Cardiovascular Effects of Aspidofractinine-Type Alkaloids from *Kopsia*

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Intravenous injection of the aspidofractinine alkaloid, kopsingine (**1**, 0.2−10.0 mg/kg) from *Kopsia teoi*, produced dose-related decreases in the mean arterial blood pressure and heart rate in anesthetized spontaneously hypertensive rats, which were similar to those seen in normotensive controls. Minor modifications in the molecular structure of kopsingine, as in kopsaporine (**2**, the 12-demethoxy derivative of kopsingine) and 14,15-dihydrokopsingine (**4**), did not significantly alter the hypotensive responses, whereas a more drastic change in the structure, as in the heptacyclic kopsidine A (**3**) and the 3-to-17 oxo-bridged compound **5**, resulted in an increase in blood pressure. The antihypertensive effects of kopsingine (**1**) and its congeners (**2** and **4**) along with the pressor effects produced by the heptacyclic oxo-bridged compounds (**5** and **3**) could be ascribed to central as well as peripheral actions.

The genus *Kopsia* (Apocynaceae) comprises some 30 species of shrubs and trees distributed mainly over Southeast Asia, India, and China, of which about 18 species occur in Malaysia.[1,2] The phytochemistry of this genus has received considerable attention, which has resulted in a considerable number of new natural products with novel structures and useful bioactivities.[3−14] Some medicinal uses have been reported, such as for the cultivated species *K. officinalis*, which is used in China for the treatment of rheumatoid arthritis, dropsy, and tonsilitis,[2] and in Malaysia, the roots of several *Kopsia* species are known to be used for poulticing ulcerated noses in tertiary syphilis.[15] As part of an investigation dealing with hypotensive principles from Malaysian plants, we have previously demonstrated that gambirine (**7**), a tetracyclic heteroyohimbine alkaloid found in abundance in *Uncaria callophylla*, substantially lowered the arterial blood pressure in normotensive anesthetized rats.[16] We have also found in preliminary studies that crude alkaloidal mixtures from several *Kopsia* species including *K. profunda, K. larutensis*, and *K. teoi* showed antihypertensive activity when tested in normotensive rats.[17] The antihypertensive activity can be traced to the presence of the various types of indole alkaloids occurring in these plants. Kopsingine (**1**) and kopsaporine (**2**, the 12-demethoxy derivative of kopsingine) are indole alkaloids of the aspidofractinine-type and constitute the major alkaloids in several Malaysian *Kopsia* species including *K. teoi* L. Allorge. Kopsingine (**1**) and kopsaporine (**2**) were first isolated from *K. singapurensis*.[18] The constitution of these alkaloids was then established based on degradative experiments carried out on kopsingine. Their structures have been recently confirmed by a detailed NMR and X-ray study on kopsingine, the predominant alkaloid present in *K. teoi*.[19] In addition to these alkaloids, many novel minor alkaloids as well as semisynthetic aspidofractinines have also been recently documented in the literature.[6,8,9,19−23] Relatively little is known about the pharmacological activities of these aspidofractinine-type alkaloids, and because the alkaloidal fraction of *K. teoi* showed antihypertensive activity in preliminary studies, we decided to obtain quantitative information regarding the cardiovascular effects of these alkaloids.

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Results and Discussion

Kopsingine (**1**) and kopsaporine (**2**) are major alkaloids obtained from *Kopsia teoi*,[19] while kopsidine A (**3**) is a minor alkaloid isolated from the leaves of the same plant.[9] Kopsidine A (**3**) and the 3-to-17 oxo-bridged compound **5** can also be obtained via an electrochemically mediated semisynthesis from **1**,[23] whereas 14,15-dihydrokopsingine (**4**) is readily available via catalytic hydrogenation (H_2, Pd/C) of kopsingine (**1**).

The basal mean arterial blood pressure (MABP) and heart rate (HR) averaged, respectively, in anesthetized spontaneously hypertensive rats (SHR) 179.4 ± 2.6 mmHg and 345.4 ± 4.2 bpm (beats per minute), and in anesthetized Wistar−Kyoto (WKY) rats 123.9 ± 2.7 mmHg and 360.0 ± 6.7 bpm on the day of the experiment.

