Analgesic Components from Bornean Medicinal Plants, *Tabernaemontana pauciflora* BLUME and *Tabernaemontana pandacaqui* POIR

Emi OKUYAMA, Li-Hon GAO and Mikio YAMAZAKI*

Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoi-cho, Inage-ku, Chiba 263, Japan. *Received December 10, 1991*

The analgesic components were isolated from a Bornean medicinal plant, *Tabernaemontana pauciflora* BLUME (syn. *Ervatamia blumeana* MARK gr.), and the major components were identified as coronaridine and 3-(2-oxopropyl)coronaridine. Four minor components were estimated to be 5*R*- and 5*S*-(2-oxopropyl)coronaridine, 3-(2-oxopropyl)voacangine and 3,3′-(oxopropyl)dicoronaridine, which might be produced during the isolation process. Voacangine was also isolated as a major component of *T. pandacaqui* POIR. Coronaridine and voacangine exhibited significant analgesic and hypothermic effects in mice at a dose of 25 mg/kg, *p.o.*, while 3-(2-oxopropyl)coronaridine was less effective. The former two compounds also revealed a surface anesthesia.

Keywords *Tabernaemontana pauciflora*; *Tabernaemontana pandacaqui*; *Ervatamia blumeana*; coronaridine; voacangine; 3-(2-oxopropyl)coronaridine; 5-(2-oxopropyl)coronaridine; 3-(2-oxopropyl)voacangine; 3,3′-(oxopropyl)dicoronaridine; analgesia

During a survey of neurotrophic components from medicinal plants,[1] the extract of *Tabernaemontana pauciflora* BLUME (*Ervatamia blumeana* MARK gr.) collected in Borneo, Malaysia, showed an analgesic effect to mice. The roots of this plant, called "Lontupak" in a local area of Sabah State, Borneo Island, have been used for treatment of toothache. Isolation and identification of the analgesic components (alkaloids) are reported in this paper. The pharmacological effects of a major alkaloid of another type of Lontupak (*T. pandacaqui* POIR) are also described.

Oral administration (*p.o.*) of the methanol extract of *T. pauciflora* showed a hypothermic effect of $-3.4\,^{\circ}C$ ($p<0.01$) as maximum difference of body temperature (ΔT_{max}) and an analgesic property of 96% ($p<0.01$) on acetic acid-induced writhing in mice at doses of 500 mg and 1 g/kg, respectively. The extract was separated by the steps shown in Chart 1. After partition of 2.05 g with ethyl acetate and water, the ethyl acetate-layer was chromatographed on Sephadex LH-20 and silica gel to obtain an alkaloid-fraction with hypothermia (ΔT_{max} $-2.8\,^{\circ}C$, 60 mg/kg) and analgesia (84% inhibition, $p<0.001$, 150 mg/kg). Large-scale separation from the methanol extract (47.0 g) resulted in the alkaloid-fraction (8.1 g). Each of the alkaloids tentatively called compounds **1**—**6** was isolated by subsequent column chromatography (Chart 2).

The major component, **1**, had a typical indole-type ultraviolet (UV) spectrum at λ_{max} ($\log\varepsilon$), 202 (3.87), 285 (3.90) and 225 (4.52) nm, and the molecular formula of $C_{21}H_{26}N_2O_2$ by the high resolution mass spectrum (HRMS). The proton and carbon-13 nuclear magnetic resonance spectra (^{1}H- and ^{13}C-NMR) and the other properties including circular dichroism (CD) were identical to those of coronaridine,[2] a known component in plants of Apocynaceae, although this is the first isolation from *T. pauciflora*.

The properties of **2** indicated that it was also a coronaridine-type compound. In the ^{1}H-NMR, the methylene signals of δ 2.81 (1H, br d, 8.5 Hz) and 2.89—2.92 (1H, m) at C-3 of **1** were not observed in **2**, but a methine signal of δ 3.33 (1H, dd, 8.5 and 4.4 Hz) and 2-oxopropyl group assigned to the signals of δ 2.51 (1H, dd, 16.2 and 8.5 Hz), 2.69 (1H, dd, 16.2 and 4.4 Hz) and 2.11 (3H, s) in the ^{1}H-NMR and the corresponding carbon signals at δ 46.70 (C-24), 208.67 (C-25) and 30.98 (C-26). The substitution with 2-oxopropyl group at C-3 position of **2** was also confirmed by observation of the cross peaks between C25(δ 208.67)–H24(δ 2.51 and 2.69) and H24(δ 2.51)–C3(δ 55.30) in the correlation spectroscopy *via* long range couplings (COLOC). Compound **2** was identified as 3-(2-oxopropyl)coronaridine.[3]

Compounds **3** and **4** had very similar spectrometric properties and had the same molecular formula of $C_{24}H_{30}N_2O_3$, which meant they were the isomers. The 2D-NMR, *viz.* HH-COSY, CH-COSY and COLOC experiments of **3** showed the presence of a methyl at δ 2.22 (3H, s) and methylene signals at δ 2.47 (1H, dd, 15.7 and

