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BIOACTIVE CONSTITUENTS FROM THE FLOWER BUDS AND PEDUNCLES OF *LINDERA MEGAPHYLLA*

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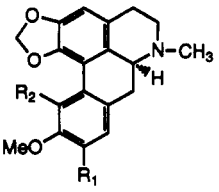
ABSTRACT.—Investigation of the flower buds and peduncles of *Lindera megaphylla* led to the isolation of six aporphine alkaloids, dicentrine, *N*-methylnanigerine, dicentrinone, dehydrodicentrine, *O*-methylbulbocapnine, and cassameridine. Three flavonoid glycosides, isoquercitrin, tiliroside, and rutin, were also isolated. *O*-Methylbulbocapnine inhibited platelet aggregation and also suppressed the contraction of the rat thoracic aorta, but to a much lesser extent than did dicentrine.

In the course of our studies on the development of naturally occurring antiplatelet agents, we have reported that dicentrine [**1**] from the roots of *Lindera megaphylla* possessed antiplatelet aggregation, vasorelaxant, and antiarrhythmic activities (1–3). In our continuing research on this plant, six aporphine alkaloids, namely, dicentrine [**1**], *N*-methylnanigerine, *O*-methylbulbocapnine [**2**] (4), dehydrodicentrine [**3**] (6), dicentrinone [**4**] (4,5), and cassameridine (7), along with three flavonoid glycosides, isoquercitrin, tiliroside, and rutin, were isolated from the flower buds and peduncles of *L. megaphylla*.

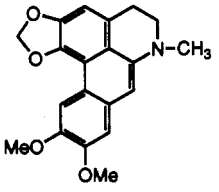
The identifications of alkaloids **2**–**4** and cassameridine were carried out by comparison of their data with published reports (4–7), and the other known compounds were identified by comparison (eims, nmr, ir, tlc) with authentic samples from this laboratory. Copies of the original spectra of these known compounds may be obtained from the senior author.

The effects on platelet aggregation were tested using washed rabbit platelets. As shown in Table 1, compounds **3** and **4**, at 300 μM, caused spontaneous aggregation of platelets in the absence of any inducers. Compound **2**, at 300 μM, exhibited 20 to 30% inhibitory activities on arachidonic acid- and collagen-induced platelet aggregation, but **1** showed complete inhibition at the same concentration.

The inhibitory effects of the isolated compounds on the contractions of the rat aorta induced by norepinephrine and high potassium are shown in Table 2. Compounds **3** and **4**, at 120 μM, had no inhibitory effect. Compound **2** exhibited inhibitory effects, at 60 and 120 μM, on norepinephrine- and high K^+-induced contractions of the rat aorta, while **1** showed a similar inhibition at 9 μM to that of 120 μM of compound **2**. This



1 R_1=OMe, R_2=H
2 R_1=H, R_2=OMe



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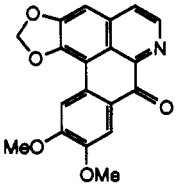

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TABLE 1. Effects of Aporphine Alkaloids **1–4** on the Aggregation of Washed Rabbit Platelets Induced by Arachidonic Acid and Collagen.[a]

Sample	Concentration (μM)	% Aggregation	
		Arachidonic acid	Collagen
Control		90.1±1.0 (9)	89.1±0.9 (8)
1	300	0.0±0.0 (4)[b]	3.1±0.6 (4)[b]
2	300	73.0±7.2 (3)[b]	60.8±8.7 (3)[b]
3	300	spontaneous aggregation	
4	300	spontaneous aggregation	
Aspirin	50	0.0±0.0 (5)[b]	85.4±3.9 (5)

[a]Platelets were preincubated with each purified compound (300 μM) or DMSO (0.5%, control) at 37° for 3 min, and arachidonic acid (100 μM) or collagen (10 μg/ml) was then added. Percentages of aggregation are presented as means ± S.E. (n) and statistical significance was evaluated by one-way ANOVA followed by the Newman-Keuls test. The ANOVA test indicated that the means between both groups (arachidonic acid and collagen) were significantly different ($p<0.01$).

[b]$P<0.01$ as compared with the respective control by using the Newman-Keuls test.

means **1** is over ten times more potent than **2**. Thus, compound **2** is an inhibitor of both platelet aggregation and vasoconstriction. The vasorelaxation is not due to the increase of prostaglandin I_2 production inasmuch as it inhibits arachidonic acid-induced platelet aggregation. Furthermore, when the endothelium of the aorta was denuded the inhibitory effect of compound **2** on vasoconstriction was unaffected. Further experiments are needed to elucidate the mechanisms of action.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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-HX 100 spectrometer.