Intravenous (iv) administration of 0.2−10.0 mg/kg of kopsingine (**1**) and kopsaporine (**2**) resulted in linear dose-related decreases in MABP and concomitant falls in HR in anesthetized SHR (Figure 1). BP and HR started to fall within 5 s of administration of kopsingine (**1**) and kopsaporine (**2**) and reached their lowest levels between 10 and 30 s. The hypotensive responses to 10 mg/kg of **1** and **2** lasted 3.9 ± 1.0 min and 3.6 ± 1.0 min, respectively. The decreases in MABP and HR induced by kopsingine and kopsaporine in the SHR were not significantly different compared with their respective normotensive WKY control groups (Figure 1) when tested by analysis of variance. In like manner, iv administration of 14,15-dihydrokopsingine (**4**, 0.2−10.0 mg/kg) also produced a dose-related decrease in MABP, which was accompanied by bradycardia in the SHR (Figure 2). The depressor responses induced by dihydrokopsingine (**4**) were not significantly different from the responses induced by kopsingine (**1**) and kopsaporine (**2**) in the SHR. In contrast to the effects of the

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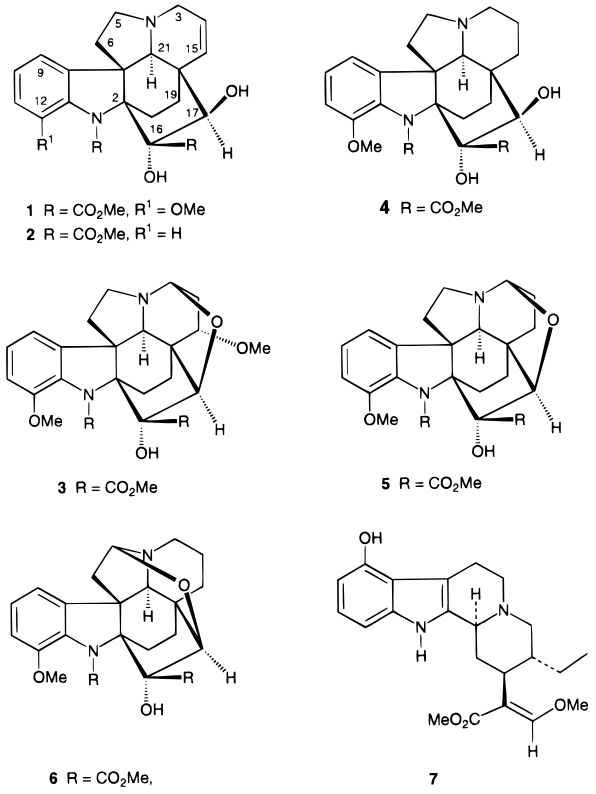

above three alkaloids, iv injections of kopsidine A (**3**) and compound (**5**) in anesthetized SHR, at doses ranging from 0.2 and 10.0 mg/kg, produced small, dose-related pressor responses (Figure 2). The pressor responses were transient and rapid in onset, lasting for only 30−50 s. The increase in BP induced by compounds **3** and **5** did not differ significantly when tested by analysis of variance. Concomitant with the increases in MABP, decreases in HR occurred in the SHR at all the six dose levels of **3** and **5** after intravenous injections (Figure 2). Compound **5** induced significantly much greater reductions in HR compared to **3** in the SHR, whether the values were expressed as an absolute ($F_{1,70} = 13.95$, $p = 0.0004$) or as a percentage change ($F_{1,70} = 11.84$, $p = 0.0010$) of the initial value. On the other hand, the reductions in HR induced by compound **5** were not significantly different from those induced by dihydrokopsingine (**4**). The similiar volume of vehicles used to dissolve the alkaloids or the drugs had no apparent effect on BP and HR when injected intravenously.

Pretreatment of a group of SHR with iv hexamethonium (20 mg/kg), at a dose known to inhibit ganglionic transmission in the rat,[24] 10 min before iv kopsingine (**1**, 10 mg/kg), significantly attenuated the antihypertensive and bradycardic actions of kopsingine (**1**, $p < 0.01$ in both cases, Student's *t*-test, Figure 3). Similarly, pretreatment with hexamethonium also significantly attenuated the depressor response to 10 mg/kg of iv dihydrokopsingine (**4**) (Student's *t*-test, $p < 0.05$). Although dihydrokopsingine-induced bradycardia tended to be attenuated by hexamethonium pretreatment, the difference was not statistically significant (Figure 3). In contrast, the pressor responses to 10 mg/kg of iv **5** were significantly potentiated by pretreatment with hexamethonium ($p < 0.05$, Student's *t*-test), while the bradycardic reponses were not significantly altered (Figure 3).