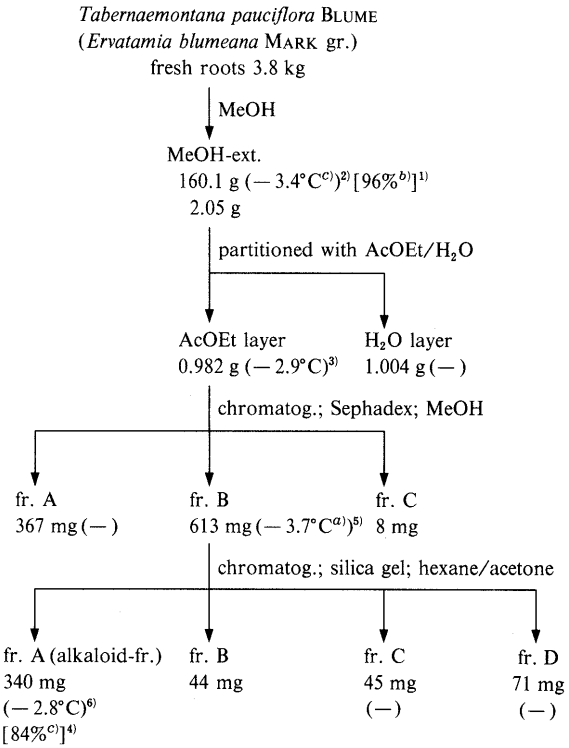


Chart 1. Isolation of the Alkaloid-fr. from *Tabernaemontana pauciflora* BLUME with an Analgesic Effect

[ ]: inhibition ratio on acetic acid-induced writhing in mice, ( ): hypothermic effect ΔT_{max}. 1) 1 g/kg, 2) 500 mg/kg, 3) 250 mg/kg, 4) 150 mg/kg, 5) 125 mg/kg, 6) 60 mg/kg, *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

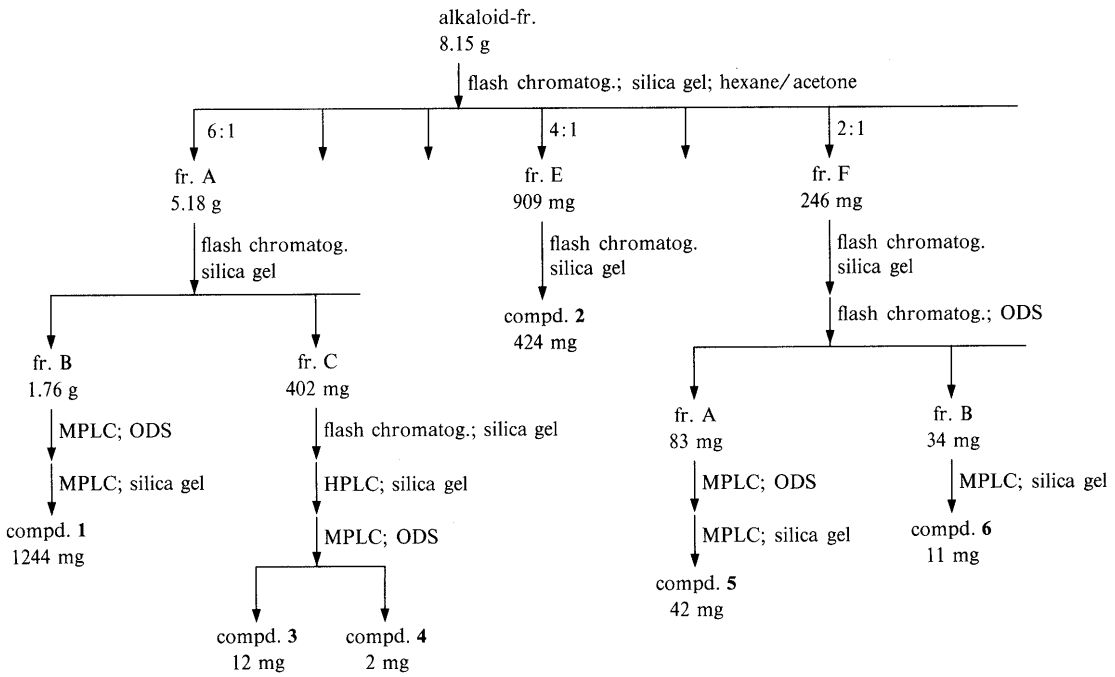
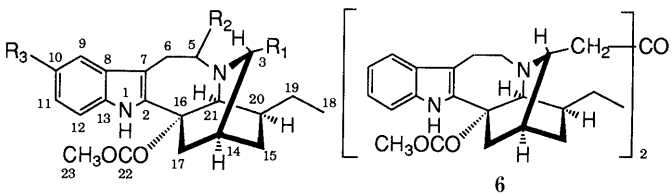
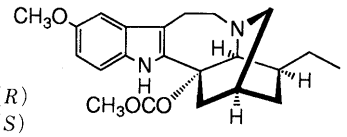


Chart 2. Isolation of **1**—**6** from the Alkaloid-fr.



