Flavonoid and Flavonoid glycoside from *Butea superba* Roxb. and their cAMP Phosphodiesterase Inhibitory Activity

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A flavonoid (3, 7, 3'-Trihydroxy-4'-methoxyflavone) (1) and a flavonoid. glycoside (3, 3'-dihydroxy-4'-methoxyflavone-7-0-(3-D-glucopyranoside) (2) were isolated from the tuber root of *Butea superba* Roxb. The structures were determined on the basis of spectral analysi6, including 2D-NMR techniques. These compounds show higher inhibitory effects on cAMP. phosphodiesterase than caffeine and theophylline.

Key words: *Butea superba*, flavonoid, flavonoid glycoside, cAMP phosphodiesterase inhibition.

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INTRODUCTION

Butea superba Roxb. is a plant in the Family Papilionaceae and has the characteristics of being a crawler that wraps itself around large trees. One branch has 3 leaves, the flowers are of a yellowish orange color and the plant grows out in the open. The long tuber root of the plant is buried under the ground like the tuber root of a yam. This plant reproduces through seeds and the propagation of its tuber root. This plant can be found growing in forests in Thailand's northern regions, eastern regions and along Kanchanaburi Province. The tuber and stem of the plant are used in medicines believed to give strength and power and increase male sexual performance. Thus, this plant has come to be known as one type of miracle herb. Since *Butea superba* Roxb. helps to enhance human health, it is therefore very interesting to investigate the chemical constituents of this plant and their biological activity. The bioactivity of each constituent was tested for an inhibitory effect towards cAMP phosphodiesterase, which has been shown to be important in controlling bodily functions and involved a wide number of diseases.o)

Table 1. IH NMR spectral data of compounds! and 1 (500 MHz DMSO).

Position	1 8H (J in Hz)	2 8H (J in Hz)
2		
3		
4		
5	7.96 d (8.8)	8.05 d (8.8)
6	6.94 dd (2.1,8.8)	7.15 dd (2.1,8.8)
7		
8	6.85 d (2.1)	7.22 d (2.4)
9		
10		
l'		
2'	8.35d(2.1)	8.40 d (2.0)
3'		
4'		
5'	6.98 d (8.8)	6.98 d (8.8)
6'	7.50 dd (2.1, 8.8)	7.52 dd (2.1,8.8)
-OCH3	3.79 s	3.78 s
1"		5.10 d (7.6)
2"		3.30 dd (7.6, 9.5)
3"		3.35 t (9.5)
4"		3.18t(9.5)
5"		3.45 m
6"a		3.45 da-(12.0, 2.5)
6"b		3.70 dd (12.0, 4.5)

Table 2. ^{13}C NMR (125 MHz DMSO) and 2D Long-Range ^{1}H-^{13}C Correlations in the HMBC Spectra of Compounds 1 and 2.

Position	1		2	
	δC	HMBC	δC	HMBC
2	158.9		159.1	
3	146.8		147.0	
4	174.6		174.8	
5	127.3	4, 7, 9	127.1	4, 7, 9
6	115.2	5, 7, 8, 10	115.7	5, 7, 8, 10
7	162.6		161.5	
8	102.1	6, 7, 9, 10	103.5	6, 7, 9, 10
9	157.4		157.1	
10	116.6		118.5	
1'	124.2		123.5	
2'	153.4	1', 3', 4', 6'	153.6	1', 3', 4', 6'
3'	157.3		157.1	
4'	146.9		146.6	
5'	113.6	1', 3', 4'	113.7	1', 4', 6'
6'	130.0	1', 2, 4', 5'	130.1	1', 3', 4', 5'
$-OCH_3$	55.2		55.2	
1"			100.1	
2"			73.2	
3"			76.5	
4"			69.8	
5"			77.2	
6"			60.8	

EXPERIMENTAL

General Experimental Procedures

All commercial grade solvents were distilled prior to use. Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. The optical rotation was determined on a JASCO DIP-370 digital polarimeter. Measurements of UV spectra were carried out on a Milton-Roy Spectronic 3000 Array UV/VIS spectrophotometer. IR spectra were recorded on a Perkin-Elmer model 1760X FT-IR spectrophotometer. Spectra of solid samples were recorded via KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded at 500.00 and 125.65 MHz, respectively, on a JEOL JNM-A500 NMR spectrometer. LREIMS (Low Resolution Electron Impact Mass Spectrometry) spetra were obtained with a Fisons Instruments model Trio 2000 mass spectrometer at 70 eV.