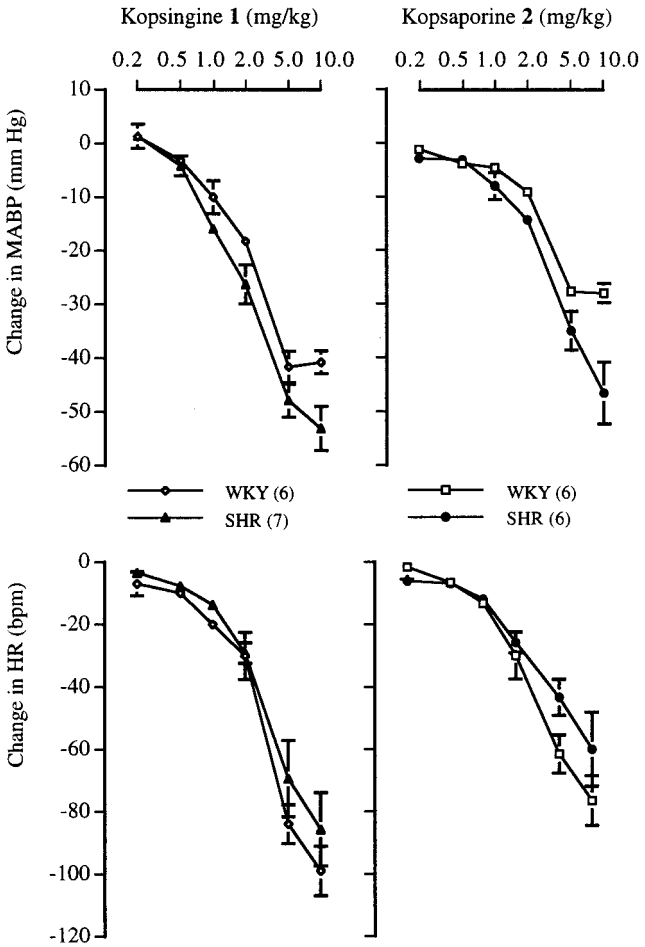

Figure 1. Effects of intravenous injections of 0.2−10.0 mg/kg of kopsingine (**1**) and kopsaporine (**2**) on MABP (mmHg) and HR (bpm) in SHR and WKY. Mean values are shown; bars indicate SEM. Number of rats is in parentheses.

Hexamethonium (20 mg/kg, iv) given alone produced a significant decrease in MABP from 175.0 ± 5.0 to 92.0 ± 2.9 mmHg ($p < 0.001$, Student's *t*-test; $n = 6$), which was maximal within 1 to 1.5 min after injection of the drug, accompanied by a reduction in HR from 352.6 ± 5.8 to 313.6 ± 11.4 bpm ($p < 0.01$, Student's *t*-test; $n = 6$). The MABP and HR at 10 min after hexamethonium (104.8 ± 6.0 mmHg and 296.8 ± 11.1 bpm, respectively) remained significantly below the predrug levels (MABP: $p < 0.001$; HR: $p < 0.01$, Student's *t*-test).

In another study, atropine (2 mg/kg, iv), at a dose known to block muscarinic receptors in the rat, was injected 10 min before iv kopsingine (**1**, 10 mg/kg). This pretreatment did not significantly alter the magnitude of the hypotensive response to kopsingine (the maximal change in MABP induced by iv kopsingine before and after atropine were −68.5 ± 1.0 mmHg and −51.0 ± 7.7 mmHg, respectively), but the bradycardic response to kopsingine was significantly antagonized (the maximal change in heart rate induced by iv kopsingine before and after atropine were −101.0 ± 5.0 bpm and −71.5 ± 7.5 bpm, respectively; $p < 0.05$, Student's *t*-test; $n = 6$). Atropine, when injected alone at 2 mg/kg, produced a maximal fall in MABP from 171.6 ± 4.2 to 155.6 ± 4.3 mmHg ($p < 0.05$, Student's *t*-test; $n = 6$) within 24 to 26 s after administration, without affecting the HR (369.0 ± 8.0 to 371.0 ± 10.5 bpm); however, 10 min after atropine administration, both the MABP and HR (160.9