PLANT MATERIAL.—The flower buds and peduncles of *Lindera megaphylla* Hemsl. (Lauraceae) were collected in July 1992, at the garden of the National Research Institute of Chinese Medicine, Taipei, Taiwan. A voucher specimen is maintained in the herbarium of our institute.

EXTRACTION AND ISOLATION.—The dried

TABLE 2. Inhibitory Effect of Aporphine Alkaloids **1–4** on the Contraction of the Rat Aorta Induced by Norepinephrine and High Potassium.[a]

Sample	Concentration (μM)	Percentage of the control contraction	
		Norepinephrine (3 μM)	KCl (60 mM)
Control		100.0±3.5 (7)	100.0±2.8 (7)
1	9	22.5±3.7 (4)[b]	77.5±2.8 (4)
2	120	22.9±0.5 (3)[b]	55.2±6.2 (3)[b]
2	60	68.1±2.4 (3)[b]	88.5±4.8 (3)
3	120	95.5±3.2 (3)	108.0±3.0 (3)
4	120	112.4±4.2 (3)	102.1±1.5 (3)
Nifedipine	1	96.5±1.5 (3)	0.0±0.0 (4)[b]

[a]Each sample was added 15 min before the addition of norepinephrine or KCl. Means±S.E. (n) are presented and statistical significance was evaluated by one-way ANOVA followed by the Newman-Keuls test. The ANOVA test indicated that the means between both groups (norepinephrine and KCl) were significantly different ($p<0.01$).

[b]$P<0.01$ as compared with the respective control by using the Newman-Keuls test.

flower buds and peduncles (1.5 kg) were refluxed with 95% EtOH and the extract was concentrated *in vacuo* and partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was chromatographed on a Si gel (70–230 mesh) column (6.5×50 cm) and successively eluted with hexane (4 liters), hexane-EtOAc (10:1, 8 liters), (5:1, 4 liters), (1:1, 4 liters), (1:5, 4 liters), (1:10, 4 liters), EtOAc (4 liters), and Me_2CO (4 liters) to yield 9 fractions (4 liters each). Fractions 6 to 8 were combined and rechromatographed on a Si gel (230–400 mesh) column (3.6×46 cm) with $CHCl_3$-MeOH (30:1→10:1) to yield dicentrinone [**4**] (35 mg), dehydrodicentrine [**3**] (24 mg), cassameridine (4 mg), dicentrine [**1**] (125 mg), and *N*-methylnandigerine (17 mg). Fraction 9 was rechromatographed on a Si gel (230–400 mesh) column (2.5×50 cm) and eluted with $CHCl_3$-MeOH (15:1) to yield *O*-methylbulbocapnine [**2**] (20 mg). The aqueous layer was chromatographed on a Diaion HP-20 (1 kg) column eluting with H_2O (10 liters), 20% MeOH (6 liters), and MeOH (6 liters), successively. The MeOH eluate was further chromatographed on a Sephadex LH-20 column (4.5×45 cm) eluting with MeOH to yield isoquercitrin (9 mg), tiliroside (3 mg), and rutin (13 mg).

PLATELET AGGREGATION.—Platelets were obtained as platelet-rich plasma from fresh rabbit blood according to the washing procedures described previously (1,8). Aggregation was measured with an aggregometer (Chrono-Log Co., Havertown, PA) using the turbidimetric method (9) and the absorbance of the platelet suspension was assigned as 0% aggregation and the absorbance of the platelet-free Tyrode solution assigned as 100% aggregation. The final concentration of the solvent (DMSO) was fixed at 0.5%.

AORTIC CONTRACTION.—The aorta was isolated from Wistar rats (250 to 300 g) according to the procedures described previously (1). Vessels were cut into rings of about 5-mm length and mounted in organ baths containing 5 ml of Krebs solution, maintained at 37°; and bubbled with a 95% O_2 and 5% CO_2 mixture. Two stainless steel hooks were inserted into the arotic lumen, one was fixed while the other was connected to a transducer. The aorta was equilibrated in the medium for 90 min with three changes of Krebs solution and maintained under an optimal tension of 1 g before specific experimental protocols were initiated; contractions were recorded isometrically via a force-displacement transducer connected to a Gould polygraph (Model 2400). The final concentration of the solvent (DMSO) was fixed at 0.1%.

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