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Chien-Chih Chen, Li-Gin Wu, Feng-Nien Ko, and Che-Ming Teng

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ANTIPLATELET AGGREGATION PRINCIPLES OF *DENDROBIUM LODDIGESII*

CHIEN-CHIH CHEN,*

National Research Institute of Chinese Medicine, 2 Lane 391, Pei-I Rd. Sec. 2, Hsintein, Taipei Hsien, Taiwan, Republic of China

LI-GIN WU,

Institute of Traditional Medicine, Yang-Ming Medical College, Taipei, Taiwan, Republic of China

FENG-NIEN KO, and CHE-MING TENG

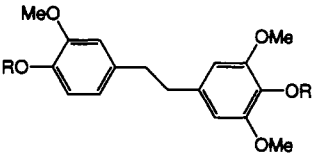
Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China

ABSTRACT.—The MeOH extract of the stem of *Dendrobium loddigesii* was found to inhibit the aggregation of rabbit platelets induced by arachidonic acid and collagen. Two active compounds, moscatilin [**1**] and moscatin [**2**], were isolated. Moscatilin diacetate [**3**] also exhibited antiplatelet aggregation activity.

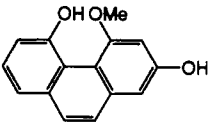
The stems of *Dendrobium* species (Orchidaceae) are used in traditional Chinese medicine as a tonic to nourish the stomach, promote the production of body fluid, and reduce fever (1). Pharmacological studies have revealed that an EtOH extract of *D. denneanum* inhibited the reverse transcriptase and DNA polymerase-α enzymes (2), and the alkaloids, shihunidine and shisunine, from *D. loddigesii*, have the ability to inhibit Na^+, K^+-ATPase (3).

In the course of our studies on the development of naturally occurring antiplatelet agents, we found that an MeOH extract of the stems of *D. loddigesii* Rolfe exhibited antiplatelet aggregation activity. The bioassay-guided isolation of the active principles and their potencies are reported herein.

In order to isolate the biologically active principles from *D. loddigesii*, the fractionated extracts were tested on the aggregation of washed rabbit platelets. As shown in Table 1, fractions 3 and 4 possessed strong inhibitory effects on the platelet aggregation induced by arachidonic acid (AA) and collagen. Platelet aggregation induced by AA was completely abolished by fraction 4 (50 μg/ml). Fraction 4 was further chromatographed on a Si gel column and two active



1 R=H
3 R=Ac



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compounds, **1** and **2**, were obtained. Through spectral analysis, chemical reaction, and comparison with authentic samples (4), compounds **1** and **2** were identified as moscatilin and moscatin, respectively. Acetylation of compound **1** with Ac_2O gave compound **3**.

The antiplatelet effects of moscatilin [**1**], moscatin [**2**], and moscatilin diacetate [**3**] were studied on the aggregation of washed rabbit platelets induced by thrombin, AA, collagen, and platelet-activating factor (PAF), and the results are shown in Table 2. Compounds **1–3** strongly inhibited both AA- and collagen-induced platelet aggregations. At 100 μg/ml, platelet aggregations induced by PAF

TABLE 1. Effect of Fractions from *Dendrobium loddigesii* on the Platelet Aggregation Induced by Thrombin (Thr), Arachidonic Acid (AA), Collagen (Col), and Platelet-Activating Factor (PAF).[a]

Fraction	Concentration (μg/ml)	% Aggregation			
		Thr	AA	Col	PAF
Control		88.0 ± 0.6 (4)	86.9 ± 2.0 (4)	89.3 ± 0.9 (4)	92.6 ± 1.7 (4)
1	200	86.4 ± 0.8 (3)	89.1 ± 1.6 (3)	89.4 ± 0.2 (3)	91.7 ± 2.3 (3)
2	200	81.8 ± 3.1 (3)[b]	85.8 ± 2.8 (3)	85.3 ± 0.6 (3)[d]	91.6 ± 1.7 (3)
3	200	86.1 ± 1.0 (3)	0.0 ± 0.0 (3)[d]	83.2 ± 1.1 (3)[d]	85.5 ± 2.8 (3)[b]
	100		30.7 ±15.5 (4)[c]		
	50		45.1 ±19.8 (4)		
4	200	83.2 ± 4.5 (3)	0.0 ± 0.0 (3)[d]	4.8 ± 3.9 (3)[d]	81.7 ± 3.8 (3)[c]
	100		0.0 ± 0.0 (3)[d]	21.3 ± 7.8 (3)[d]	
	50		0.0 ± 0.0 (3)[d]	67.3 ±13.8 (3)	
	20		48.1 ±19.7 (3)	84.1 ± 2.9 (3)	
5	200	79.0 ± 4.2 (3)	72.2 ± 5.8 (3)[b]	7.6 ± 3.6 (3)[d]	79.2 ± 2.2 (3)
6	200	84.7 ± 2.8 (3)	91.9 ± 0.7 (3)	80.3 ± 2.3 (3)[c]	87.2 ± 0.8 (3)[b]