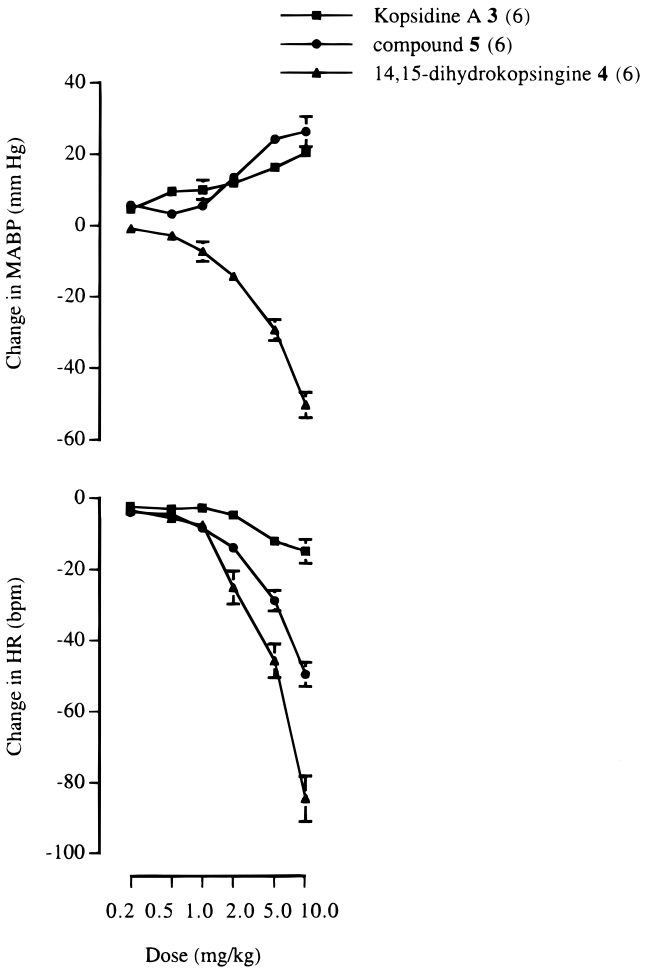

Figure 2. Effects of intravenous injections of 0.2−10.0 mg/kg of 14,15-dihydrokopsingine (**4**), kopsidine A (**3**), and compound **5** on MABP and HR in SHR. Mean values are shown; bars indicate SEM. Number of rats is in parentheses.

± 3.2 mmHg and 368.0 ± 9.0 bpm, respectively) were not significantly different from that of predrug levels.

In other experiments, prior treatment with the adrenergic blocker, phentolamine (3 mg/kg, iv), caused reversal of the depressor actions of 10 mg/kg of iv kopsingine (**1**) and dihydrokopsingine (**4**) into pressor actions. The maximal change in MABP in response to **1** and **4** before pretreatment were, respectively, −56.5 ± 7.8 and −44.7 ± 6.6 mmHg and at 10 min after pretreatment were, respectively, 30.7 ± 2.3 and 18.2 ± 2.8 mmHg. In contrast, the bradycardic effects of these alkaloids were not significantly altered by phentolamine pretreatment. The maximal change in HR in response to **1** and **4** before pretreatment were, respectively, −76.0 ± 7.3 and −49.2 ± 7.0 bpm and at 10 min after pretreatment were, respectively, −66.7 ± 10.0 and −52.5 ± 11.2 bpm.

Phentolamine (3 mg/kg, iv) given alone resulted in a rapid and marked decrease in MABP from 179.9 ± 5.2 to 106.7 ± 7.2 mmHg ($p < 0.001$, Student's *t*-test; $n = 6$), accompanied by an insignificant increase in HR from 342.0 ± 11.4 to 372.5 ±14.6 bpm. The MABP and HR at 10 min after phentolamine (108.4 ± 5.1 mmHg and 356.7 ± 7.4 bpm, respectively) remained significantly reduced compared to the predrug levels ($p < 0.001$ in both cases).