6



7

1 : $R_1=R_2=R_3=H$
2 : $R_1=CH_2COCH_3$, $R_2=R_3=H$
3 : $R_1=R_3=H$, $R_2=CH_2COCH_3(R)$
4 : $R_1=R_3=H$, $R_2=CH_2COCH_3(S)$
5 : $R_1=CH_2COCH_3$, $R_2=H$, $R_3=OCH_3$

Fig. 1. Structures of **1**—**7**

4.4) and 2.73 (1H, dd, 15.7 and 9.1) in ^{1}H-NMR, and δ 30.93 (C-26), 208.19 (C-25) and 51.78 (C-24) in ^{13}C-NMR which were assigned to the oxopropyl group attached to C-5. In 2D nuclear Overhauser spectroscopy (NOESY), the cross peaks were observed between δ 3.44 (H-5, ddt, 12.1, 9.1 and *ca.* 4.7 Hz) and 2.50 (H-17 pro-*R*, br d, 13.4 Hz) and between δ 2.91 (H-6 pro-*R*, dd, 15.4 and 12.1 Hz) and 3.75 (H-21, s). The chemical structure of **3** is thus estimated as 5*R*-(2-oxopropyl)coronaridine (Fig. 1). Compound **4** also seemed to have an oxopropyl substituent at C-5 by comparing it with **3** in the ^{1}H-NMR. The 5*S*-configuration of **4** was supported by the cross peaks between δ 3.87—3.92 (H-5, m) and 3.43 (H-21, br s) and between δ 3.09 (H-6 pro-*S*, dd, 16.8 and 10.7 Hz) and 2.79 (H-3 pro-*S*, br d, 8.5 Hz) in the NOESY.

The ^{1}H- and ^{13}C-NMR of **5** were similar to those of **2** except for the aromatic signals. A methoxy signal bonded to the aromatic moiety of **5** was observed at δ 3.84(s) in the ^{1}H-NMR and its position was assigned by COLOC. The comparison with the ^{1}H- and ^{13}C-NMR of voacangine with those of **5** confirmed that **5** had 10-methoxyindole moiety.[2b,4] These data suggested that **5** was 3-(2-oxopropyl)voacangine.

Compound **6** was also similar to **2** in the ^{13}C-NMR, but the methyl signal of oxopropyl group was not observed. The dimerized structure shown in Fig. 1 was indicated by the molecular formula of **6**, $C_{45}H_{54}N_4O_5$, and the MS fragment pattern which was almost identical to that of **2** except for the molecular ion. The ^{1}H- and ^{13}C-NMR were assigned by 2D-NMR techniques (Tables I and II).

Since most of the minor components had an oxopropyl group we reexamined the extract of Lontupak (*T. pauciflora*). The thin layer chromatography (TLC) comparison of the methanol-extract and the acetone-extract suggested that the components having an oxopropyl moiety might be artifacts because of insertion of acetone during the purification process.

The alkaloid fraction of the other type of Lontupak (*T. pandacaqui* Poir) was separated from the methanol-extract. The fraction was further purified without acetone to give **7** as a major component, which was identified as voacangine.[2b,4]

The pharmacological activities of **1**, **2** and **7** [coronaridine, 3-(2-oxopropyl)coronaridine and voacangine, respectively] were tested in mice. Oral administration of 50 mg/kg of **1** and **7** indicated the hypothermic effects of ΔT_{max} −2.0 °C ($p<0.001$, 1 h) and −1.8 °C ($p<0.05$, 1 h), respectively, whereas no significant effect was observed at a dose of 100 mg/kg of **2**. The analgesic effects of these three compounds are shown in Fig. 2. The inhibitions of **1** and **7** at a dose of 50 mg/kg were more than 90% on acetic acid-induced writhing, however, **2** reduced the writhing less (35% inhibition, $p<0.05$) at the same dose. The analgesic effect of coronaridine in rats was briefly reported by Kupchan *et al.*[5] Compounds **1** and **2** did not suppress the locomotor activity enhanced by methamphetamine at an oral dose of 50 mg/kg, and a large amount of **1**, in fact, showed an increase because of jumping behavior.

