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ISOLATION OF NEW ISOFLAVONOLIGNANS, BUTESUPERINS A and B, FROM A THAI MIRACLE HERB, *BUTEA SUPERBA*

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Abstract – Two new isoflavonolignans, designated as butesuperins A and B, were isolated from the active fraction of the root of *Butea superba* for PDE inhibition together with eight known isoflavone derivatives.

INTRODUCTION

Butea superba (BS) is a herb in the family Papilionaceae (Leguminosae), and has the characteristics of being a crawler that wraps itself around large trees.[1] One branch has three leaves and the flowers are of a yellowish orange color. This plant can be found growing in forests in Thailand (known as several local names, *eg* 'Thong-khruea' in the central region)[2] and India (known as 'Palaslata' in Hindi).[3] The head

and body of the plant are locally used as medicines for strength and power, and, in addition, are considered to help male-sexual performance.[4] Thus, this plant has come to be known as one type of miracle herbs in Thailand. From BS, chalcone, flavanone, and 3-hydroxyflavone (flavanol) derivatives have been isolated as its chemical constituents until now.[5] The expected medicinal effect of BS could be primarily caused by increasing the relaxation of phosphodiesterases (PDEs). In fact, 4'-methoxy-3,7,3'-trihydroxyflavone (**1**) and its glycoside (**2**) (Figure 1) had been found to show PDE-inhibition activity for cAMP, albeit with weak activity.[5d]

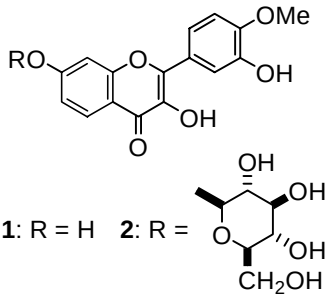


Figure 1. The PDE-inhibitory active flavones isolated from BS

Furthermore, during our isolation studies of bioactive components from BS it was reported that an oral administration of powdered tubers of this plant to volunteers with erectile dysfunction (ED) resulted in appreciable improvement in 82.4% of ED patients without changes in blood testosterone level.[6] Flavonoids and/or their glycosides mentioned above appears to act primarily by increasing the relaxation of PDEs. However, detailed information on the regulation of cyclic nucleotides *via* the PDE inhibition, in addition to the identification of real active principles, is not known yet. We focused on the platelet activating factor (PAF)-induced platelet aggregation by dual inhibitions of PDE3A and PDE5 for the bioassay-guided isolation of active principles from BS. In this paper we describe the isolation of new isoflavonolignans, designated as butesuperin A (**8**) and butesuperin B (**9**), from the bioactive fraction of the root of BS together with known isoflavone derivatives.

RESULTS AND DISCUSSION

After the removal of sucrose precipitated during evaporation of the solvent of the ethanol extract of the root of BS, the residual syrupy mass was separated by column chromatography under normal phase condition (NP-CC) using hexane, chloroform, acetone, and methanol as eluents. Preliminary evaluation of each fraction indicates that activity is mainly concentrated to the acetone eluent, of which the least polar eluent in the next column chromatography under reverse phase condition (RP-CC) was obtained as the most active fraction. Thus, the active fraction was subjected to further bioassay-guided separation by

combination of NP-CC and preparative TLC (PTLC), as shown in Chart 1, resulting in the isolation of