Plant Materials

The tubers of *Butea superba* Roxb. were collected from Amphur Muang, Lumpang Province, Thailand in May 1997. Botanical identification was claimed through comparison with a voucher specimen No. BKF 70163 in the herbarium collection of Royal Forest Department of Thailand.

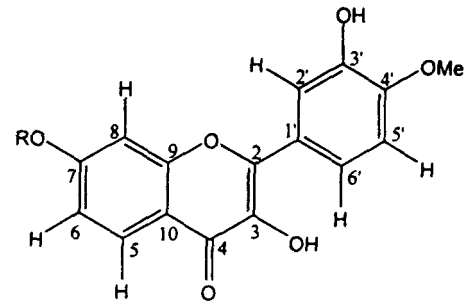
Extraction and Isolation

Powdered sun dried roots (16.0 kg) of *Butea superba* were repeatedly extracted with MeOH (5x10 L). The MeOH extracts were filtered and evaporated under reduced pressure to obtain a dark-red gummy residue (106.0 g) of MeOH crude extract. This

MeOH crude extract was subsequently re-extracted with hexane and then $CHCl_3$ to leave the final insoluble residue (72.0 g). The hexane and $CHCl_3$ extract fractions were evaporated under reduced pressure to give a hexane crude extract (21.0 g) and $CHCl_3$ crude extract (12.0 g), respectively. The $CHCl_3$ crude extract (12.0 g) was subjected to silica gel column chromatography using gradient elution with $CHCl_3$ and MeOH in a stepwise fashion. Compound 1 was eluted with 5% MeOH in $CHCl_3$. Similar fractions were combined and the solvent was removed under reduced pressure to give compound 1 (135.0 mg) after recrystallization from MeOH and $CHCl_3$. The final residue (72.0 g) was separated by column chromatography on Silica gel using gradient elution with increasing amounts of MeOH in $CHCl_3$ to give compound 2 (60.0 mg) from 10% of MeOH in $CHCl_3$ fraction. Compound 2 was recrystallized from MeOH and $CHCl_3$.

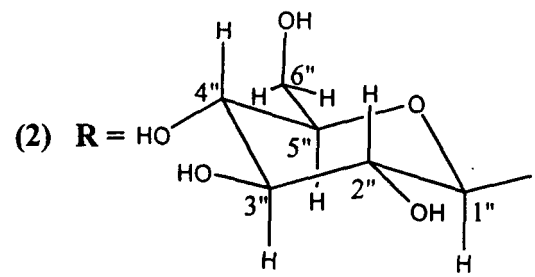
Flavonoid (3, 7, 3'-Trihydroxy-4'-methoxyflavone) (compound 1): pale yellow needle crystal, mp 258-260°C (d) [lit 288(d), but no spectroscopic data for verification], (found: C, 64.0; H, 3.9, $C_{16}H_{12}O_6$ required: C, 64.0; H, 4.0); UV λ_{max} EtOH nm 254, 316 and 365; IR ν^{KBr}_{max} cm^{-1}; 3340-3000, 2940, 1650, 1594, 1575, 1500, 1450, 1380, 1260, 1090, 1020, 790; ^1H and ^{13}C-NMR Table 1; EIMS m/z (rel. int.) 300[M^+] (25), 282 (30), 268 (100), 253 (25), 132 (60).