TABLE I. ^{1}H-NMR Spectral Data (in $CDCl_3$) of **1**—**7**

H	1	2	3	4	5	6[k]	7
1	7.76 br s	7.74 br s	7.95 br s	7.67 br s	7.61 br s	7.74 br s	7.64 br s
3 pro-*S*	2.81 br d (8.5)	3.33 dd (8.5, 4.4)[c]	2.70 br d (8.2)	2.79 br d (8.5)	3.34 dd (8.5, 4.4)[c]	3.27 br dd (8.5, 4.4)[c]	2.81 br d (8.5)
3 pro-*R*	2.89—2.92 m		3.02—3.04 m	2.85 dt (8.5, 3.3)			2.91 ddd (8.5, 3.7, 2.6)
5 pro-*S*	3.36—3.42 m	3.26—3.31 m	3.40 ddt (12.1, 9.1, *ca.*4.7)[c]	3.87—3.92 m[c]	3.21—3.31 m	3.19—3.29 m	3.35—3.42 m
5 pro-*R*	3.15—3.23 m	3.21 dd (11.0, 5.5)			3.11—3.23 m[h]	3.11—3.18 m[l]	3.17—3.24 m
6 pro-*S*	2.98—3.04 m[a]	3.15—3.19 m	3.04 dd (15.4, 5.3)	3.09 dd (16.8, 10.7)	3.11—3.23 m[h]	3.11—3.18 m[l]	3.11—3.17 m
6 pro-*R*	3.15—3.23 m[a]	2.99 ddd (17.0, 6.1, 5.5)	2.91 dd (15.4, 12.1)	3.05 dd (16.8, 5.8)	2.91—2.98 m	2.93—3.00 m	2.92—2.99 m
9	7.47 br d (8.0)	7.46 br d (8.0)	7.48 br d (7.4)	7.43 br d (7.7)	6.91 d (2.4)	7.44 br d (7.9)	6.92 d (2.4)
10	7.08 ddd (8.0, 7.1, 1.1)	7.08 ddd (8.0, 7.1, 0.8)	7.09 t-like (7.4)	7.08 ddd (7.7, 7.1, 1.1)		7.06 ddd (7.9, 7.2, 1.2)	
11	7.14 ddd (8.0, 7.1, 1.1)	7.15 ddd (8.0, 7.1, 1.1)	7.14 td-like (7.4, 1.1)	7.16 ddd (8.0, 7.1, 1.1)	6.81 dd (8.8, 2.4)	7.13 ddd (7.9, 7.2, 1.3)	6.80 dd (8.6, 2.4)
12	7.24 dd (8.0, 1.1)	7.24 br d (8.0)	7.27 br d (7.4)	7.23 br d (8.0)	7.13 dd (8.8, 0.5)	7.22 br d (7.9)	7.13 dd (8.6, 0.4)
14	1.88 br s	1.70 br d (1.7)	1.80 br s	1.92 br s	1.70 br d (1.1)	1.63 br s	1.87 br s
15 pro-*S*	1.13 ddt (12.4, 7.2, 1.9)	1.53—1.61 m[b,e]	1.03 br dd (12.2, 7.3)	1.05 br dd (12.6, 7.2)	1.53—1.62 m[i]	1.47—1.56 m[m]	1.12 ddt (12.6, 7.1, 2.1)
15 pro-*R*	1.73 m	1.21—1.30 m[b,d]	1.67—1.72 m	1.71—1.77 m[g]	1.20—1.31 m[j]	1.14—1.20 m	1.70—1.77 m
17 pro-*S*	2.58 dd (13.5, 3.0)	2.65 dd (13.8, 1.9)	2.13 ddd (13.4, 4.1, 2.2)	2.58 br d (13.8)	2.64 dd (13.6, 1.9)	2.61 dd (13.6, 1.8)	2.57 br dd (11.8, 2.5)
17 pro-*R*	1.90 ddd (13.5, 3.3, 2.5)	1.98 ddd (13.8, 4.0, 2.6)	2.50 br d (13.4)	1.71—1.77 m[g]	1.96 ddd (13.6, 4.0, 2.4)	1.92 ddd (13.6, 6.4, 3.2)	1.87—1.91 m
18-H_3	0.90 t (7.4)	0.89 t (7.4)	0.94 t (7.4)	0.86 t (7.4)	0.89 t (7.4)	0.86 t (7.3)	0.90 t (7.3)
19	1.40—1.47 m	1.40—1.46 m	1.35—1.47 m[f]	1.47 ddq (13.8, 7.2, 7.2)	1.42 ddq (13.2, *ca.*7.2, *ca.*7.2)	1.36 ddq (13.7, 7.3, 6.1)	1.44 ddq (13.8, 6.4, 6.4)
19	1.53—1.60 m	1.53—1.61 m[e]	1.49—1.58 m	1.34 ddq (13.8, 6.9, 6.9)	1.53—1.62 m[i]	1.47—1.56 m[m]	1.53—1.60 m
20	1.32—1.35 m	1.21—1.30 m[d]	1.35—1.47 m[f]	1.24—1.28 m	1.20—1.31 m[j]	1.26 dd (7.2, 7.2)	1.28—1.36 m
21	3.56 br s	3.58 br s	3.75 br s	3.43 br s	3.56 br s	3.55 br s	3.54 br s
23-H_3	3.71 s	3.70 s	3.64 s	3.75 s	3.70 s	3.68 s	3.71 s
24		2.69 dd (16.2, 4.4)	2.47 dd (15.5, 4.4)	2.83 dd (14.2, 10.3)	2.69 dd (16.2, 4.4)	2.63 dd (16.0, 4.4)	
24		2.51 dd (16.2, 8.5)	2.73 dd (15.5, 8.8)	2.52 dd (14.2, 4.8)	2.51 dd (16.2, 8.5)	2.44 dd (16.0, 8.5)	
26-H_3		2.11 s	2.22 s	2.24 s	2.11 s		
27-H_3					3.84 s		3.85 s

a, b) Interchangeable. *c*) Methine signals. *d*—*j, l, m*) Overlapped with each other. *k*) H-*n'* was identical to H-*n*.