Our study of the antihypertensive activity observed in the basic fraction of *K. teoi* initially focused on the

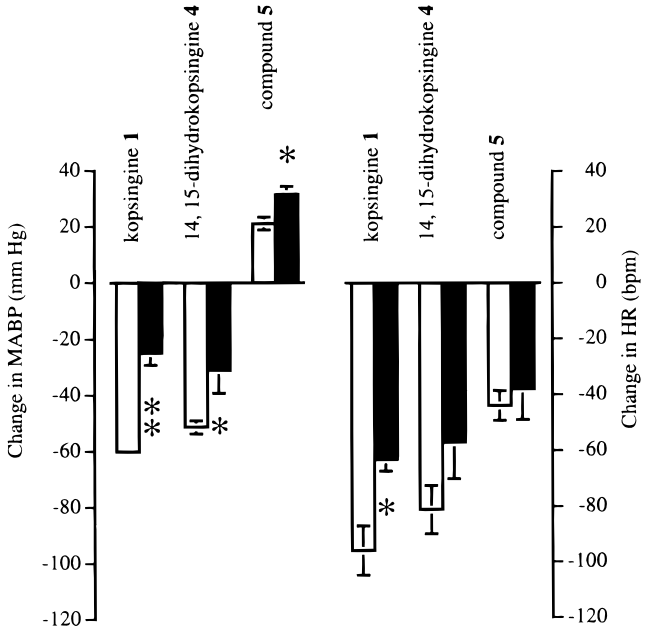

Figure 3. Maximal changes in MABP and HR in response to intravenous injections of kopsingine (**1**, 10 mg/kg), 14,15-dihydrokopsingine (**4**, 10 mg/kg), and compound **5** (10 mg/kg) before □ and after ■ ganglionic blockade with hexamethonium (20 mg/kg, iv) in SHR. Values represent means of 6 rats in each group; bars, SEM. *$p < 0.05$, **$p < 0.01$ significantly different from values obtained before hexamethonium pretreatment.

predominant alkaloid present, that is, kopsingine (**1**), which showed dose-dependent hypotensive activity in both SHR and normotensive WKY rats. Because the 12-demethoxy derivative **2** was also available in sufficient quantity, and various other derivatives were accessible, either by simple reactions (**4**) or by previously reported semisynthesis from kopsingine (**1**) (**3** and **5**),[23] we were presented with the opportunity of evaluating how modifications in the basic structure of the aspidofractinine framework could affect the cardiovascular effects of these indole alkaloids. As the results have shown, it appears that minor modifications in the aspidofractinine skeleton, such as removal of the aromatic methoxy substituent (**2**) or removal of unsaturation in the piperidine ring (**4**), did not appreciably alter the cardiovascular effects of these compounds when compared to kopsingine (**1**). These compounds also show essentially similar dose-dependent hypotension and bradycardia similar to that elicited by kopsingine (**1**) (Figure 1). A more drastic modification of the molecular skeleton, however, such as an intramolecular closure to form the heptacyclic oxo-bridged derivatives **3** and **5** produced a pronounced alteration in the cardiovascular effect of kopsingine (**1**); these compounds now elicited small elevations in BP (Figure 2) instead of the depressor responses observed for **1**.

In a preliminary attempt to further define the origin of these responses, we investigated the effects of hexamethonium, atropine, and phentolamine pretreatment. Autonomic ganglionic blockade by hexamethonium apparently attenuated the depressor and bradycardic effects of **1**, **4**, and presumably **2**. It appears that these structurally related alkaloids may, at least in part, exert their cardiovascular effects by acting at a site within the central nervous system, for the integrity of an intact

peripheral nervous system is required. As the bradycardic effect of **1** was significantly attenuated by atropine, the efferent vagus nerve thus appears to contribute to the bradycardia seen. The observation that atropine had no effect on **1**-induced hypotension suggests that the fall in blood pressure was not secondary to decreased cardiac output owing to the bradycardia; however, because **1** and **4** still had substantial residual effects in causing moderate decreases in MABP and HR following ganglionic blockade, the involvement of peripheral effects or nonspecific effects in mediating the remaining responses cannot be completely ruled out. In this respect, it is interesting to observe that the depressor effects of intravenous **1** and **4** were completely abolished by pretreatment with iv phentolamine, but only minor effects were observed on the cardiac component. These results indicate that the alkaloid-induced decreases in BP appear to be mediated by peripheral α-adrenoceptors and lend further support to the notion that these alkaloids may also have peripheral actions. The pressor effects of **1** and **4**, however, which were unmasked after phentolamine pretreatment, are unlikely to be due to a baroreceptor-reflex action causing vasoconstriction, as the peripheral α-adrenoceptors have already been blocked by phentolamine.

The observations that inhibition of peripheral ganglionic transmission with hexamethonium caused a further increase in pressor response to **5** (and presumably **3**), but had no effect on bradycardia, favor direct actions of these oxo-bridged heptacyclic alkaloids on the periphery. In addition, these alkaloids may also inhibit sympathetic tone to the periphery as the pressor effect of **5** was potentiated following ganglionic blockade.

