reynolds *et al.*, 1986), cycloeucalenol (**22**) (Kocor and Pyrek, 1973; Khuong-Huu *et al.*, 1975; Wen *et al.*, 1986), melilotigenin C (**25**) (Macias *et al.*, 1998), a mixture of β-sitosterol (**12**) and stigmasterol (**13**), a mixture of stigmast-4-en-3-one (**19**) and stigmasta-4,22-dien-3-one (**20**) (Tandon and Rastogi, 1976; Mahato and Banerjee, 1980; Greca *et al.*, 1990), a mixture of 3β-hydroxystigmast-5-en-7-one (**23**) and 3β-hydroxystigmast-5,22-dien-7-one (**24**) (Greca *et al.*, 1990; Notaro *et al.*, 1992; Achenbach and Schwinn, 1995) and alkyl *trans*-ferulates (**11**). The structures of compounds tested for bioactivities are presented in Fig. 1. Compounds **4-6**, **8** and **14-18** were first isolated from *E. stricta* whereas compounds **15**, **16**, **21**, **22**, **25**, a mixture of **19** and **20**, and a mixture of **23** and **24** were first isolated from *E. subumbrans*. Moreover, this is the first report regarding the isolation of compounds **15** and **19-25** from the genus *Erythrina*.

The isolated compounds **2**, **4-8**, **10**, **14-16** and **22** from *E. stricta* roots and *E. subumbrans* stems were tested for antiplasmodial, antimycobacterial and cytotoxic activities. The results are shown in Table I. Compounds **2**, **4**, **5**, **8**, **10** and **16** exhibited moderate antiplasmodial activity against *Plasmodium falciparum* (IC_{50} 2.5-5.5 μg/mL), whereas compounds **6**, **7**, **14**, **15** and **22** were inactive. With the exception of compound **15**, the rest of the compounds tested showed varying antimycobacterial activity

Table I. Antiplasmodial, antimycobacterial and cytotoxic activities of some isolated compounds

Compound	Antiplasmodial (IC_{50}, μg/mL)	Antimycobacterial (MIC, μg/mL)	Cytotoxicity (IC_{50}, μg/mL)		
			KB	BC	NCI-H187
2	3.4	25	5.1	2.9	2.1
4	5.5	50	Inactive[c]	13.9	Inactive[c]
5	3.8	12.5	6.9	4.2	4.1
6	Inactive[a]	50	11.1	13.7	Inactive[c]
7	Inactive[a]	12.5	14.9	12.5	2.6
8	2.5	12.5	12.8	14.2	5.0
10	3.2	12.5	4.5	12.5	2.9
14	Inactive[a]	50	Inactive[c]	Inactive[c]	Inactive[c]
15	Inactive[a]	Inactive[b]	Inactive[c]	Inactive[c]	Inactive[c]
16	4.6	200	Inactive[c]	Inactive[c]	Inactive[c]
22	Inactive[a]	200	Inactive[c]	Inactive[c]	Inactive[c]

[a] Inactive at 10 μg/mL; [b] Inactive at >200 μg/mL; [c] Inactive at 20 μg/mL.