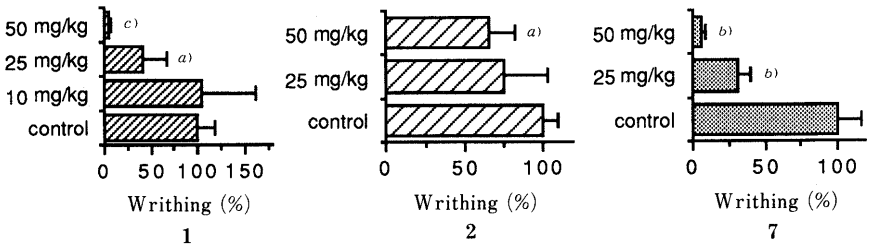


Fig. 2. Analgesic Effects of **1**, **2** and **7** on Acetic Acid-Induced Writhing in Mice

$n=7$—8, *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

These two compounds were not significantly effective on pentobarbital-induced hypnosis.

Lontupak is using as a medicine for toothache, so that **1** and **7** were also considered to have local anesthetic effect. The results of blink tests in guinea pigs revealed that 0.2% application of **1** and **6** provided surface anesthesia (Fig. 3). Infiltration anesthesia was measured by intracutaneous injection of **1** in guinea pigs. The twitch responses grandually disappeared as the injected part was bruised, so that it seemed **1** imparted some toxicity rather than acting as an infiltration anesthetic.

From these data, it is considered that coronaridine and voacangine are the major analgesic principles in Lontupak.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. UV and infrared (IR) spectra were recorded on Hitachi U 3400, Hitachi IR 260-10 and Nicolet 5ZDX spectrometers, respectively. Optical rotations were measured with a JASCO DIP-140 polarimeter, and CD and ORD were obtained by JASCO J-500 and J-20 polarimeters, respectively. ^{1}H- and ^{13}C-NMR were recorded on a JEOL GSX 500 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet,

TABLE II. ^{13}C-NMR Spectral Data (in $CDCl_3$) of **1**—**7**

C	1	2	3	5	6[a]	7
2	136.59	136.55	136.01	137.48	136.45	137.56
3	51.57	55.30	56.56	55.18	55.14	51.57
5	53.13	51.50	59.08	51.50	51.46	53.16
6	22.10	22.06	27.32	22.18	22.02	22.23
7	110.29	110.14	109.48	109.95	110.10	110.15
8	128.80	128.80	127.87	129.21	128.76	129.23
9	118.42	118.46	118.18	100.86	118.44	100.86
10	119.19	119.29	119.29	154.05	119.25	154.04
11	121.89	122.03	121.79	111.95	122.00	111.81
12	110.32	110.39	110.53	111.13	110.32	111.07
13	135.48	135.54	135.68	130.64	135.48	130.58
14	27.38	30.79	27.94	30.83	30.76	27.38
15	32.04	26.98	32.42	26.98	26.94	32.05
16	55.09	54.85	53.81	54.91	54.77	55.17
17	36.50	37.70	36.47	37.73	37.62	36.58
18	11.62	11.72	11.52	11.71	11.64	11.65
19	26.74	26.81	26.93	26.81	26.73	26.78
20	39.13	38.48	38.40	38.48	38.41	39.14
21	57.45	58.25	50.60	58.34	58.17	57.52
22	175.74	175.60	174.85	175.57	175.52	175.71
23	52.53	52.63	52.51	52.63	52.58	52.56
24		46.70	51.78	46.75	46.79	
25		208.67	208.19	208.68	210.36	
26		30.98	30.93	31.02		
27				56.05		56.06

a) C-*n*′ was identical to C-*n*.

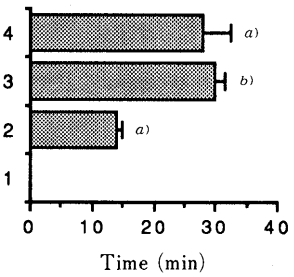


Fig. 3. Local Anesthetic Effects of **1** and **7** on Blink Test in Guinea Pigs

$n=5$, 1. control, 2. 1% procaine, 3. 0.2% **1**, 4. 0.2% **7**, *a*) $p<0.01$, *b*) $p<0.001$.

d=doublet, t=triplet, m=multiplet, br=broad. Hitachi M-60 was used for EIMS, and Hitachi RMO-7M for HRMS. Column chromatographies were performed on Sephadex LH-20, Wakogel C-200. Nakarai Silica gel 60 and Silica gel CQ-3 (Fuji Gel). Pre-packed columns, Kusano CPO-HS-221-21 and Senshu Pak SSC-Silica-5251-N, were used for MPLC and high performance liquid chromatography (HPLC), respectively.

