Phytochemistry, Vol. 30, No. 10, pp. 3353–3356, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00
© 1991 Pergamon Press plc

PICROTOXANE TERPENOIDS FROM *PICRODENDRON BACCATUM*

KAZUO KOIKE, TAICHI OHMOTO,* TAKATOSHI KAWAI† and TADASHI SATO†

School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274, Japan; †Tsukuba Research Laboratories, Eisai Co., Ltd, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-26, Japan

(*Received* 15 *February* 1991)

Key Word Index—*Picrodendron baccatum*; Simaroubaceae; bark; picrodendrin E; picrodendrin F; picrodendrin I; picrotoxane; X-ray crystallography; CD exiton coupling.

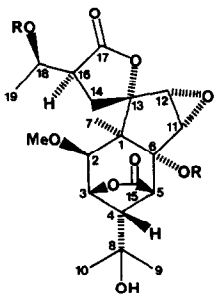
Abstract—Three new picrotoxane type terpenoids, picrodendrins E, F and I, were isolated from the bark of *Picrodendron baccatum*. The structures were determined by spectroscopic evidence and X-ray crystallographic analysis.

INTRODUCTION

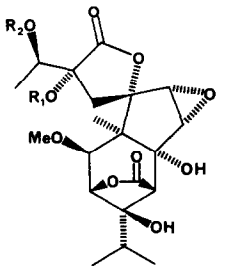
Picrodendron baccatum Klug et Urban, has the common name in the Dominican Republic, 'mata becerro' which means 'calf killer', and it used to kill bed bugs and lice in Dominica. In previous phytochemical studies of the plant collected in Indonesia, we have isolated four new picrotoxane terpenoids [1, 2]. In our continuing studies of this plant, we have now isolated three new picrotoxane terpenoids, picrodendrins E (**1**), F (**2**) and I (**3**).

RESULTS AND DISCUSSION

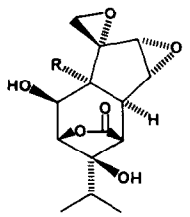
Picrodendrin F (**2**), $C_{20}H_{28}O_{10}$, was obtained as prisms. Its IR spectrum showed the presence of hydroxyl (ν_{max} 3450 cm^{-1}) and γ-lactone (ν_{max} 1775 and 1765 cm^{-1}) groups. Comparisons of the ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of **2** with those of picrodendrin A (**4**) [1] indicated that **2** has a picrotoxane skeleton. In particular, ^1H and ^{13}C NMR signals due to the cyclohexane, cyclopentane and γ-lactone systems of **2** resonanced at almost the same frequencies as the corresponding ones in the spectrum of **4**, while the remaining ^1H and ^{13}C signals of **2** observed at major different positions. The long-range ^{13}C–^1H correlation spectroscopy (COSY) study of **2** was examined to clarify the connectivity of each resonance in the spectrum of **2**. Thus, the long-range ^{13}C–^1H couplings observed between a γ-lactone carbonyl carbon (C-17, δ180.01) and both unequivalent methylene protons (H-14, δ3.36 and 4.44) and a methine proton (H-18, δ4.71), between an oxygen-bearing carbon (C-16, δ80.42) and both the methylene protons (H-14) and a methyl protons (H-19, δ1.61), and between a spiro carbon (C-13, δ90.31) and the methylene protons (H-18). These correlations were given by the long-range couplings established the spiro γ-lactone group of the molecule. Thus, the planar structure **2** is proposed for picrodendrin F. In order to confirm the proposed structure and to determine its stereochemistry, a single crystal X-ray analysis of its 18-*p*-bromobenzoate (**2a**) was conducted. The X-ray crystallographic analysis was carried out on a single crystal obtained from a methanol–water solution. However, the crystal structure is unusual in that the two molecules of the asymmetric unit have different conformations, as shown in Fig. 1. Nevertheless, the relative stereostructure of picrodendrin F (**2**) has been determined. Secondly, we applied the CD exciton chirality method [3] to the picrodendrin F 16,18-di-*p*-bromobenzoate (**2b**). The CD spectrum of **2b** showed