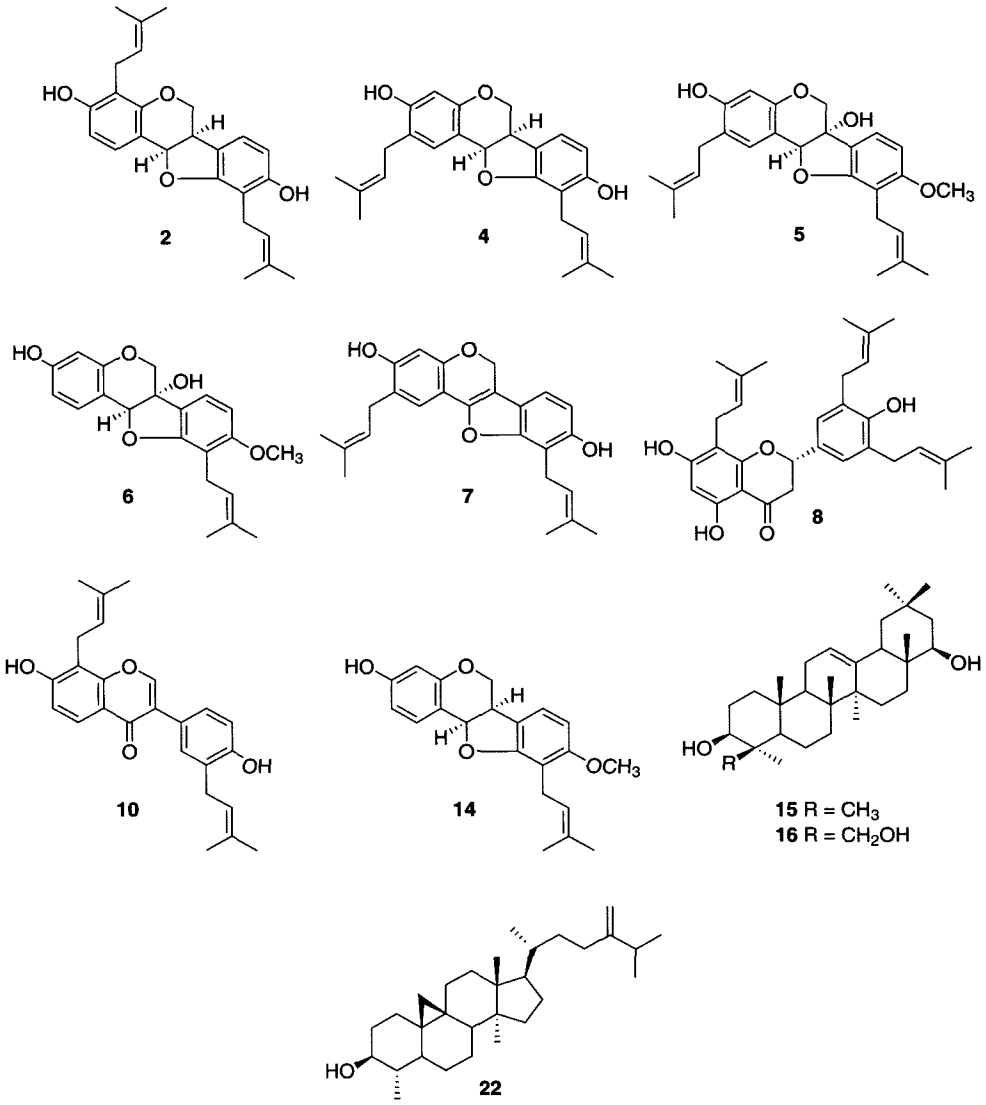

Fig. 1. Structures of some pterocarpanoids, flavonoids and triterpenoids isolated from *E. stricta* and *E. subumbrans*

against *Mycobacterium tuberculosis* (MIC 12.5-200 μg/mL), with **5**, **7**, **8** and **10** being the most active compounds (MIC 12.5 μg/mL). For cytotoxic activity, compounds **2**, **5**-**8** and **10** exhibited strong to weak activity against the KB cells (IC_{50} 4.5-14.9 μg/mL), whereas **2**, **4**-**8** and **10** showed strong to weak activity against the BC cells (IC_{50} 2.9-14.2 μg/mL). In addition, compounds **2**, **5**, **7**, **8** and **10** were strongly active against the NCI-H187 cells (IC_{50} 2.1-5.0 μg/mL).

For pterocarpans **2** and **4**, it should be noted that the position of the prenyl group at C-4 in **2** played an important role to enhance all bioactivity potency. However, with regard to 6a-hydroxypterocarpans **5** and **6**, it appears that the presence of the prenyl group at C-2 position of **5** tended to enhance all the bioactivities tested. Comparing pterocarpan **4** and the corresponding pterocarpene **7**, it seems that **7** possessing the C=C bond between C-6a and C-11a showed much higher antimycobacterial activity and cytotoxic activity against the KB and NCI-H187 cells but showed no antiplasmodial activity. Furthermore, the presence of 6a-hydroxyl group in **6** slightly enhanced cytotoxic activity against the KB and BC cells as compared to the corresponding pterocarpan **14**. In contrast, the presence of 6a-hydroxyl group in **6** did not seem to affect either antiplasmodial activity or cytotoxic activity against the NCI-H187 cells. However, the effect of the methoxyl group at C-9 position in **5**, **6** and **14** on the biological activity is uncertain.

From the overall results, it could be concluded that among the tested compounds, 5-hydroxysophoranone (**8**) exhibited the highest antiplasmodial activity (IC_{50} 2.5 μg/mL) whereas **8** showed the same level of antimycobacterial activity (MIC 12.5 μg/mL) as erystagallin A (**5**), erycristagallin (**7**) and erysubin F (**10**). For cytotoxicity, ery-

braedin A (**2**) appeared to be the most potent compound against the NCI-H187 and BC cells with the IC_{50} values of 2.1 and 2.9 μg/mL, respectively, whereas **10** was the most potent compound against the KB cells with the IC_{50} value of 4.5 μg/mL. Among the triterpenes tested, only soyasapogenol B (**16**) showed moderate antiplasmodial activity with the IC_{50} value of 4.6 μg/mL.

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