[a]Platelets were preincubated with each fraction or the solvent (0.5% DMSO, control) at 37° for 3 min, then thrombin (0.1 U/ml), AA (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added. Percentages of aggregation are presented as means ± S.E. (n) and statistical significance was evaluated by Student's t test.

[b]$P<0.05$ as compared with control values.

[c]$P<0.01$ as compared with control values.

[d]$P<0.001$ as compared with control values.

were also significantly inhibited by these three compounds. As shown in Figure 1, the antiplatelet actions of compounds **1**–**3** inhibited AA-induced platelet aggregation in a concentration-dependent manner. Compound **3** showed greater antiplatelet effect than the other two substances. In AA-induced platelet ag-

TABLE 2. Effect of Purified Components from *Dendrobium loddigesii* on the Platelet Aggregation Induced by Thrombin (Thr), Arachidonic Acid (AA), Collagen (Col), and Platelet-Activating Factor (PAF).[a]

Compound	Concentration (μg/ml)	% Aggregation			
		Thr	AA	Col	PAF
Control		89.3 ± 1.9	84.0 ± 6.7	86.4 ± 3.6	86.3 ± 2.7
1	100	85.3 ± 3.7	0.0 ± 0.0[d]	0.0 ± 0.0[d]	24.7 ± 2.9[d]
	50		0.0 ± 0.0[d]	0.0 ± 0.0[d]	72.1 ± 4.3[b]
	20		36.4 ±16.3[b]	19.6 ±16.0[c]	
	10		52.0 ±16.6	42.3 ±17.5[b]	
	5		78.7 ± 9.0	58.4 ±15.1	
	2			78.5 ± 6.3	
2	100	41.0 ± 9.5[d]	0.0 ± 0.0[d]	0.0 ± 0.0[d]	24.7 ± 2.9[d]
	50		0.0 ± 0.0[d]	0.0 ± 0.0[d]	72.1 ± 4.3[b]
	20		0.0 ± 0.0[d]	19.6 ±16.0[c]	
	10		22.8 ±18.6[c]	42.3 ±17.5[b]	
	5		87.6 ± 3.0	58.4 ±15.1	
	2			78.5 ± 6.3	
3	100	93.4 ± 2.8	0.0 ± 0.0[d]	12.8 ± 6.3[d]	36.0 ± 7.8[d]
	20		0.0 ± 0.0[d]		
	10		0.0 ± 0.0[d]		
	5		33.9 ± 8.1[d]		
	2		83.8 ± 1.2		

[a]Platelets were preincubated with each compound or the solvent (0.5% DMSO, control) at 37° for 3 min, then thrombin (0.1 U/ml), AA (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added. Percentages of aggregation are presented as means ± S.E. ($n=3$) and statistical significance was evaluated by Student's t test.

[b]$P<0.05$ as compared with control values.

[c]$P<0.01$ as compared with control values.

[d]$P<0.001$ as compared with control values.

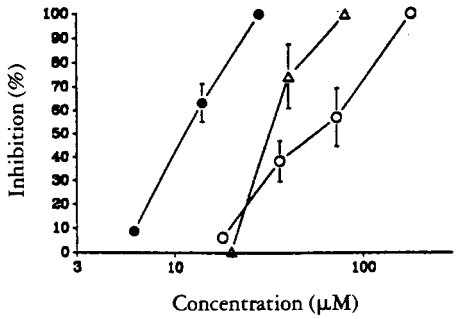

FIGURE 1. The effects of compounds **1** (—○—), **2** (—Δ—), and **3** (—●—) on the platelet aggregation induced by arachidonic acid. Percent inhibitions are expressed as means ± S.E. (n=3).

gregation the IC_{50} values for **1–3** were determined as 61.8, 37.2, and 11.2 μM, respectively.