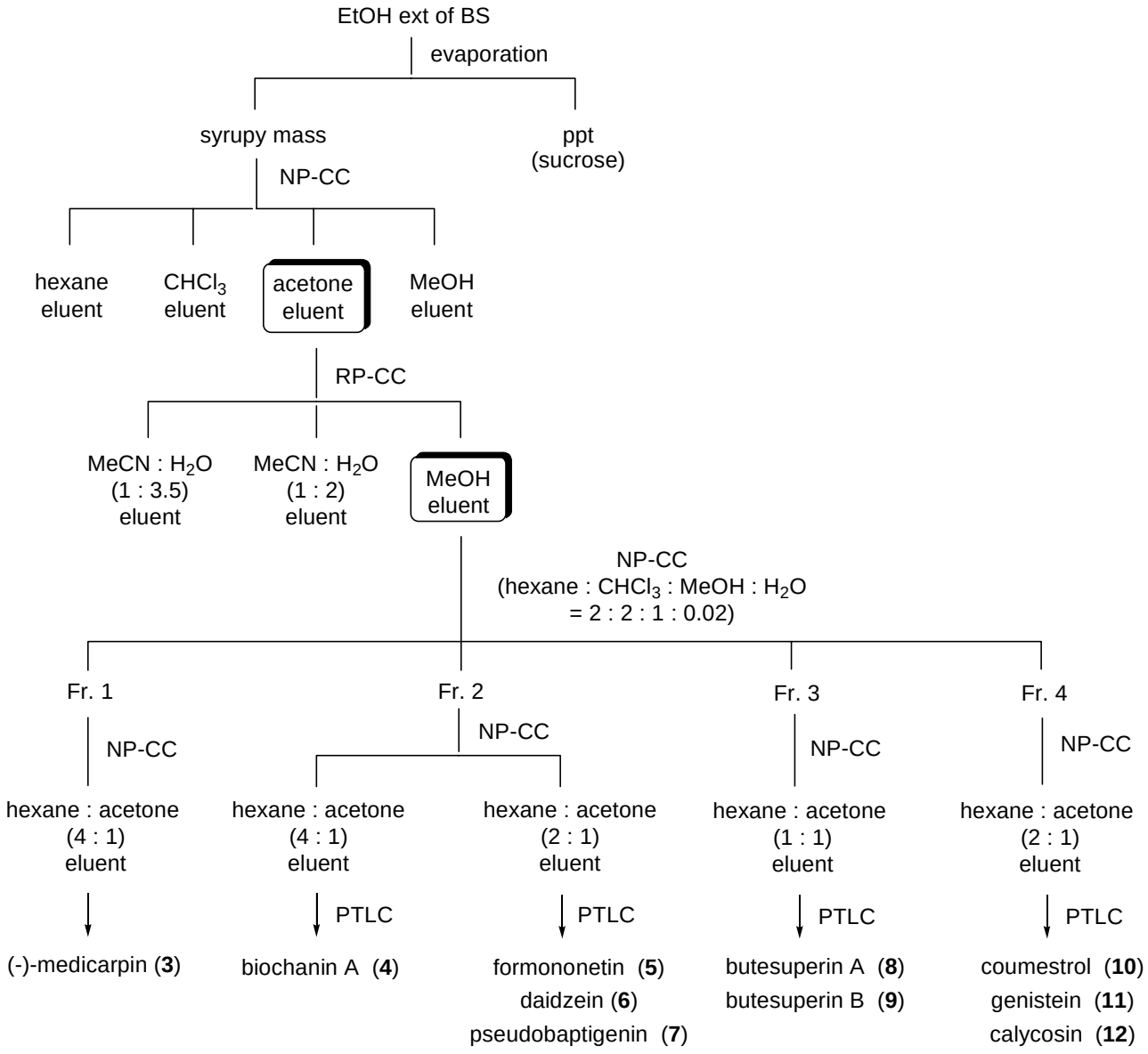


Chart 1. Flow chart of the separation of isoflavones from BS

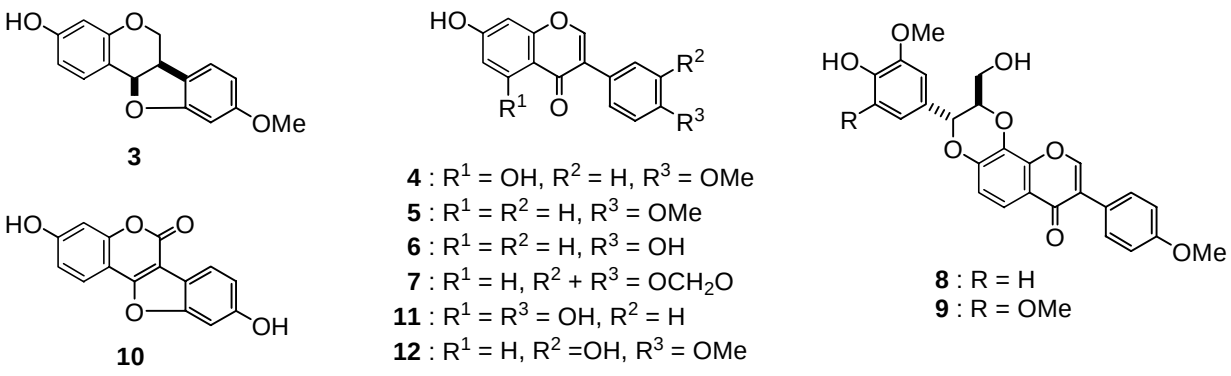
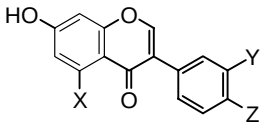


Figure 2. The isolated isoflavone derivatives from BS in this study

ten isoflavone derivatives including two new isoflvaonolignans (Figure 2).

