Chingazumianine, a Novel Dichlorinated Alkaloid from *Corydalis koidzumiana*

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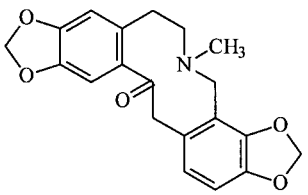
A dichlorinated alkaloid with a novel skeleton, chingazumianine (**1**), was isolated from the herb *Corydalis koidzumiana*, and its structure elucidated by spectroscopic data and X-ray crystallographic analysis. In citrate-treated human platelet-rich plasma (PRP), known compounds, *i.e.*, protopine (**2**), (±)-tetrahydropalmatine (**3**), and palmatine (**4**), isolated from this plant, showed significant inhibition of secondary aggregation induced by adrenaline in a concentration-dependent manner, suggesting that the antiplatelet effects of these compounds is mainly due to an inhibitory effect on thromboxane formation.

1. Introduction. – Previously, several alkaloids belonging to tetrahydroprotoberberine, benzophenanthridine, protopine, morphinandienone, and benzylisoquinoline types from *Corydalis koidzumiana* OHWI (Fumariaceae) have been reported [1–4]. In a continued search for novel bioactive constituents from plants, a dichlorinated alkaloid with a novel skeleton, chingazumianine (**1**), and four known alkaloids, protopine (**2**), (±)-tetrahydropalmatine (**3**), (+)-corybulbine, and palmatine (**4**), were isolated. In the present paper, the structure characterization of **1**, elucidated by spectroscopic methods and X-ray analysis, and the antiplatelet effects of the major constituents **2**–**4** are reported.

2. Results and Discussion. – Compound **1**, a colorless prism, gives a positive test with *Dragendroff*'s reagent and possesses the molecular formula $C_{23}H_{23}Cl_2NO_6$, as determined from HR-FAB-MS (*m*/*z* 480.0973 ($[M+1]^+$), ±0.8 amu error), LR-MS (M^+ at *m*/*z* 479) with the characteristic isotope distribution for Cl_2 [5], and from ^{1}H- and ^{13}C-counting in the NMR spectra. IR Absorptions were indicative of a conjugated C=O group (1680 and 1660 cm^{-1}) and an aromatic ring (1600 and 1560 cm^{-1}). The ^{13}C-NMR spectrum of **1** exhibited signals for all 23 C-atoms, among which were 2 C=O groups of an acetophenone and amide moiety, 12 aromatic C-atoms, 2 tetrasubstituted olefinic C-atoms (>C=CCl_2) [6], 2 aliphatic C-atoms (phenyl–CH_2– and –CH_2N<), 4 MeO groups, and 1 Me group of the acetophenone moiety (*Table 1*). The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) showed that **1** had two tetrasubstituted benzene moieties.



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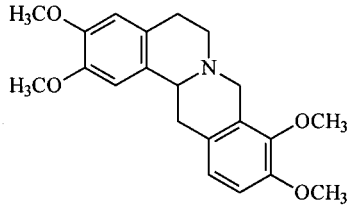

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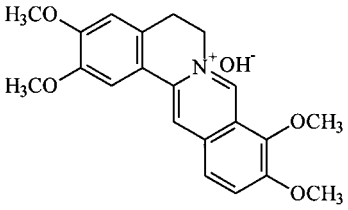

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Analysis of ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY (*Fig. 1*) data established the partial structures **a**–**c** and their connectivities. Consequently, chingazumianine (**1**) was characterized as 2-[1-(6-acetyl-2,3-dimethoxyphenyl)-2,2-dichloroethenyl]-3,4-dihydro-6,7-dimethoxyisoquinoline-1(2*H*)-one (**1**).

The ^{1}H-NMR spectrum of **1** showed 2 CH_2 signals at δ 2.80 (*m*, 1 H) and 3.08 (*m*, 1 H), and δ 3.60 (br. *s*, 1 H) and 3.90 (*m*, 1 H), an acetyl signal at δ 2.56 (*s*), 4 MeO signals at δ 3.85 (*s*, 3 H) and 3.90 (*s*, 9 H), and 4 aromatic-proton signals at δ 6.60 (*s*, 1 H), 6.95 (*d*, J = 8.4, 1 H); 7.52 (*d*, J = 8.4, 1 H), and 7.59 (*s*, 1 H). The

Table 1. *^{13}C-* (100 MHz) *and ^{1}H-NMR* (400 MHz) *Data* (δ in ppm, J in Hz) *for Chingazumianine* (**1**) *in $CDCl_3$*[a]). Arbitrary numbering.