Exogenous arachidonic acid can be converted into prostaglandin endoperoxides by platelet cyclooxygenase and then, in turn, converted by thromboxane synthase to thromboxane A_2, which is an important mediator for platelet aggregation. Collagen may also trigger platelet aggregation by increasing the formation of thromboxane A_2 (5). The antiplatelet effects of moscatilin and moscatin may be due to the inhibition of thromboxane A_2 formation because the platelet aggregations induced by arachidonic acid and collagen were most easily inhibited. Since the diacetate derivative of moscatilin [**3**] is about 5 times more potent than moscatilin [**1**] itself, this may imply that since **3** is more lipophilic it is able to penetrate across the platelet membrane to reach the site of action intracellularly.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured on Yanaco micro-melting point apparatus and were uncorrected. The ir spectra were recorded on a Jasco Ir-100 spectrometer. ^{1}H-Nmr spectra were taken on a Bruker AM-300 WB (300 MHz) Ft-nmr instrument. Eims spectra were recorded on a JEOL JMS-HX100 spectrometer.

PLANT MATERIAL.—The stems of *D. loddigesii* Rolfe were obtained at a market in Taipei under the direction of Mr. M.T. Kao of the National Research Institute of Chinese Medicine, where voucher specimens are maintained.

EXTRACTION AND ISOLATION.—The air-dried stems of the plant (3 kg) were extracted with MeOH, and the extract was concentrated *in vacuo* and fractionated into $CHCl_3$-soluble and $CHCl_3$-insoluble fractions. The $CHCl_3$-soluble fraction was applied to a column of Si gel. The following fractions were eluted, in order, with the indicated solvent systems: fraction 1 (n-hexane-CH_2Cl_2, 1:1), fraction 2 (CH_2Cl_2), fraction 3 (CH_2Cl_2-Me_2CO, 10:1), fraction 4 (CH_2Cl_2-Me_2CO, 5:1), fraction 5 (CH_2Cl_2-Me_2CO, 31:1), and fraction 6 (Me_2CO). Fraction 4 was further chromatographed on a Si gel column, using a solvent gradient system (n-hexane-EtOAc, 4:1→3:1) to yield moscatilin (**1**, colorless needles, 620 mg) and moscatin (**2**, colorless crystals, 25 mg). Identification of compounds **1** and **2** was based on the comparison of eims and ^{1}H-nmr data with those reported (4) and with authentic samples.

Moscatilin diacetate [**3**].—Moscatilin [**1**] was acetylated with Ac_2O/pyridine at room temperature for 24 h. The usual workup gave compound **3**, mp 105–106°; eims m/z 346 [M^+]; ^{1}H nmr ($CDCl_3$) δ 2.28 (3H, s, -$OCOCH_3$), 2.30 (3H, s, -$OCOCH_3$), 2.86 (4H, s, H_2-α and H_2-α′), 3.66 (3H, s, OCH_3-3′), 3.75 (6H, s, OCH_3-3 and OCH_3-5), 6.33 (2H, s, H-2 and H-6), 6.65 (1H, d, J=1.8 Hz, H-2′), 6.73 (1H, dd, J=1.8 and 8.3 Hz, H-6′), 6.91 (1H, d, J=8.3 Hz, H-5′).

Platelet aggregation tests.—Washed rabbit platelets were obtained from EDTA-anticoagulated platelet-rich plasma according to the procedures described previously (6). Platelets were counted by a Coulter Counter (Model ZM), adjusted to 4.5×10^8 platelets/ml, and suspended in Tyrode solution containing (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2) with bovine serum albumin (0.35%). Aggregation was measured by the turbidimetric method (7), designed with the absorbance of platelets in suspension at 0% aggregation and the absorbance of platelet-free Tyrode solution as 100% aggregation. The aggregation was measured by a Lumi-aggregometer (Chrono-Log Co.) connected to dual channel recorders. The platelet suspension was stirred at 1200 rpm. Platelets were preincubated with the test compounds or the solvent (DMSO) in the control experiment for 3 min before the addition of aggregation inducers. To eliminate the effect of the solvent on the aggregation, the final concentration of DMSO was fixed at 0.5%.

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