The structures of eight known isoflavones were determined by comparison of their ^{1}H-NMR spectral data (in some cases, additionally ^{13}C-NMR spectral data) with the reported ones and they were, in the order of polarity, (-)-medicarpin (**3**),[7] biochanin A (**4**), [8] formononetin (**5**),[7] daidzein (**6**),[9] pseudobaptigenin (**7**),[10] coumestrol (**10**),[9] genistein (**11**),[9] and calycosin (**12**).[11] The ^{1}H-NMR spectral data of the isoflavone isolates except coumestane skeletons (**3**) and (**10**), are given in Table 1. Among the isoflavone isolates 4'-hydroxyisoflavones, daidzein (**6**) and genistein (**11**), were isolated as a pair of the corresponding 4'-methoxy derivatives, formononetin (**5**) and biochanin A (**4**), respectively. The characteristic relation can be easily deduced by signal patterns in ^{1}H-NMR spectra due to a 3-aryl substituent. Thus, lower field-shifted signals due to aromatic protons of the 3-aryl group in the range of 0.09-0.14 ppm are observed in the 4'-OMe series than in the 4'-OH series (see, Table 1).

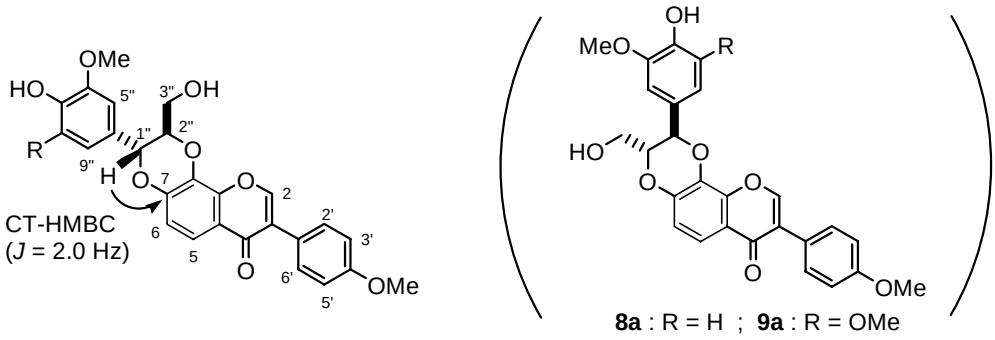
Table 1. ^{1}H-NMR spectral data of the isoflavone isolates from BS except coumestane skeletons [400 MHz, in $(CD_3)_2CO$].



#C	X = Y = H		X = OH, Y = H		X = H	
	6 : Z = OH	**5** : Z = OMe	**11** : Z = OH	**4** : Z = OMe	**7** : Y + Z = OCH_2O	**10** : Y = OH, Z = OMe
2	8.14 (s)	8.17 (s)	8.13 (s)	8.20 (s)	8.19 (s)	8.15 (s)
5	8.05 (d, *J*=8.8)	8.05 (d, *J*=8.8)	-	-	8.06 (d, *J*=8.8)	8.06 (d, *J*=8.8)
6	6.98 (dd, *J*=8.8, 2.0)	6.98 (dd, *J*=8.8, 2.0)	6.26 (d, *J*=2.0)	6.28 (d, *J*=2.0)	7.00 (dd, *J*=8.8, 2.0)	6.99 (d, *J*=8.4, 2.0)
8	6.89 (d, *J*=2.0)	6.89 (d, *J*=2.0)	6.39 (d, *J*=2.0)	6.42 (d, *J*=2.0)	6.90 (d, *J*=2.0)	6.89 (d, *J*=2.0)
2'	7.46 (d, *J*=8.8)	7.55 (d, *J*=8.8)	7.40 (d, *J*=8.8)	7.54 (d, *J*=9.2)	7.16 (d, *J*=2.0)	7.15 (d, *J*=2.0)
3'	6.88 (d, *J*=8.8)	6.97 (d, *J*=8.8)	6.87 (d, *J*=8.8)	6.99 (d, *J*=8.8)	-	-
5'	6.88 (d, *J*=8.8)	6.97 (d, *J*=8.8)	6.87 (d, *J*=8.8)	6.99 (d, *J*=8.8)	6.89 (d, *J*=8.8)	6.97 (d, *J*=8.4)
6'	7.46 (d, *J*=8.8)	7.55 (d, *J*=8.9)	7.40 (d, *J*=8.8)	7.54 (d, *J*=9.2)	7.08 (d, *J*=8.8, 2.0)	7.06 (dd, *J*=8.4, 2.0)