Flavonoid glycoside (3,5'-Dihydroxy-4'-methoxyflavone-7-O-β-D-glucopyranoside) (compound 2); white amorphous solid; mp 210-212°C, (found: C, 57.0; H, 4.5, $C_{22}H_{22}O_{11}$ requires: C, 57.1; H, 4.8); $[\alpha]^{25}_D$+9.5 (c 1.05, MeOH); UV λ_{max} EtOH nm 267, 290 and 355; ν^{KBr}_{max} cm^{-1}; 3600-3100, 2900, 1650, 1550, 1450, 1300, 1260, 1060-1030, 891, 800; ^1H and ^{13}C-NMR Table 1 and 2; EIMS m/z (rel. int.) 282 (20), 268 (100), 253 (23), 133 (12), 132 (90).



(1) R = H

Scheme 1



Scheme 2

Bioassay

Phosphodiesterase activity was determined from the amount of inorganic phosphate liberated during the reaction by the malachite green method.[2, 3] Phosphodiesterase assay solutions were prepared as follows: (a) the enzyme solution contains phosphodiesterase (0.037 unit mL^{-1}), 5-nucleotidase (1.67 unit mL^{-1}), $MgCl_2$ (5 mM) and Tris HCl (0.2 M); (b) reaction mixture A contains malachite green (0.33 mM), polyvinyl alcohol (3.87g L^{-1}) and ammonium molybdate in 6 M HCl (8.33 mM). The test sample was dissolved in 1.5 % dimethyl sulfoxide in water. The reaction was performed by the addition of cAMP (10 mM, 100 μL) to the enzyme solution (400 μL) at 30°C. Then the sample solution (500 μL), reagent mixture A (1.0 mL) and 25% sodium citrate (200 μL) were added to the above solution successively at 5 minute intervals. The absorbance of the colour complex was

observed at 630 nm using a UV/VIS spectrophotometer referred against a mixed reagent blank. A calibration curve, obtained by this procedure using potassium dihydrogen phosphate solution of a known concentration, was used to determine the amount of phosphate present in the assay. For a control experiment, dimethyl sulfoxide was added instead of the solution of the sample to minimize the effects of the solvent and theophylline and caffeine were used as positive controls for phosphodiesterase assay. The IC_{50} valves of compound (1) and (2) were determined from the calibration curve of sample concentration against cAMP phosphodiesterase activity.

RESULTS AND DISCUSSION

The 3, 7, 3'-Trihydroxy-4'-methoxyflavonone (compound 1) was obtained from a chloroform soluble crude extract from the root of *Butea superba* by silica-gel column chromatography using a gradient elution with chloroform and methanol. The IR spectrum of 1 showed a broad absorption band between 3000 and 3340 cm^{-1} of OH stretching and a strong absorption band at 1650 cm^{-1}, which was consistent with a conjugated carbonyl group. The carbon-carbon double bond stretching vibration of the aromatic phenyl group was also observed at 1594, 1575 and 1500 cm^{-1}. The UV spectrum exhibited absorption maxima at 254 and 365 nm which are characteristic absorption bands of a flavone skeleton.[4] The molecular formula of $C_{16}H_{12}O_6$ was established for compound 1 from the elemental analysis, LREIMS and ^1H and ^{13}C NMR data (Tables 1 and 2). The ^1H and ^{13}C NMR spectra together with 2D NMR experiments allowed the complete structure of compound 1 to be established. The occurrence of flavonoid (1) was clearly determined from the ^1H 500 MHz NMR spectrum which displayed six aromatic protons at δ 6.85 (d, J=2.1 Hz), 6.94 (dd, J=2.1, 8.8 Hz), 6.98 (d, J=8.8 Hz), 7.50 (dd, J=2.1, 8.8 Hz), 7.96 (d, J=8.8 Hz), and 8.35 (d, J=2.1 Hz) and one methyl proton singlet at 3.79 ppm. Detailed analysis of the 2D ^1H and ^{13}C NMR spectrum, including COSY, NOESY, HMQC and HMBC supported the structure of 3, 7, 3'-Trihydroxy-4'-methoxyflavone (1). Although compound 1 is a known compound isolated as a minor constituent of quebracho tannin extract,[5] its ^1H and ^{13}C NMR spectral data have not been published before.