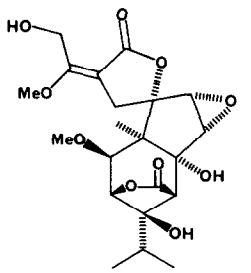
1 R=H
1a R=Ac



2 R_1=R_2=H
2a R_1=H, R_2=p-Br-Bz
2b R_1=R_2=p-Br-Bz



3 R=CH_2OH
5 R=CH_3



4

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of compounds **1–5** (in pyridine-d_5)

H	1	2	4	3	5
2	4.38 *br s*	3.78 *br s*	3.70 *s*	5.14 *d* (5.5)*	4.20 *d* (4.4)*
3	5.24 *br d* (4.0)	5.19 *br s*	5.15 *d* (1.1)	5.15 *br s*	5.00 *d* (1.1)
4	2.81 *t* (4.0)				
5	3.39 *d* (4.0)	3.47 *d* (1.1)	3.47 *d* (1.1)	3.21 *dd* (3.1, 0.9)	3.12 *dd* (3.1, 1.1)
6				3.43 *d* (3.1)	2.69 *d* (3.1)
7	1.76 *s*	1.94 *s*	1.89 *s*	4.57 *dd* (11.0, 4.0)*	1.89 *s*
				4.84 *dd* (11.0, 4.0)*	
8		2.78 *sept* (6.6)	2.74 *sept* (6.2)	2.92 *sept* (6.7)	2.04 *sept* (6.8)
9	1.54 *s*	1.50 *d* (6.6)	1.48 *d* (6.2)	1.19 *d* (6.7)	1.18 *d* (6.8)
10	1.47 *s*	1.25 *d* (6.6)	1.21 *d* (6.2)	1.11 *d* (6.7)	1.08 *d* (6.8)
11	4.17 *d* (2.8)	4.17 *d* (2.9)	4.18 *d* (2.9)	4.14 *d* (2.9)	4.07 *d* (2.9)
12	4.11 *d* (2.8)	4.77 *d* (2.9)	3.66 *d* (2.9)	3.62 *d* (2.9)	3.56 *d* (2.9)
14α	4.17 *dd* (14.0, 12.0)	4.44 *d* (14.7)	4.74 *d* (17.2)	4.84 *d* (6.2)	4.80 *d* (6.2)
14β	2.63 *dd* (14.0, 7.0)	3.36 *d* (14.7)	3.34 *d* (17.2)	3.11 *d* (6.2)	3.11 *d* (6.2)
16	3.14 *m*				
18	4.27 *m*	4.71 *q* (6.2)			
19	1.63 *d* (6.4)	1.61 *d* (6.2)	5.29 *dd* (13.2, 4.0)*		
			5.37 *dd* (13.2, 4.0)*		
OMe-2	3.37 *s*	3.41 *s*	3.96 *s*		
OMe-18			3.31 *s*		
OH-2				7.53 *d* (5.5)*	7.43 *d* (4.4)*
OH-7				7.04 *t* (4.0)*	
OH-18	6.73 *d* (4.0)*				
OH-19			7.35 *d* (4.0)*		

Figures in parentheses are coupling constants in Hz.
*The coupling disappeared on addition of D_2O.

Table 2. ^{13}C NMR spectral data of compounds **1–5** (in pyridine-d_5)

C	1	2	4	3	5
1	55.49	53.84	52.99	46.44	40.83
2	85.29	87.76	87.89	68.66	76.22
3	77.89	82.88	83.15	90.34	89.44
4	54.75	82.27	82.21	81.14	80.94
5	49.52	57.82	57.82	51.42	51.48
6	77.21	78.08	78.17	38.49	45.16
7	24.40	25.23	25.12	63.61	27.70
8	68.12	31.03	31.03	27.76	27.70
9	30.48	18.26	18.24	16.32	16.52
10	28.65	15.64	15.63	15.35	15.27
11	63.53	63.02	62.85	58.03	57.18
12	62.43	63.66	63.17	60.91	60.83
13	91.50	90.31	89.33	66.33	67.28
14	33.07	37.51	34.25	50.60	52.04
15	174.72	175.77	175.83	178.12	177.63
16	48.25	80.42	104.27		
17	177.13	180.01	170.09		
18	68.25	70.16	166.34		
19	21.02	18.15	53.63		
OMe-2	59.19	58.77	59.08		
OMe-18			55.75		