Plant Material The fresh roots of Lontupak were collected in Tenghilan forest, Sabah, Borneo, Malaysia in October, 1989. The plant was first identified as *Ervatamia blumeana* WARK gr. by Mr. Julius Kulip, Forest Research Center (FRC), Forestry Department, Sabah. In our report of reference 1*a*, *E. blumeana* was used for Lontupac. After that, however, FRC suggested that *Tabernaemontana pauciflora* BLUME synonimous with *E. blumeana* was now preferable. The other type of Lontupak collected near Kudat, Sabah in November, 1990, was identified as *T. pandacaqui* POIR. According to further information of FRC, in some areas of Sabah, the local name Lontupak also refers to a plant of the family Meliaceae, and *T. pauciflora* and *T. pandacaqui* were called Lampada. The specimens of the plants are kept in the herbarium of FRC.

Extraction and Separation Fresh roots (3.8 kg) were extracted with methanol (31 l) at room temperature. The small part (2.95 g) of the extract (the total weight: 160.1 g) was partitioned with ethyl acetate and water, and the organic part (982 mg) showed the activity. It was chromatographed on Sephadex LH-20 with methanol as an eluent to give active fr. B (613 mg), which was further separated on silica gel chromatography. The hexane eluate (alkaloid fr., 340 mg) was obtained as the active fraction. Large scale preparation from the extract of 46.9 g proceeded in a similar manner as above and obtained the alkaloid fraction (8.15 g). The fraction was flash chromatographed on silica gel eluted with hexane–acetone to yield fr. A, 5.18 g (6:1 eluate), fr. E. 909 mg (4:1 eluate) and fr. F, 246 mg (2:1 eluate). Fraction A was re-chromatographed on silica gel, and the hexane–acetone 8:1 eluate gave **1** (1244 mg) after further purification by MPLCs on ODS with methanol–water, 4:1, and on silica gel with hexane–ethyl acetate 12:1. The hexane–acetone, 6:1 eluate (402 mg) from fr. A was separated by flash chromatography (silica gel, hexane–acetone, 8:1), HPLC (silica gel, hexane–ethyl acetate, 6:1) and MPLC (ODS, methanol–water, 3:1) to give **3** (12 mg) and **4** (2 mg). Compound **2** (424 mg) was purified by the flash chromatography of fr. E on silica gel with hexane–ethyl acetate (8:1) eluent. After flash chromatographies of fr. F on silica gel (hexane–ethyl acetate, 8:1—6:1) and ODS (methanol–water, 4:1—6:1), **5** (42 mg) and **6** (11 mg) were isolated by following MPLCs on ODS (methanol–water, 5:2) and/or silica gel (hexane–ethyl acetate, 6:1).

The dried roots of *T. pandacaqui* (250 g) were extracted with methanol. The extract (15.3 g) was partitioned with ethyl acetate and water. The organic part was separated by Sephadex LH-20 with methanol, followed by repeated silica gel column chromatography (hexane–ethyl acetate) and MPLC on ODS (methanol–water, 6:1). Compound **7** (130 mg) was obtained as a major alkaloid.

Compound **1**: White powder, mp 52—54 °C (lit.[5] mp 92—93 °C), $[\alpha]_D^{26}$ −41° ($c=2.0$, $CHCl_3$) (lit.[2d] $[\alpha]_D^{20}$ −44°, $CHCl_3$). HRMS *m/z*: 338.1994 (M^+, 338.1993 Calcd for $C_{21}H_{26}N_2O_2$), 253.1142 (253.1102 Calcd for $C_{16}H_{15}NO_2$), 214.0870 (214.0868 Calcd for $C_{13}H_{12}NO_2$), 136.1116 (136.1124 Calcd for $C_9H_{14}N$). EIMS *m/z* (%): 338 (M^+, 100), 323 (27), 253 (11), 214 (31), 169 (21), 136 (84), 124 (45). UV (EtOH) nm ($\log\varepsilon$): 225 (4.49), 284 (3.94), 292 (3.90). IR (KBr) cm^{-1}: 3380, 2925, 2860, 1710, 1458, 1260, 1080, 742. CD (MeOH) nm ($\Delta\varepsilon$): 243 (+3.5), 268 (−4.0).

Compound **2**: White powder, mp 140—142 °C, $[\alpha]_D^{26}$ −24° ($c=2.0$, $CHCl_3$) (lit.[3b] mp 140 °C, $[\alpha]_D^{22}$ −27°, $CHCl_3$). EIMS *m/z* (%): 394 (M^+, 50), 337 (100), 264 (19), 229 (18), 214 (10), 168 (18), 154 (18), 136 (10). UV (EtOH) nm ($\log\varepsilon$): 225 (4.53), 284 (3.94), 292 (3.91). IR (KBr) cm^{-1}: 3300, 2950, 2860, 1730, 1695, 1465, 1230, 1085, 1010, 745. CD (MeOH) nm ($\Delta\varepsilon$): 243 (+2.2), 275 (−2.9).