Our results therefore show that kopsingine (**1**) and its congeners **2** and **4** (in which there have been minor modifications to the basic aspidofractinine structure) were equally effective in lowering the arterial BP and HR in a dose-dependent manner. Although decreases of BP and HR in parallel may be due to an effect on central nervous function, it is also possible that the alkaloids produced a vasodilation with a direct cardioinhibition. On the other hand, the heptacyclic oxo-bridged compounds **3** and **5** (in which there have been a more drastic modification of the molecular framework), produced small, dose-dependent pressor responses that could be due to both central and peripheral actions inasmuch as the pressor effects of **5** were not inhibited after ganglionic blockade but instead became more pronounced.

Experimental Section

General Experimental Procedures. All melting points were uncorrected. Mass spectra were obtained on a VG ProSpec spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-GSX 270 spectrometer at 270 and 67.8 MHz, respectively.

Plant Material. Details of collection of plant material (*Kopsia teoi* L. Allorge), deposition of voucher specimens, and extraction and isolation of alkaloids have been described previously.[19] The structures of kopsingine (**1**), kopsaporine (**2**), and kopsidine A (**3**) have been established by spectral methods and, in the case of kopsingine, confirmed by X-ray analysis.[9,19,23]

Synthesis of Compounds. 14,15-Dihydrokopsingine (**4**), was prepared via catalytic hydrogenation of kopsingine as follows. Kopsingine (**1**, 500 mg, 1 mmol) in 15 mL CH_2Cl_2 was stirred over Pd/C (150 mg) under a hydrogen atmosphere (H_2 balloon) at room temperature for 2 h. The mixture was then filtered over Si gel to provide **4** in quantitative yield.

14,15-Dihydrokopsingine 4: mp 239 °C (dec); EIMS, *m/z* (rel int) 458 [M$^+$] (100), 430 (10), 399 (24), 370 (42), 355 (14), 341 (21), 315 (20), and 301 (38); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.93 (br t, J = 12 Hz, H-19), 1.28 (dd, J = 14, 7, H-15), 1.38 (ddd, J = 13, 12, 8, H-18), 1.49−1.65 (m, H-19), 1.65−1.78 (m, H-14), 1.70−1.77 (m, H-6), 1.99−2.08 (m, H-15 and H-18), 2.15−2.20 (m, H-14), 2.52−2.61 (m, H-3 and H-5), 2.68 (d, J = 2 Hz, H-21), 2.78 (dd, J = 8, 6 Hz, H-5), 3.11−3.22 (m, H-3 and H-6), ca. 3.78 (H-17), 3.78 (s, CO_2Me), 3.82 (s, 12-OMe and NCO_2Me), 5.63 (s, 16-OH), 6.79 (d, J = 7 Hz, H-11), 6.82 (d, J = 8 Hz, H-9), 7.02 (dd, J = 8, 7 Hz, H-10), and 8.07 (d, J = 6 Hz, 17-OH); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 24.3 (C-14), 27.3 (C-18), 28.8 (C-19), 35.2 (C-15), 35.7 (C-20), 40.9 (C-6), 49.0 (C-3), 49.1 (C-5), 51.8 (CO_2Me), 52.9 (NCO_2Me), 56.0 (12-OMe), 57.1 (C-7), 70.0 (C-21), 76.2 (C-2), 80.9 (C-16), 85.2 (C-17), 111.8 (C-9), 113.0 (C-11), 124.8 (C-10), 128.3 (C-13), 144.6 (C-8), 149.3 (C-12), 155.5 (NCO_2Me), and 172.1 (CO_2Me).

Compound **5** was prepared via electrochemical oxidation of 14,15-dihydrokopsingine (**4**) as follows:[23] compound **4** (200 mg, 0.4 mmol) in 40 mL of mixed solvent (30% CH_2Cl_2−MeCN) containing Et_4ClO_4 (0.1M) and 2,6-lutidine (86 mg, 0.8 mmol) was placed in a divided cell under nitrogen. The anodic potential was maintained at 0.84 V vs Ag/AgCl and the electrolysis continued until 2.1 F mol^{-1} had been transferred. The progress of the electrolysis was also monitored by TLC as well as cyclic voltammetry. The solution was then evaporated to dryness, and CH_2Cl_2 (12 mL) was added. The precipitated electrolyte was then filtered off, and the residue was washed with CH_2Cl_2. The solvent was then removed under reduced pressure, and the resulting mixture containing compounds **5** and **6** was filtered over Si gel followed by further partitioning using centrifugal chromatography (SiO_2, 1% MeOH−$CHCl_3$) to afford compounds **5** (20%) and **6** (55%).