Butesuperins A (**8**) and B (**9**) were isolated as optical active new isoflavonolignans with retusin [7,8-dihydroxy-3-(4-methoxyphenyl)benzopyran-4-one][12] structure as a common isoflavone unit (see, Table 2) and their molecular formulae were determined to be $C_{26}H_{22}O_8$ and $C_{27}H_{24}O_9$, respectively, by HRFABMS spectra. Further examination of NMR spectra (Table 2) indicates that the remaining lignan core (C_6-C_3) is coniferyl (4-hydroxy-3-methoxycinnamyl) alcohol in **8** and sinapyl (3,5-dimethoxy-4-hydroxycinnamyl) alcohol in **9**, respectively. Thus, different signal patterns between **8** and **9** in the ^{1}H-NMR spectra come from the C_6 unit of the lignan core; an ABM type aromatic system

Table 2. NMR spectral data of butesuperins A (**8**) and B (**9**).

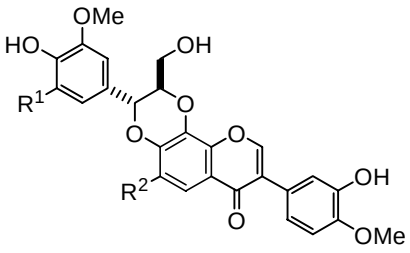


8a : R = H ; **9a** : R = OMe

#C	**8** : R = H[a]		**9** : R = OMe[b]	
	δ_H	δ_C	δ_H	δ_C
2	8.24 (s)	152.88	8.24 (s)	152.90
3	-	124.61	-	124.58
4	-	175.23	-	175.24
4a	-	118.57	-	119.63
5	7.66 (d, *J*=8.8)	115.21	7.66 (d, *J*=8.8)	115.23
6	7.01 (d, *J*=8.8)	117.43	7.02 (d, *J*=8.8)	117.43
7	-	148.08	-	148.05
8	-	132.18	-	132.85
8a	-	146.89	-	146.22
1'	-	124.94	-	124.94
2' (6')	7.54 (d, *J*=8.8)	130.66	7.54 (d, *J*=8.8)	130.66
3' (5')	6.96 (d, *J*=8.8)	113.99	6.96 (d, *J*=8.8)	114.01
4'	-	159.79	-	160.14
4'-OMe	3.80 (s)	55.15	3.80 (s)	55.15
1"	5.15 (d, *J*=8.0)	79.32	5.13 (d, *J*=8.0)	79.31
2"	4.27 (m)	77.35	4.29 (m)	77.64
3"	3.57 (dd, *J*=12.6, 4.0) 3.88 (dd, *J*=12.6, 2.4)	61.14	3.59 (dd, *J*=12.4, 3.6) 3.89 (dd, *J*=12.4, 2.4)	61.15
4"	-	127.96	-	126.84
5"	7.13 (d, *J*=2.0)	115.46	6.84 (s)	105.86
6"	-	147.88	-	148.48
7"	-	147.23	-	147.88
8"	6.87 (d, *J*=8.0)	111.61	-	148.48
9"	6.97 (dd, *J*=8.0, 2.0)	121.33	6.84 (s)	105.86
OMe	3.84 (3H, s)	55.94	3.82 (6H, s)	56.32

[a]Measured in CD_3OD and assignments were based on 2D experiments. [b]Measured in $(CD_3)_2CO$.

[δ 6.87 (1H, d, *J* = 8.0 Hz), 6.97 (1H, dd, *J* = 8.0, 2.0 Hz), 7.13 (1H, d, *J* = 2.0 Hz)] with a methoxy group [δ 3.84 (3H, s)] in the former and a symmetrical A_2 one [δ 6.84 (2H, s)] with two methoxy group [δ 3.82 (6H, s)] in the latter. The *trans* stereochemistry between the methine hydrogens (1”-H and 2”-H) is reasonably deduced by the coupling constant (*J* = 8.0 Hz).[13] No positive information on discrimination of the structures (**8**) and (**9**) from regioisomeric **8a** and **9a** was given from heteronuclear multiple bond correlation (HMBC) of butesuperin A using parameters (long-range *J* = 5 and 8 Hz); however, application of constant time HMBC (CT-HMBC) technique[14] (long-range *J* = 2 Hz), which was recently developed by one (K. F.) of us for improving separation of cross peaks, showed the presence of the cross peak between 1”-H and C7, allowing us to depict the former **8** and **9** as the structures of butesuperins A and B. It is noteworthy that only three isoflavonolignans [xanthocercins A (**13**)[15a] and B (**14**)[15a] and 5-methoxyxanthocercin A (**15**)[15b]], structurally related to our new components, have been reported as natural sources until now, in spite of many examples of flavonolignans,[16] to our knowledge.