Compound **3**: White powder, mp 52—57 °C, $[\alpha]_D^{20}$ −60° ($c=0.12$, EtOH). HRMS *m/z*: 394.2234 (M^+, 394.2254 Calcd for $C_{24}H_{30}N_2O_3$), 337.1905 (337.1914 Calcd for $C_{21}H_{25}N_2O_2$), 214.0882 (214.0867 Calcd for $C_{13}H_{12}NO_2$), 192.1379 (192.1387 Calcd for $C_{12}H_{18}NO$), 154.0662 (154.0657 Calcd for $C_{11}H_8N$), 148.1114 (148.1125 Calcd for $C_{10}H_{14}N$), 136.1133 (136.1126 Calcd for $C_9H_{14}N$). EIMS *m/z* (%): 394 (M^+, 100), 351 (22), 337 (31), 214 (12), 192 (42), 179 (37), 167 (19), 154 (30), 148 (81), 136 (18). UV (EtOH) nm ($\log\varepsilon$): 225 (4.50), 286 (3.90), 293 (3.87). IR (KBr) cm^{-1}: 3370, 2920, 2860, 1710, 1460, 1240, 1155, 745. CD (EtOH) nm ($\Delta\varepsilon$): 249 (+4.0), 289 (−6.2).

Compound **4**: White powder, mp 169—171 °C, $[\alpha]_D^{20}$ −72° ($c=0.051$, EtOH). HRMS *m/z*: 394.2269 (M^+, 394.2254 Calcd for $C_{23}H_{30}N_2O_3$), 337.1935 (337.1915 Calcd for$C_{21}H_{25}N_2O_2$), 214.0894 (214.0867 Calcd for $C_{13}H_{12}NO_2$), 192.1366 (192.1387 Calcd for $C_{12}H_{18}NO$), 154.0662 (154.0656 Calcd for $C_{11}H_8N$), 148.1123 (148.1099 Calcd for $C_7H_{16}O_3$), 136.1113 (136.1125 Calcd for $C_9H_{14}N$). EIMS *m/z* (%): 394 (M^+, 100), 351 (20), 337 (22), 192 (36), 179 (34), 168 (12), 154 (20), 148 (63), 136 (14). UV (EtOH) nm ($\log\varepsilon$): 225 (4.53), 283 (3.90), 291 (3.85). IR (KBr) cm^{-1}: 3451, 1713. CD (EtOH) nm ($\Delta\varepsilon$): 240 (+2.1), 280 (−3.0).

Compound **5**: White powder, mp 68—70 °C, $[\alpha]_{400}^{20}$ −20° ($c=0.16$, EtOH). HRMS *m/z*: 424.2365 (M^+, 424.2360 Calcd for $C_{25}H_{32}N_2O_4$), 367.2025 (367.2020 Calcd for $C_{22}H_{27}N_2O_3$), 264.1597 (264.1597 Calcd for $C_{15}H_{20}NO_3$), 244.0978 (244.0973 Calcd for $C_{14}H_{14}NO_3$), 192.1363 (192.1386 Calcd for $C_{12}H_{18}NO$), 136.1128 (136.1125 Calcd for $C_9H_{14}N$). EIMS *m/z* (%): 424 (M^+, 58), 367 (100), 264 (22), 259 (16), 244 (13), 192 (14), 184 (17), 160 (12), 136 (12). UV (EtOH) nm ($\log\varepsilon$): 224 (4.43), 284 (3.94). IR (KBr) cm^{-1}: 3370, 2925, 2860, 1702, 1485, 1450, 1220, 1140, 822, 795. CD (EtOH) nm ($\Delta\varepsilon$): 253 (+1.5), 282 (−3.2), 312 (+0.6).

Compound **6**: White powder, mp 136—138 °C, $[\alpha]_{400}^{26}$ +23° ($c=0.13$, EtOH). HRMS *m/z*: 730.4073 (M^+, 730.4090 Calcd for $C_{45}H_{54}N_4O_5$), 394.2226 (394.2254 Calcd for $C_{24}H_{30}N_2O_3$), 337.1904 (337.1913 Calcd for $C_{21}H_{25}N_2O_2$), 229.1066 (229.1102 Calcd for $C_{14}H_{15}NO_2$), 168.0829 (168.0812 Calcd for $C_{12}H_{10}N$). EIMS *m/z* (%): 730 (M^+, 7), 394 (16), 337 (100), 229 (18), 168 (15), 154 (12), 136 (10). UV (EtOH) nm ($\log\varepsilon$): 226 (4.78), 285 (4.16), 293 (4.12). IR ($CHCl_3$) cm^{-1}: 3440, 1710. CD (EtOH) nm ($\Delta\varepsilon$): 240 (+3.9), 280 (−4.9), 309 (+1.5).

