Bioactive Alkaloids from *Illigera luzonensis*

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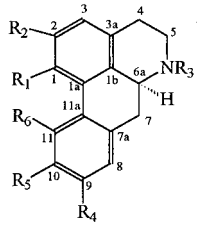
Using antiplatelet aggregation as a guide to fractionation, seven aporphines, actinodaphnine (**1**), *N*-methylactinodaphnine (**2**), launobine (**3**), dicentrine (**4**), *O*-methylbulbocapnine (**5**), hernovine (**7**), and bulbocapnine (**9**), and two oxoaporphines, dicentrinone (**6**) and liriodenine (**8**), were isolated from the stems of *Illigera luzonensis*. Among them, compounds **2**, **4**, **5**, **8**, and **9** were isolated for the first time from this species. Moreover, compounds **1**−**5**, **7**, and **8** showed significant antiplatelet aggregation and compounds **1** and **6** exhibited significant vasorelaxant activities, respectively.

Illigera luzonensis Merr. (Hernandiaceae) is a scandent shrub growing in the southern part of Taiwan.[1] In previous phytochemical studies, actinodaphnine (**1**) and launobine (**3**) have been isolated from the stems and roots of *I. luzonensis*,[2,3] and other alkaloids have been isolated from *Illigera* species.[4−6] Pharmacological studies have revealed that extracts of this species exhibit significant biological activities, e.g., antispasmodic, analgesic, antifebrile, and local anesthetic effects.[7]

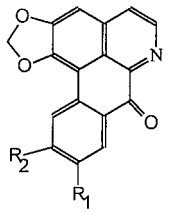
In the course of our studies on bioactive natural substances, the MeOH extract of the stems of *I. luzonensis* was found to exhibit significant vasorelaxation activity as well as inhibitory effects on platelet aggregation induced by several aggregating agents. Bioassay-directed fractionation led to the isolation of nine aporphine alkaloids. Their isolation, identification, and biological activities are reported herein.

The MeOH extract of the stems of *I. luzonensis* was fractionated by solvent partitioning guided by *in vitro* antiplatelet aggregation and vasorelaxation activity tests. Further separation and purification by silica gel column chromatography furnished eight active compounds, actinodaphnine (**1**),[8] *N*-methylactinodaphnine (**2**),[9] launobine (**3**),[3] dicentrine (**4**),[10] *O*-methylbulbocapnine (**5**),[10] dicentrinone (**6**),[10] hernovine (**7**),[11] and liriodenine (**8**),[12,13] in addition to one inactive compound, bulbocapnine (**9**).[14]

The antiplatelet effects of the compounds were studied on the aggregation of washed rabbit platelets induced by either ADP (20 μM), arachidonic acid (AA) (100 μM), collagen (10 μg/mL) or PAF (3.6 nM) and on the vasorelaxing action of rat thoracic aorta induced by either high K^+(80 mM) or norepinephrine (3 μM). The results are shown in Tables 1−3. As indicated in Table



1 $R_1 + R_2$= -OCH_2O- , $R_3 = R_6$ =H, R_4=OH, R_5= OCH_3
2 $R_1 + R_2$= -OCH_2O- , R_3=CH_3, R_4=OH, R_5= OCH_3, R_6=H
3 $R_1 + R_2$= -OCH_2O- , R_3=R_4=H, R_5= OCH_3, R_6=OH
4 $R_1 + R_2$= -OCH_2O- , R_3=CH_3, R_4=R_5= OCH_3, R_6=H
5 $R_1 + R_2$= -OCH_2O- , R_3=CH_3, R_4=H, R_5=R_6=OCH_3
7 R_1= R_6= OCH_3, R_2= R_5= OH, R_3=R_4=H
9 $R_1 + R_2$= -OCH_2O- , R_3=CH_3, R_4=H, R_5= OCH_3, R_6=OH