13 : R^1 = OMe, R^2 = H
14 : R^1 = R^2 = H
15 : R^1 = R^2 = OMe

CONCLUSION

In conclusion, new isoflavonolignans designated as butesuperins A and B were isolated from the active fractions of BS for PDE inhibition together with known isoflavone derivatives. The detailed effect of each isolate on the PAF-induced platelet aggregation is in progress and the results will be reported elsewhere in the near future.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a micro melting point hot-stage instrument (Yanagimoto) and are uncorrected. IR and UV spectra were recorded on a JASCO IR-700 and a JACSO V-503 spectrophotometers, respectively. Specific rotation, $[\alpha]_D$, was recorded on a JASCO P-1020 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM ECP 400, JEOL JNM-500α, and Varian INOVA-500 (for C-T HMBC) spectrometers. FABMS and HRFABMS spectra were recorded on a JEOL JMX-HX 110A spectrometer with a direct inlet system. For column

chromatography silica gel 60 (70-230 mesh ASTM; Kanto) for NP-CC and LiChroprep RP-18 (40-63 μM; Merck) for RP-CC, respectively, were used, while for PTLC silica gel GF254 (Merck) was used.

Extraction and Isolation. The root (2.4 kg) of *Butea superba*, which was collected at Cha Cheng Chao Province in Thailand in 2002, was extracted with EtOH (8 L x 3) under reflux for 8 h (each). After the removal of sucrose (19.2 g) precipitated during evaporation of the solvent the remaining syrupy mass (25 g) was subjected to NP-CC as shown in Chart 1 to give hexane (0.3 g), $CHCl_3$ (2 g), acetone (10 g) and MeOH eluents (10 g). A part (0.78 g) of the acetone eluent was subjected to RP-CC under medium pressure conditions using aqueous acetonitrile [MeCN : H_2O = 1 : 3.5 (0.07 g), MeCN : H_2O = 1 : 2 (0.07 g)] followed by methanol (0.53 g). The methanol fraction was separated by NP-CC with a mixed solvent of hexane-$CHCl_3$-MeOH-H_2O (2 : 2: 1 : 0.02) to give four fractions [Fr. 1 (0.03 g), Fr. 2 (0.05 g), Fr. 3 (0.02 g), and Fr. 4 (0.04 g)] containing isoflavone derivatives. These fractions were further separated by NP-CC using a mixed solvent of hexane-acetone. (-)-Medicarpine (**3**) {$[\alpha]_D^{24}$ -87.9° (*c* 0.05, MeOH)} (0.003 g, 0.0016%) from Fr. 1 (hexane : acetone = 4 : 1), biochanin A (**4**) (0.002 g, 0.0011%; hexane : acetone = 4 : 1), formonetin (**5**) (0.005 g, 0.0027%; hexane : acetone = 2 : 1), daizein (**6**) (0.001 g, 0.0005%; hexane : acetone = 2 : 1), and pseudobaptigenin (**7**) (0.001 g, 0.0005%; hexane : acetone = 2 : 1) from Fr. 2 followed by PTLC, and coumestrol (**10**) (0.002 g, 0.0011%), genistein (**11**) (0.001 g, 0.0005%), and calycosin (**12**) (0.002 g, 0.0011%) from Fr. 4 (hexane : acetone = 2 : 1) followed by PTLC were isolated, respectively, as known isoflavone derivatives. Butesuperin A (**8**) (0.002 g, 0.0011%) and butesuperin B (**9**) (0.002 g, 0.0011%) were isolated from Fr. 3 (hexane : acetone = 1 : 1) followed by PTLC.

Butesuperin A (8). Colorless powder, mp 135-136 °C (washed with Et_2O-hexane), IR (ATR) ν_{max} 3386, 1705 cm^{-1}; UV (MeOH) λ_{max} (log ε) 259 (4.23); ^1H- and ^{13}C-NMR, see Table 2; HRFABMS *m/z*: 463.1387 (MH^+) (Calcd for $C_{26}H_{23}O_8$: 463.1393); $[\alpha]_D^{21}$ +13.2° (*c* = 0.02, acetone).

Butesuperin B (9). Colorless powder, mp 116-118 °C (washed with Et_2O-hexane), IR (ATR) ν_{max} 3383, 1705 cm^{-1}; UV (MeOH) λ_{max} (log ε) 259 (4.25); ^1H- and ^{13}C-NMR, see Table 2; HRFABMS *m/z*: 493.1484 (MH^+) (Calcd for $C_{27}H_{25}O_9$: 493.1499); $[\alpha]_D^{21}$ +3.8° (*c* = 0.02, acetone).

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