a pair of typical exciton split Cotton effects with opposite signals centred upon the UV absorption of the *p*-bromobenzoate chromophore: $\Delta\varepsilon$ +15.4 at 254 nm and $\Delta\varepsilon$ −8.6 at 236 nm. The chirality of the vicinal *p*-bromobenzoyl groups was determined as positive. Thus, the absolute configuration at C-18 has been confirmed as *R*. From the above results, the absolute stereostructure of picrodendrin F was determined to be **2**.

Picrodendrin E (**1**), $C_{20}H_{28}O_9$, was obtained as prisms. The IR spectrum showed the presence of hydroxyl

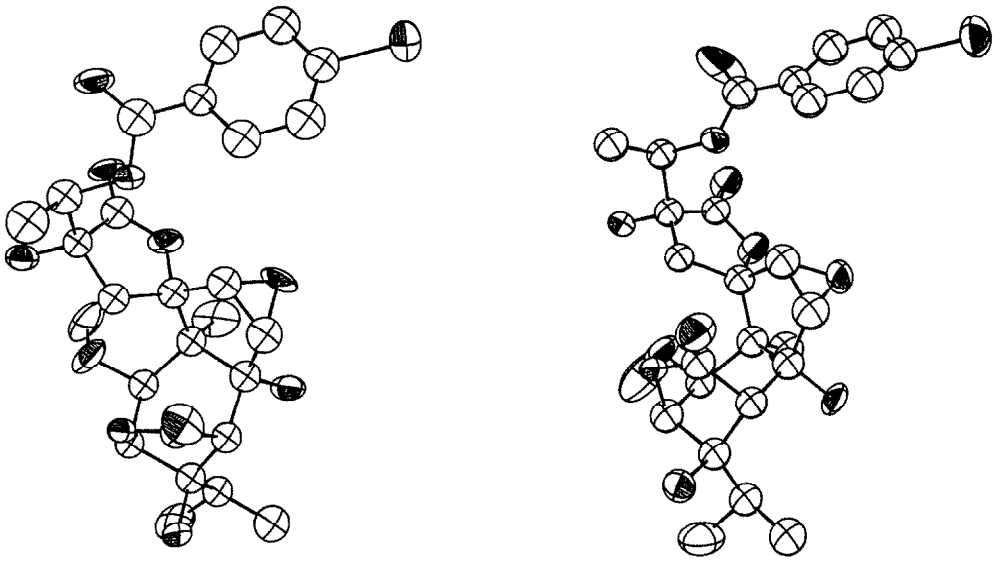


Fig. 1. Computer-generated drawings of the final X-ray model for the *p*-bromobenzoate **2a**.

(ν_{max} 3450 cm^{-1}) and γ-lactone (ν_{max} 1790 and 1750 cm^{-1}) groups. The M_r of **1** was 16 mass units lower than that of **2**, suggesting one less hydroxyl group than that of **2**. The ^1H and ^{13}C NMR spectra of **1** were very similar to those of **2**, except that an isopropyl group in **2** was replaced by a hydroxyisopropyl group. The ^1H–^1H COSY suggested the presence of two isolated structure units, ■–C(2)H–C(3)H–C(4)H–C(5)H–■ and ■–C(14)H_2–C(16) H–C(18)H(OH)–C(19)H_3 in the formula. The ^1H NMR spectrum of the acetyl derivative **1a**, H-5, H-11 and H-18 gave clear downfield shifts ($\Delta\delta$ = 0.87, 0.87 and 1.07 ppm, respectively), thus revealing that the remaining two of three hydroxyl groups were located at C-6 and C-18. The stereochemistry of **1** was determined by NOE measurements (See Experimental). These observations indicated that all chiral centres are compatible with those of picrodendrin F (**2**). Thus, the structure of picrodendrin E is **1**.