Compound **7**: White powder, mp 143—144 °C, $[\alpha]_D^{12}$ −39° ($c=2.0$,

$CHCl_3$) (lit.[2f] mp 137—138 °C, $[\alpha]_D$ −42°, $CHCl_3$). EIMS *m/z* (%): 368 (M^+, 100), 353 (18), 244 (27), 184 (32), 136 (87), 124 (31). UV (EtOH) nm (log ε): 225 (4.45), 288 (3.97). IR (KBr) cm^{-1}: 3450, 2948, 2870, 1715, 1495, 1460, 1235, 1040, 835, 820. CD (MeOH) nm (Δε): 251 (+2.3), 281 (−5.1), 314 (+0.7).

Pharmacological Assay Male mice (Std: ddY, 5 w., 22—33 g) and male guinea pigs (Hartley, 4—8 w., 300—400 g) propagated at Shizuoka Agricultural Cooperative Association (Hamamatsu, Japan) were housed at 24—25 °C with a 12 h dark: 12 h light cycle for at least 5 d before experimentation, and allowed free access to food and water. Test samples were suspended in normal saline with 5% gum arabic or 2% Tween 80.

Hypothermic Effect: Rectal temperatures were measured for 6 h after administration of the test samples by thermistor (Takara Instrumental Co., Ltd.)

Analgesic Activity: An acetic acid-induced writhing method[6] was used. The test samples were administered 30 min before the i.p. injection of 0.7% acetic acid. After 5 min, the number of squirms by each mouse was counted during the following 15 min.

Local Anesthetics: Blink reflex in guinea pigs was used.[7] Each test solution adjusted to pH 5—6 was dropped into the conjunctival sac. The cornea was stimulated three times at the intervals of 5 min using a mandoline line. The experiment was continued until blinks were observed all three times, although the anesthetic activity was judged to be negative when the stimulant did not cause blinks at least twice in the three time-trials.

Statistics: Statistical significance was evaluated by the Student's *t* test.

Acknowledgements The authors are especially grateful to Datuk M. Munang and Mr. J. Kulip, Department of Forestry, Sabah, Malaysia for identification of plants and for making this project possible. They also thank Mr. T. Fukai of Borneo Biomass Research Corp. Co., Ltd., Mr. N. Yajima of Asama Chemical Co., Ltd., Mr. W. Bingku, Mr. L. Bingku, and Dr. F. Ikegami and Dr. H. Fujimoto of this faculty for helping in the collection of the medicinal plants, Ms. M. Ohtsuka of this faculty for first screening, and the staff of the Analytical Center of this University for measurement of NMR and MS. Professor K. Watanabe kindly directed the blink test.

References

1) *a*) E. Okuyama, L. Gao, S. Nishimura and M. Yamazaki, *J. Pharmacobio-Dyn.*, **15**, s-11 (1992); *b*) E. Okuyama, S. Nishimura and M. Yamazaki, *Chem. Pharm. Bull.*, **39**, 405 (1991); *c*) M. Yamazaki, Y. Maebayashi, N. Iwase and T. Kaneko, *ibid.*, **36**, 2075 (1988).
2) *a*) K. Bláha, Z. Koblicová and J. Trojánek, *Tetrahedron Lett.*, **1972**, 2763; *b*) E. Wenkert, D. W. Cochran, H. E. Gottlieb, E. W. Hagaman, R. B. Filho, F. J. de A. Matos and M. I. L. M. Madruga, *Helv. Chim. Acta*, **59**, 2437 (1976); *c*) C. Kan, H.-P. Husson, S.-K. Kan and M. Lounasmaa, *Planta Medica*, **41**, 72 (1981); *d*) A. R. Araujo, C. Kascheres, F. Fujiwara and A. J. Marsaioli, *Phytochemistry*, **23**, 2356 (1984); *e*) T. A. Van Beek, R. Verpoorte, A. B. Svendsen and R. Fokkens, *J. Nat. Prod.*, **48**, 400 (1985); *f*) I. W. Southo and J. Buckingham, "Dictionary of Alkaloids," Chapman and Hall, New York, 1989.
3) *a*) N. di G. D. Monache, I. L. D'Albuguerque, F. D. Monache and G. B. M.-Bettolo, *Atti Accad. Naz. Lincei, Cl. Sci. Fis., Mat. Natur., Rend.*, **52**, 375 (1972); *b*) P. Perera, F. Sandberg, T. A. van Beek and R. Verpoorte, *Planta Medica*, **50**, 251 (1984).
4) S. P. Gunasekera, G. A. Cordell and N. R. Farnsworth, *Phytochemistry*, **19**, 1213 (1980).
5) S. M. Kupchan, A. Bright and E. Macko, *J. Pharm. Sci.*, **52**, 598 (1963).
6) B. A. Whittle, *Br. J. Pharmacol. Chemother.*, **22**, 236 (1964).
7) K. Kubota, "Experimental Pharmacology," Nankodo, Tokyo, 1987.