	δ (C)	δ (H)		δ (C)	δ (H)
H–C(1)	109.0	6.60 (*s*)	C(2′)	147.8	
C(2)	148.0		C(3′)	156.6	
C(3)	152.0		H–C(4′)	111.7	6.95 (*d*, J = 8.4)
H–C(4)	110.9	7.59 (*s*)	H–C(5′)	125.6	7.52 (*d*, J = 8.4)
C(4a)	132.7		C(6′)	133.1	
C(5)	161.9		Me*CO*	199.0	
CH_2(7)	47.7	3.60 (br. *s*) 3.90 (*m*)	*Me*CO	28.3	2.56 (*s*)
CH_2(8)	28.2	2.80 (*m*) 3.80 (*m*)	Cl_2C=*C*	133.1[b])	
C(8a)	122.1		Cl_2*C*=C	128.2[b])	
C(1′)	128.02		MeO	55.9, 56.0, 61.2	3.85, 3.90

[a]) All assignments were confirmed by ^{1}H,^{1}H COSY, HMQC, HMBC, and NOESY data. [b]) Attributions may be reversed.

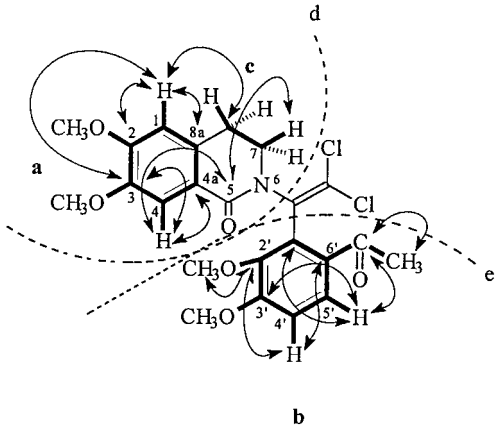


Fig. 1. *Structure of* **1**, *partial structures* **a**–**c** (in boldface) *showing some key* $^{1}H,^{13}C$ *long-range correlations* (HMBC), *and fragmentation patterns.* Bold and dotted lines represent $^{1}H,^{1}H$ and $^{1}H,^{13}C$ spin systems identified by $^{1}H,^{1}H$ COSY, HMQC, and HMBC experiments. Arbitrary numbering.

HMBC *H*–C(1)/C(8), H–C(7)/C(5), and H–C(4)/C(5) confirmed the connectivity of the partial structures **a** and **c** (arbitrary numbering). The ^{1}H- and ^{13}C-NMR signals of **1** were assigned by COSY, HMQC, HMBC, and NOESY data. In the EI-MS of **1**, the base peak at *m*/*z* 444 was attributed to the fragment $[M - Cl]^+$. This and characteristic peaks at *m*/*z* 408 ($[444 - Cl - H]^+$), 209 ($[444 - \mathbf{d} - CHO]^+$, and 205 $[M - \mathbf{e} - CH_2{=}CCl_2]^+$ (see *Fig. 1*) supported the structure of **1**.

The structure of **1** was further supported by a X-ray crystallographic analysis (*Fig. 2*). The resulting C–Cl bond distances are also consistent with these assignments.