Picrodendrin I (**3**) was obtained as plates. Its IR spectrum showed the presence of hydroxyl (ν_{max} 3495 cm^{-1}) and γ-lactone (ν_{max} 1760 cm^{-1}) groups. From the mass spectral and elementary analysis, the molecular formula was concluded to be $C_{15}H_{20}O_7$, suggesting that it has one more hydroxyl group than that of picrodendrin D (**5**) [2]. Comparisons of the ^1H and ^{13}C NMR spectral data with those of **5** indicated that a methyl group at C-1 in **5** was replaced by a hydroxymethyl group in **3**. By exchanging the hydrogen of the hydroxyl group at δ7.04 (1H, J = 4.0 Hz) with D_2O, each doublet of doublets of the unequivalent methylene protons of the hydroxymethyl group at δ4.57 and 4.84 (each 1H, *dd*, J = 11.0 and 4.0 Hz) was transformed into each doublet (J = 11.0 Hz). Therefore, the structure of picrodendrin I is **3**. The stereochemistry of **3** was determined by NOE measurements (see Experimental). These observations indicated that all chiral centres are compatible with those of picrodendrin D (**5**). Thus, the structure of picrodendrin I is **3**.

EXPERIMENTAL

General. Mps: uncorr. ^1H and ^{13}C NMR (400 and 100 MHz, respectively) chemical shifts are given as δ (ppm) with TMS as int. standard. Silica gel (BW-820 MH, Fuji Davison) and Diaion-HP20 (Mitsubishi Kasei) were used for CC and silica gel (CQ-3, 24 mm i.d. × 320 mm, Fuji Gel) was used for low-pressure LC.

Extraction. Dried bark (8 kg) of *Picrodendron baccatum* collected in Indonesia, in September, 1986 was extracted with MeOH (49 l) at room temp. The MeOH extract was concd under red. pres. to give a residue (1.9 kg), to which an equal vol. of H_2O was added. The aq. soln was extracted successively with $CHCl_3$, EtOAc and *n*-BuOH. Each fr. was concd to give $CHCl_3$ (80 g), EtOAc (260 g), *n*-BuOH (300 g) and H_2O-soluble (1260 g) frs. The EtOAc fr. (260 g) was chromatographed on Diaion HP-20 (2 kg) and eluted with H_2O, H_2O–MeOH and MeOH. The crude picrotoxane terpenoids frs were obtained by eluting with H_2O–MeOH (5:1) and further purified by low-pressure LC (silica gel CQ-3, solvent system C_6H_6–EtOAc, 9:1) to afford picrodendrins E (**1**, 150 mg), F (**2**, 40 mg) and I (**3**, 30 mg).

Picrodendrin E (**1**). Prisms (MeOH–H_2O), mp 245°, $[\alpha]_D^{24}$ −36.3° (pyridine; *c* 2.2). IR ν_{max}^{KBr} cm^{-1}: 3450, 3300, 3000, 1790, 1750, 1640, 1270, 1120, 1010, 905. MS *m/z* (rel. int.): 413 ([M + H]$^+$, 28), 394 (8), 368 (3), 350 (4), 318 (2), 292 (17), 277 (36), 263 (5), 241 (8), 227 (17), 59 (100). ^1H and ^{13}C NMR: Tables 1 and 2, respectively. HR-MS *m/z* 413.1817 (calcd for $C_{20}H_{29}O_9$ [M + H]$^+$, 413.1812). ^1H NOE difference spectra: irradiated proton→observed proton (NOE enhancement %); δ1.76 (H-7)→δ4.38 (H-3, 11) and 5.24 (H-3, 5); δ1.54 (H-10)→δ5.24 (H-3, 7) and 3.39 (H-5, 6); δ4.17 (H-11)→δ3.39 (H-5, 13), δ4.17 (H-11)→δ3.39 (H-5, 13) and 4.11 (H-12, 5); δ2.63 (Hβ-14)→δ4.11 (H-12, 17), 4.17 (Hα-14, 29) and 4.27 (H-18, 5).