The 3, 3'-Dihydroxy-4'-methoxyflavone-7-O-β-D-glucopyranoside (compound 2) was isolated as an amorphous white solid powder from MeOH extract residue by silica gel column chromatography using $CHCl_3$-MeOH as the eluent. The IR spectrum of compound 2 displayed absorption at 3100-3600 cm^{-1} of OH stretching, and strong absorption at 2900 cm^{-1} of C-H stretching. The absorption band of a conjugated carbonyl group appeared at 1650 cm^{-1}. The molecular formula of compound 2 was assigned as $C_{22}H_{22}O_{11}$ based on elemental analysis and ^1H and ^{13}C NMR (Table 1 and 2) while its EI-MS showed no molecular ion peak pointing to its glycoside nature. The ^1H NMR spectrum of compound 2 in DMSO showed the presence of a sugar moiety by one proton doublet at δ 5.10 (J=7.6 Hz, H-1"), one proton doublet of doublets at δ 3.30 (J=7.6, 9.5 Hz, H-2"), one proton triplet at δ 3.35 (J=9.5 Hz, H-3"), one proton triplet at δ 3.18 (J=9.5 Hz, H-4") and one proton multiplet at δ 3.45 (H-5"). Two protons of C-6" included one proton showed doublet of doublets at δ 3.45 (J=12.0, 2.5 Hz, H-6"a) and another one proton showed doublet of doublets at δ 3.70 (J=12.0, 4.5 Hz, H-6"b). The chemical shifts at δ 6.98, 7.15, 7.22, 7.52, 8.05 and 8.40 were assigned to flavonoid protons at C-5', C-6, C-8, C-6', C-5 and C-2' respectively, on the basis of their similarity to signals observed for compound 1.

Their ^1H and ^{13}C-NMR spectra indicate that glycoside 2 has a glycone portion identical to that in compound 1. In the ^1H NMR spectra (Table 1), an unusual pattern of 7-O-glycosylation was indicated by downfield shifts of H-6 (ca. +0.21 ppm) and H-8 (ca. +0.36 ppm) with respect to compound 1. Similarly, in the ^{13}C-NMR

spectra of compound 2 (Table 2), 7-O-glycosylation was confirmed by the diagnostic[6] upfield shift of C-7 (-0.24 ppm) and by downfield shifts of the ortho-related C-8 (+1.45 ppm) and C-6 (+0.55 ppm) and para-related C-10 (+1.99 ppm) carbon with respect to compound 1. The ^{1}H and ^{13}C NMR data indicated the β-configuration at an anomeric position for the glucopyranosyl unit (Tables 1 and 2). Therefore, the structure of compound 2 was assigned as 3,3'-dihydroxy-4'-methoxyflavone-7-O-β-D-glucopyranoside.

From the bioactivity testing of compound 1 and compound 2, it was found that both of these compounds were effective in inhibiting cAMP phosphodieterase. These two compounds have IC_{50} = 190 and 58 μg/mL, respectively. The cAMP phosphodiesterase inhibition of both these compounds was more effective than those of theophylline (IC_{50} = 615 μg/mL) and caffein (IC_{50} = 420 μg/mL). The cAMP phosphodiesterase enzyme has a main function in the hydrolysis of the intracellular cAMP. Substances that inhibit cAMP phosphodiesterase are therefore capable of stimulating the functioning of the central nervous system (CNS) and stimulating the functioning of cells.[7] Thus, papaverin, dipyridamole, caffeine and theophylline are effective when the cAMP phosphodiesterase is inhibited.[8, 9] Furthermore, substances that have an effect in inhibiting cAMP phosphodesterase also take part in controlling numerous severe diseases, including diabetes,[10-13] hypertension,[14, 15] asthma,[16] hepatomas,[17] psoriasis[18] and possibly cancer.[19, 20] In addition, substances that inhibit phosphodiesterase are shown to have effects in controlling platelet-aggregation inhibition.[21-26]

Therefore, *Butea superba* Roxb. is composed of flavonoids that are effective in inhibiting the cAMP phosphodiesterase enzyme, which are very beneficial to the human body. At least, when taking this herb, the body will begin to feel healthier. This herb will also help control the numerous diseases mentioned beforehand.

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