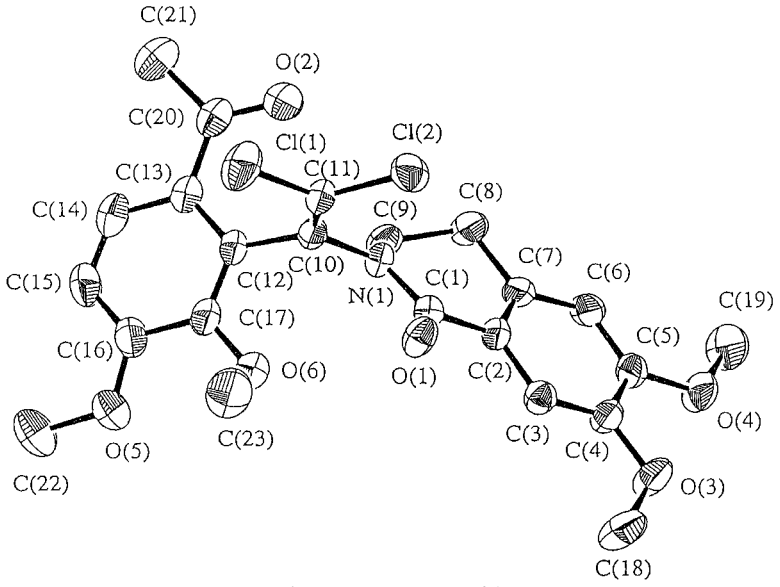
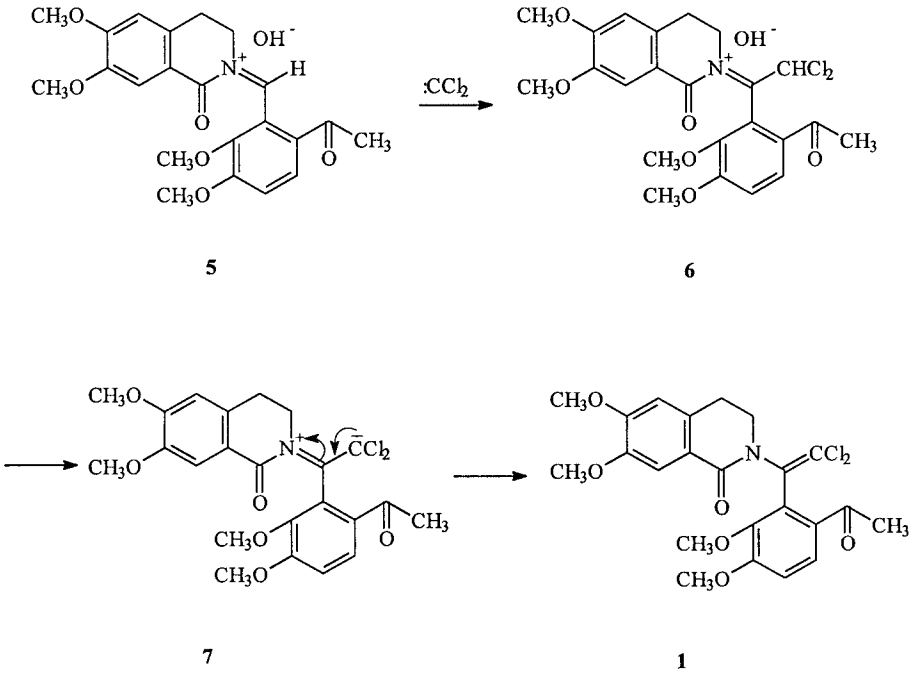


Fig. 2. *ORTEP View of* **1**

The isolation of alkaloids containing a Cl-atom from plants has previously been reported [7][8]. Alkaloids incorporating two Cl-atoms isolated from plants are rather unusual. As in the case of **1**, it is likely that **1** is an artifact formed during experimental work. Compound **5**, a precursor, reacted presumably with dichlorocarbene formed from $CHCl_3$ to yield an intermediate **6** [9]. The latter may undergo dehydration and electron transfer to afford **1** (*Scheme*). Chlorination may occur in this plant because it was collected at the seacoast of the northeast part of Taiwan. Therefore, **1** may also be assumed to be a real metabolite of this plant. More experiments are needed to clarify these assumptions.

Scheme



5 **6**

7 **1**

The antiplatelet effects of **2**–**4** were studied on the aggregation of human PRP induced by ADP (20 μM), collagen (10 μg/ml), and adrenaline (5 μM). In human PRP, adrenaline caused biphasic aggregation (see *Fig. 3*). As shown in *Table 2* and *Fig. 3*, **2**–**4** showed significant antiplatelet effects in a concentration-dependent manner. Compounds **2**–**4** prevented the secondary-phase aggregation at low concentrations, but not the primary-phase aggregation induced by adrenaline, and completely abolished the secondary-phase aggregation at high concentrations. The secondary-phase aggregation induced by adrenaline in human PRP is known to depend on the generation of thromboxane A_2 and on the release of ADP and to be inhibited by cyclooxygenase inhibitors, such as aspirin and indomethacin [10][11]. This result indicates that the antiplatelet effect of **2**–**4** is partially due to an inhibitory effect on thromboxane A_2 formation. More experiments are required to evaluate the antiplatelet effect and the exact mechanism of action.