Compound 5: EIMS, *m/z* (rel int) 456 [M$^+$] (42), 428 (34), 427 (56), and 338 (54); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.16−1.20 (m, H-18), 1.40−1.59 (m, H-14 and H-15), 1.61−1.70 (m, H-6 and H-18), 1.94−2.00 (m, H-15 and 2 × H-19), 2.04−2.08 (m, H-14), 2.98−3.02 (m, H-6), 3.05−3.15 (m, 2 × H-5), 3.40 (d, J = 2 Hz, H-21), 3.78 (s, CO_2Me), 3.82 (s, 12-OMe), 3.82 (s, NCO_2Me), 3.87 (d, J = 2 Hz, H-17), 4.38 (t, J = 2 Hz, H-3), 6.06 (s, 16-OH), 6.79 (d, J = 7 Hz, H-11), 6.82 (d, J = 8 Hz, H-9), and 7.00 (dd, J = 8, 7 Hz, H-10); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 24.9 (C-18), 25.8 (C-14), 26.7 (C-15), 28.0 (C-19), 32.8 (C-20), 40.7 (C-6), 52.2 (CO_2Me), 53.0 (NCO_2-Me), 53.1 (C-5), 56.2 (12-OMe), 59.0 (C-7), 65.6 (C-21), 76.3 (C-2), 77.2 (C-16), 81.9 (C-17), 86.3 (C-3), 112.2 (C-9), 112.9 (C-11), 124.9 (C-10), 128.2 (C-13), 143.8 (C-8), 148.8 (C-12), 156.0 (NCO_2Me), and 171.4 (CO_2Me).

Animals. Male SHR and male WKY normotensive rats of 2.5−3.5 months of age were anesthetized with the sodium salt of thiobutabarbital (Inactin, RBI; 100

mg/kg ip). This anesthetic was chosen as it has no depressor effects in rats.[25]

Recording of Blood Pressure. The trachea was cannulated, and the animal respired spontaneously with moist oxygen supplement. The left jugular vein was cannulated for intravenous injections of alkaloids. Systemic BP was measured with a Statham p23Db pressure tranducer connected to a cannula (PE 50, Clay Adams) inserted into the left carotid artery. The tranducer was connected to the Grass Polygraph (Model 7D). MABP was calculated as diastolic BP + 1/3 pulse pressure. HR was obtained directly from the pulsatile BP. Body temperature was maintained at 37.5 °C with a heating lamp. About 1 h was allowed to elapse after surgery for equilibration of the preparation.

Calculation. Responses were measured as maximal changes in MABP and HR from the baseline levels. The changes in MABP and HR were expressed as mmHg and bpm, respectively. All data are given as mean ± standard error of the mean. Two-way analysis of variance[26] was used for comparison of the maximal changes in MABP and HR between groups of animals. Student's *t*-test for paired values was used to compare the maximal responses to intravenous injections of alkaloids obtained before and after pretreatment with hexamethonium, atropine, or phentolamine. A probability of 0.05 or less was considered significant.

Drugs. The drugs used in this study were atropine sulfate (Sigma), hexamethonium bromide (Sigma), and phentolamine mesylate (RBI). All the drug solutions were prepared in 0.9% saline.

The alkaloids were solubilized in 0.2 M HCl, and the pH was adjusted to ca. 5 with 0.2 M NaOH. Sodium chloride salt was finally added to obtain a solution of 0.9% in NaCl. Further dilutions were made with 0.9% saline.[16]

Alkaloid at doses of 0.2, 0.5, 1.0, 2.0, 5.0, and 10 mg/kg was administered intravenously. After administration of each dose, BP was allowed to return to baseline before injection of the next dose. The effects of hexamethonium (20 mg/kg, iv), atropine (2 mg/kg, iv), and phentolamine (3 mg/kg, iv) on the cardiovascular responses to the alkaloids were examined in separate groups of animals. A dose of 10 mg/kg of alkaloid was administered intravenously before and 10 min after pretreatment with hexamethonium, atropine, or phentolamine. The total volume of injection was always 0.3 mL. Each test group consisted of 6 or 7 animals.