6 R_1=R_2=OCH_3
8 R_1=R_2=H

1, at a concentration of 100 μg/mL, compound **1** completely inhibited platelet aggregation induced by all four agents (ADP, AA, collagen, and PAF). Compound **2** showed complete inhibition of ADP-, AA-, and collagen-induced platelet aggregation. Compound **4** completely inhibited platelet aggregation induced by AA and was a potent inhibitor of ADP-, collagen-, and PAF-induced platelet aggregation. Compound **5** completely inhibited platelet aggregation induced by AA and collagen. Compound **7** completely inhibited platelet aggregation induced by collagen, and compound **8** was a potent inhibitor of ADP- and collagen-induced platelet ag-

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Table 1. Effects of Test Compounds on the Platelet Aggregation Induced by ADP, AA, Collagen, and PAF in Washed Rabbit Platelets[a]

	aggregation (%)			
compd[b]	ADP (20 μM)	AA (100 μM)	collagen.(10 μg/mL)	PAF (3.6 nM)
1	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
2	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	68.1 ± 6.2*
3	84.2 ± 0.5	11.8 ± 1.7***	29.9 ± 1.3***	70.7 ± 1.1*
4[16]	4.0 ± 3.2***	0.0 ± 0.0***	3.2 ± 2.6***	7.6 ± 3.2***
5	47.9 ± 8.0**	0.0 ± 0.0***	0.0 ± 0.0***	75.1 ± 6.7
7	87.2 ± 2.6	47.0 ± 16.6**	0.0 ± 0.0***	64.1 ± 3.7*
8	5.4 ± 4.4***	40.5 ± 18.1**	5.3 ± 3.1***	84.1 ± 1.7
9	86.8 ± 0.6	89.3 ± 1.7	83.2 ± 1.4	86.6 ± 1.3
aspirin	77.9 ± 1.9	0.0 ± 0.0	87.8 ± 1.5	90.4 ± 1.1
control	79.8 ± 1.8	88.4 ± 1.1	88.5 ± 0.4	90.5 ± 1.1

[a] Platelets were preincubated with DMSO (0.5%, control), aspirin, or test compounds at 37 °C for 3 min, and then ADP (20 μM), AA (100 μM), collagen (10 μg/mL), or PAF (3.6 nM) was added. Percentages of aggregation are presented as means ± SE (n = 3−5). *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the respective control. [b] The concentration of eact test compound was 100 μg/mL, except for **8** (25 μg/mL); aspirin was 25 μg/mL.

Table 2. Effects of Test Compounds on the Platelet Aggregation Induced by AA in Washed Rabbit Platelets[a]

compd	concen (μg/mL)	aggregation (%)
1	100	0.0 ± 0.0***
	50	0.0 ± 0.0***
	20	42.7 ± 7.0**
	10	60.5 ± 5.1*
	5	80.3 ± 2.6
2	100	0.0 ± 0.0***
	20	0.0 ± 0.0***
	10	16.2 ± 8.3***
	5	51.4 ± 10.8*
	2	69.1 ± 7.8
	1	82.5 ± 2.7
4	100	0.0 ± 0.0***
	20	0.0 ± 0.0***
	10	55.1 ± 12.7*
	5	77.7 ± 5.8
	2	83.4 ± 2.1
5	100	0.0 ± 0.0***
	50	0.0 ± 0.0***
	20	50.4 ± 5.8*
	10	72.5 ± 3.6
	5	83.4 ± 2.0
aspirin	100	0.0 ± 0.0***
control		88.4 ± 1.1

[a] Platelets were preincubated with DMSO (0.5%, control), aspirin, or test compounds at 37 °C for 3 min, and then AA (100 μM) was added. Percentages of aggregation are presented as means ± SE (n = 3−5). *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the respective control.