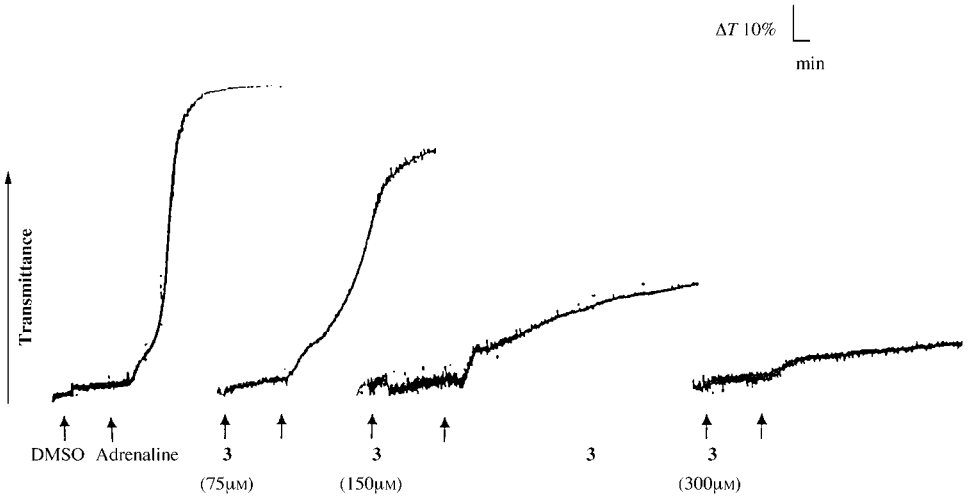


Fig. 3. *Effect of* **3** *on aggregation of human platelet-rich plasma* (PRP) *induced by adrenaline.* PRP was incubated with **3** at various concentration of with DMSO (0.5%, control) for 3 min, then the inducer adrenaline (5 μM) was added to trigger the aggregation.

Table 2. *Effect of Constituents,* **2**–**4** *on Aggregation of Human Platelet-Rich Plasma* (PRP) *Induced by ADP, Collagen, or Adrenaline*[a])

Compound	Conc. [μM]	Platelet aggregation [%]		
		ADP	Collagen	Adrenaline
Control		92.1 ± 0.1	90.2 ± 0.2	94.1 ± 0.3
2	75	–[b])	–[b])	88.9 ± 0.4[c])
	150	89.7 ± 1.1	21.4 ± 0.8[d])	9.2 ± 0.8[d])
	300	0.0 ± 0.0[e])	0.0 ± 0.0[e])	0.0 ± 0.0[e])
3	75	–[b])	–[b])	90.2 ± 0.9
	150	95.5 ± 0.5	–[b])	44.5 ± 2.9[d])
	300	90.9 ± 1.1	90.0 ± 1.8	16.1 ± 3.5[d])
4	75	–[b])	–[b])	96.6 ± 1.0[c])
	150	–[b])	97.9 ± 1.2	25.5 ± 1.7[d])
	300	84.7 ± 2.6	88.4 ± 1.1	12.9 ± 0.7[d])
Aspirin	50	84.4 ± 1.2	74.0 ± 3.2	29.5 ± 1.0[d])

[a]) PRP was preincubated with DMSO (0.5%, control), various concentrations of **2**–**4**, or aspirin at 37° for 3 min, and ADP (20 μM), collagen (10 μg/ml), or adrenaline (5 μM) were then added. Values are presented as the mean ± s.e.m. ($n = 3-4$). [b]) Not determined. [c]) $P < 0.05$ compared with control. [d]) $P < 0.01$ compared with control. [e]) $P < 0.001$ compared with control.