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References and Notes

(1) Markgraf, F. *Blumea* **1972**, *20*, 416−425.
(2) Sevenet, T.; Allorge, L.; David, B.; Awang, K.; Hadi, A. H. A.; Kan-Fan, C.; Quirion, J. C.; Remy, F.; Schaller, H.; Teo, L. E. *J. Ethnopharmacol.* **1994**, *41*, 147−183.
(3) Kam, T. S.; Yoganathan, K.; Koyano, T.; Komiyama, K. *Tetrahedron Lett.* **1996**, *37*, 5765−5768.
(4) Kam, T. S.; Yoganathan, K.; Chen, W. *Tetrahedron Lett.* **1996**, *37*, 3603−3606.
(5) Kam, T. S.; Yoganathan, K.; Li, H. Y. *Tetrahedron Lett.* **1996**, *37*, 8811−8814.
(6) Kam, T. S.; Yoganathan, K.; Chen, W. *J. Nat. Prod.* **1996**, *59*, 1109−1112.
(7) Kam, T. S.; Yoganathan, K.; Chuah C. H. *Tetrahedron Lett.* **1995**, *36*, 759−762.
(8) Kam, T. S.; Yoganathan, K.; Chuah, C. H. *Tetrahedron Lett.* **1994**, *35*, 4457−4460.
(9) Kam, T. S.; Yoganathan, K.; Chuah, C. H. *Tetrahedron Lett.* **1993**, *34*, 1819−1822.
(10) Uzir, S.; Mustapha, A. M.; Hadi, A. H. A.; Awang, K.; Wiart, C.; Gallard, J. C.; Pais, M. *Tetrahedron Lett.* **1997**, *38*, 1571−1574.
(11) Awang, K.; Sevenet, T.; Hadi, A. H. A.; David, B.; Pais, M. *Tetrahedron Lett.* **1992**, *33*, 2493−2496.
(12) Homberger, K.; Hesse, M. *Helv. Chim. Acta* **1982**, *65*, 2548−2557.
(13) Homberger, K.; Hesse, M. *Helv. Chim. Acta* **1984**, *67*, 237−248.
(14) Ruangrungsi, N.; Likhitwitayawuid, K.; Jongbunprasert, V.; Ponglux, D.; Aimi, N.; Ogata, K.; Yasuoka, M.; Haginiwa, J.; Sakai, S. *Tetrahedron Lett.* **1987**, *28*, 3679−3682.
(15) Burkhill, I. H. *A Dictionary of the Economic Products of the Malay Peninsula*; Ministry of Agriculture and Cooperatives: Kuala Lumpur, Malaysia, 1966.
(16) Mok, J. S. L.; Chang, P.; Lee, K. H.; Kam, T. S.; Goh, S. H. *J. Ethnopharmacol.* **1992,** *36*, 219−223.
(17) Mok, J. S. L.; Yoganathan, K.; Kam, T. S. *Abstracts of the 11th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology*, Langkawi, Kedah, Malaysia, 1995.
(18) Thomas, D. W.; Biemann, K.; Kiang, A. K.; Amarasingham, R. D. *J. Am. Chem. Soc.* **1967**, *89*, 3235−3242.
(19) Kam, T. S.; Yoganathan, K.; Chuah, C. H.; Chen Wei *Phytochemistry* **1993,** *32*, 1343−1346.
(20) Kam, T. S.; Yoganathan, K. *Phytochemistry* **1996**, *42*, 539−541.
(21) Kam, T. S.; Yoganathan, K. *Nat. Prod. Lett.* **1997**, *10*, 69−74.
(22) Varea, T.; Kan, C.; Reny, F.; Sevenet, T.; Quiron, J. C.; Husson, H. P.; Hadi, H. A. *J. Nat. Prod.* **1993**, *56*, 2166−2169.
(23) Kam, T. S.; Lim, T. M.; Tan, G. H. *Tetrahedron Lett.* **1995,** *36*, 1327−1330.
(24) Hahn, R. A. *Life Sci.* **1981,** *29*, 2501−2509.
(25) Munoz-Ramirez, H.; Khosla, M. C.; Bumpus, F. M.; Khairallah, P. A. *Am. J. Physiol.* **1978,** *234*, H477−H453.
(26) SAS User's Guide Statistic, Version 5; SAS Institute Inc.: Cary, NC, 1985.

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