gregation. Compound **3** showed significant inhibition of both AA- and collagen-induced platelet aggregation. Compounds **2** and **4** gave complete inhibitory effects against AA-induced platelet aggregation even at 20 μM (Table 2). Compounds **1** and **5** gave complete inhibitory effects against AA at 50 μM. Aspirin was used as a reference control, and it completely inhibited AA-induced platelet aggregation but not those by other inducers. Thus, the mechanism of antiplatelet effects of these aporphine and oxoaporphine alkaloids appear to be different from that of aspirin, a cyclooxygenase inhibitor.[15]

Contraction of the vascular smooth muscle can be induced by an α_1-adrenoceptor agonist (e.g., norepinephrine) that is blocked by the antagonist prazosin.[16] The high K^+-induced contraction of vascular smooth muscles is the result of an increase in Ca^{2+} influx through voltage-dependent Ca^{2+}-channels that are blocked by dihydropyridines (e.g., nifedipine).[17] As indicated in Table 3, compound **1** significantly inhibited the high K^+- and norepinephrine-induced contractions.

Table 3. Effects of Test Compounds on High Potassium- and Norepinephrine-Induced Phasic and Tonic Contractions of Rat Thoracic Aortas[a]

compd[b]	K^+ (80 mM)	NE (3 μM)-phasic	NE (3 μM)-tonic
1	41.9 ± 2.5**	40.6 ± 1.5**	15.5 ± 0.1***
2	112.9 ± 0.6	225.0 ± 80.0	98.9 ± 6.3
6	111.1 ± 2.8	89.7 ± 8.4	64.0 ± 3.3*
nifedipine	0.0 ± 0.0		
prazosin		0.0 ± 0.0	0.0 ± 0.0
control	100	100	100

[a] Rat aortas were perincubated with various compounds, DMSO (0.1%, control), nifedipine, or prazosin at 37 °C for 15 min, an then high potassium (K^+, 80 mM) or norepinephrine (NE, 3 μM) was added. Percentages of the control contraction were calculated and presented as means ± SE (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the respective control. [b] The concentration of each test compound was 100 μg/mL; nifedipine and prazosin were 1 μM.

Compound **6** slightly inhibited norepinephrine-induced contractions without affecting high K^+-induced contraction. However, compounds **1** and **6** did not affect the resting tension during the 15 min incubation period.

Of the isolated alkaloids, actinodaphnine (**1**) and dicentrine (**4**) are the most potent compounds on the platelet aggregation induced by ADP, AA, collagen, and PAF in washed rabbit platelets. Compound **4** was previously reported to be a platelet aggregation and norepinephrine-induced aortic contraction antagonist isolated from *Lindera megaphylla*.[18] Further **4** was recently reported to be an α_1-adrenoceptor antagonist.[19,20] It is interesting that the six molecules of the aporphines **1**−**5** and **9** are similar in size and the potency of the alkaloids is sensitive to small structural changes in the molecules, suggesting the action at a specific receptor site.

Experimental Section

General Experimental Procedures. The experimental procedures were carried out as previously described.[21]

Plant Material. The stems of *I. luzonensis* were collected from Ping Tung Hsien, Taiwan, in November 1991. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The stems of *I. luzonensis* (2.1 kg) were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and partitioned to yield $CHCl_3$ and aqueous

extracts. Extraction was guided by bioassays in antiplatelet aggregation and vasorelaxation activity tests. The bioactive $CHCl_3$ extract (36.0 g) was chromatographed over silica gel (1500 g) and eluted with mixtures of *n*-hexane/$CHCl_3$/MeOH of increasing polarities to yield 50 fractions (120 mL each). Fractions (98 mg) eluted with 100% $CHCl_3$ were further purified by silica gel column chromatography and preparative TLC [$CHCl_3$−MeOH (50:1)] to give actinodaphnine (**1**) and *N*-methylactinophnine (**2**). Fractions (235 mg) eluted from $CHCl_3$−MeOH (35:1) were further separated and purified by silica gel column chromatography with $CHCl_3$−MeOH (20:1) to give launobine (**3**), dicentrine (**4**), *O*-methylbulbocapnine (**5**), and bulbocapnine (**9**). Fractions (196 mg) eluted from $CHCl_3$−MeOH (20:1) were further separated and purified by silica gel column chromatography with $CHCl_3$−MeOH (15:1) to yield hernovine (**7**). Finally, fractions (90 mg) eluted from $CHCl_3$−MeOH (45:1) were further separated and purified by silica gel column chromatography with $CHCl_3$−MeOH (25:1) to yield dicentrinone (**6**) and liriodenine (**8**).