Compound **2** also showed significant antiplatelet effects on ADP- and collagen-induced platelet aggregation in human PRP. In rabbit platelets, **2** inhibited thromboxane formation and phosphoinositide breakdown and then led to the decrease of intracellular calcium concentration [12]. This result indicates that **2** in human PRP may also inhibit phosphoinositide breakdown and then lead to decreased intracellular calcium concentration. More experiments are required to evaluate the exact mechanism of action of **2**.

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Experimental Part

General. M.p.: uncorrected. UV Spectra: *Jasco-UV/VIS* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi-260-30* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian-Unity-400* spectrometer; at 400 and 100 MHz, resp. MS: *JMS-HX-100* mass spectrometer; *m/z* (rel. %).

Plant Material. Herb (3.6 kg) of *Corydalis koidzumiana* were collected at Ching Shan Beach, Taipei Hsien, Taiwan, in September 1998. A voucher specimen is deposited in the laboratory of Medicinal Chemistry.

Extraction and Isolation. Fresh herb (3.6 kg) was extracted with MeOH at r.t. The 3% AcOH-soluble fraction of the MeOH extract was alkalinized with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on Al_2O_3. Elution with cyclohexane/Me_2CO 4:1 afforded (±)-tetrahidropalmatine (**3**; 17.2 mg) and palmatine (**4**; 28.7 mg). Elution with cyclohexane/Me_2CO 3:2 afforded **1** (10.1 mg). Elution with cyclohexane/Me_2CO 1:1 afforded protopine (**2**; 1.3 g). Elution with cyclohexane/Me_2CO 1:2 afforded (+)-corybulbine. The known compounds were identified by spectroscopic methods and comparison with reported data or authentic samples [13].

Chingazumianine (*=2-[1-(6-Acetyl-2,3-dimethoxyphenyl)-2,2-dichloroethenyl]-3,4-dihydro-6,7-dimethoxyisoquinolin-1(2*H*)-one*; **1**). Colorless needles: M.p. 195–196° ($CHCl_3$/MeOH). IR (KBr): 1680, 1660, 1600, 1560. UV (MeOH): 220 (4.57), 260 (4.33), 302 (4.20). ^1H- and ^{13}C-NMR: *Table 1*. EI-MS (70 eV): 483 (0.48, $[M+4]^+$), 481 (2.67, $[M+2]^+$), 479 (3.77, M^+), 444 (100, $[M-Cl]^+$), 408 (11, $[M-2Cl-H]^+$), 380 (18), 209 (75, $[444-\mathbf{d}-CHO]^+$), 205 (33, $[M-\mathbf{e}-CH_2{=}CCl_2]^+$), 191 (33), 175 (25). HR-FAB-MS: 480.0973 ($[M+1]^+$, $C_{23}H_{24}Cl_2NO_6^+$; calc. 480.0981).

X-Ray Analysis[1]). X-Ray crystal analysis was performed with a single crystal (colorless, 0.20 × 0.42 × 0.94 mm) obtained from $CHCl_3$/MeOH. X-Ray diffraction data were collected on a *Rigaku-AFC7S* diffractometer with graphite-monochromated MoK_α radiation. The structure was solved by and expanded with *Fourier* techniques [14]. All non-H atoms were refined anisotropically by full-matrix least-squares techniques. All calculations were performed with the TeXsan crystallographic software package of *Molecular Structure Corporation*. The crystal data were as follows: $C_{23}H_{23}Cl_2NO_6$, triclinic, π(#2); $a=10.487(1)$, $b=10.670(2)$, $c=10.932(2)$ Å, $\alpha=72.92(1)°$, $\beta=98.08(1)°$, $\gamma=102.32(1)°$, and $V=1138.3(3)$ Å^3; $Z=2$; $R=3.8\%$, $Rw=3.1\%$ for 4258 independent reflections.

Platelet Aggregation. Human platelet-rich plasma (PRP) was obtained from the supernatant after the centrifugation of blood mixed with 3.8% sodium citrate (1:9 to blood). All glassware was siliconized. Aggregation was measured by a turbidimetric method [15]. The absorbance of PRP was taken as 0% aggregation and the absorbance of platelet-poor plasma (PPP) as 100% aggregation. The aggregation was measured by a *Lumi*-aggregometer (*Chrono-Log*, USA).

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[1]) Crystallographic data (excluding structure factor) for **1** have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 144467. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

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