Actinodaphnine (1): colorless needles (23 mg); mp 206−207 °C; $[\alpha]^{24}_D$ +63° (*c* 1.0, $CHCl_3$); $[M]^+$ 311; spectral data consistent with literature values.[8]

***N*-Methylactinodaphnine (2):** colorless needles (19 mg); mp 220−223 °C; $[\alpha]^{24}_D$ +32° (*c* 0.60, $CHCl_3$); $[M]^+$ 325; spectral data consistent with literature values.[9]

Launobine (3): colorless needles (11 mg); mp 212−213 °C; $[\alpha]^{24}_D$ +190° (*c* 0.90, $CHCl_3$); $[M]^+$ 311; spectral data consistent with literature values.[3]

Dicentrine (4): colorless prisms (18 mg); mp 167−168 °C; $[\alpha]^{24}_D$ +68° (*c* 0.66, $CHCl_3$); $[M]^+$ 339; spectral data consistent with literature values.[10]

***O*-Methylbulbocapnine (5):** colorless needles (8 mg); mp 130−131 °C; $[\alpha]^{24}_D$ +246° (*c* 0.61, $CHCl_3$); $[M]^+$ 339; spectral data consistent with literature values.[10]

Dicentrinone (6): pale yellow needles (21 mg); mp 293−295 °C; $[M]^+$ 335; spectral data consistent with literature values.[10]

Hernovine (7): colorless granular crystals (21 mg); mp 233−234 °C; $[\alpha]^{24}_D$ +140° (*c* 0.52, MeOH); $[M]^+$ 313; spectral data consistent with literature values.[11]

Liriodenine (8): yellow needles (17 mg); mp 285−287 °C; $[M]^+$ 275; spectral data consistent with literature values.[12,13]

Bulbocapnine (9): colorless needles (12 mg); mp 200−202 °C; $[\alpha]^{24}_D$ +225° (*c* 0.85, $CHCl_3$); $[M]^+$ 325; spectral data consistent with literature values.[14]

Assay Methods for Platelet Aggregation and Aortic Contraction. Platelet Aggregation. Rabbit washed platelets were obtained from EDTA-anticoagulated blood according to our previously described method.[22] Platelets were suspended in Tyrode's solution, and the numbers were adjusted to 4.5×10^8 platelets/mL. Aggregation was measured by the turbidimetric method using a Lumi-aggregometer (Chrono-Log Co.). The absorbance of platelet suspension was taken as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation.

Aortic Contraction. Rat thoracic aorta was isolated and cut into rings of about 5 mm in length and mounted in organ baths containing 5 mL of Krebs solution at 37 °C and oxygenated with 95% O_2−5% CO_2. Aortas were equilibrated in the medium for 90 min, and 1 g of resting tension was given. Contractions were recorded isometrically via a force-displacement transducer connected to a Grass polygraph. Aortas were allowed to equilibrate for 15 min with each compound before the generation of a contraction by adding norepinephrine (3 μM) or high K^+ (80 mM). Three min later, the tissue was washed with three changes of Krebs solution before the next test experiment.[23]

Data Analysis. The experimental results are expressed as means ± SE and accompanied by the number of observations. A one-way analysis of variance (ANOVA) was used for multiple comparison, and if there was significant variation between treatment groups, then the mean values for inhibitors were compared with those for control by the Student's *t* test, and *P* values of less than 0.05 were considered to be statistically significant.

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