Acetylation of compound **1**. Compound **1** (20 mg) was treated with Ac_2O and pyridine at room temp. for 10 hr, and the reaction mixt. worked-up as usual and then recrystallized from MeOH to give diacetate (**1a**, 20 mg) as prisms, mp 199°, $[\alpha]_D^{25}$ +24.8 (pyridine; *c* 1.9). IR ν_{max}^{KBr} cm^{-1}: 3560, 2970, 1780, 1740, 1240, 1205, 1170, 1105, 1080, 1005, 980, 815. MS *m/z* (rel. int.): 497 ([M

$+H]^+$, 4), 436 (17), 419 (22), 391 (7), 359 (11), 59 (100). ^1H NMR (C_5D_5N): δ1.35 (3H, *s*, H-9), 1.49 (3H, *s*, H-10), 1.50 (3H, *d*, J = 6.7 Hz, H-19), 1.78 (3H, *s*, H-7), 1.92, 2.12 (each 3H, *s*, Ac-6, Ac-18), 2.31 (1H, *dd*, J = 14.5, 8.0 Hz, H-14β), 2.80 (1H, *t*, J = 4.4 Hz, H-4), 3.35 (1H, *m*, H-16), 3.36 (3H, *s*, H-20), 3.90 (1H, *d*, J = 2.5 Hz, H-12), 4.08 (1H, *dd*, J = 14.5, 11.2 Hz, H-14α), 4.26 (1H, *d*, J = 4.4 Hz, H-5), 4.56 (1H, *br s*, H-2), 5.04 (1H, *d*, J = 2.5 Hz, H-11), 5.31 (1H, *br d*, J = 4.4 Hz, H-3), 5.34 (1H, *m*, H-18).

Picrodendrin-F (**2**). Prisms (MeOH–H_2O), mp 165–167°, $[\alpha]_D^{18}$ −29.0° (pyridine; *c* 0.6). IR ν_{max}^{KBr} cm^{-1}: 3450, 1775, 1765, 1245, 1080, 990. MS *m/z* (rel. int.): 429 ($[M+H]^+$, (2), 384 (28), 348 (23), 320 (57), 295 (13), 253 (87), 71 (73), 43 (100). ^1H and ^{13}C NMR: Tables 1 and 2, respectively. HR-MS *m/z* 429.1796 (Calcd for $C_{20}H_{29}O_{10}$ $[M+H]^+$, 429.1761).

p-*Bromobenzoylation of compound* **2**. A soln of **2** (20 mg) in dry pyridine (2 ml) was treated with *p*-bromobenzoylchloride (50 mg). The whole mixt. was stirred at 80° for 6 hr. The reaction mix. was then partitioned into a mixt. of $CHCl_3$ and H_2O, and the organic layer was taken, washed with a satd aq. $NaHCO_3$ soln and brine, dried over $MgSO_4$, and then evapd *in vacuo*. The crude product (35 mg) was purified by MPLC (silica gel CQ-3, CH_2Cl_2–MeOH (9:1), detect 254 nm) to give the 18-*p*-bromobenzoate (**2a**, 20 mg). Compound **2a**: prisms (MeOH–H_2O), mp 109–110°. IR ν_{max}^{KBr} cm^{-1}: 3420 (br), 1760, 1750, 1285, 1260, 1245, 1150. FAB-MS *m/z* 613, 611. ^1H NMR (pyridine-d_5): δ1.22 (3H, *d*, J = 6.6 Hz, H-9), 1.49 (3H, *d*, J = 6.6 Hz, H-10), 1.71 (3H, *d*, J = 6.2 Hz, H-19), 1.94 (3H, *s*, H-7), 2.76 (1H, *sept*, J = 6.6 Hz, H-8), 3.31 (1H, *d*, J = 15.1 Hz, H-14β), 3.40 (3H, *s*, OMe-2), 3.51 (1H, *d*, J = 1.0 Hz, H-5), 3.78 (1H, *br s*, H-2), 4.28 (1H, *d*, J = 2.9 Hz, H-11), 4.32 (1H, *d*, J = 2.9 Hz, H-12), 4.60 (1H, *d*, J = 15.1 Hz, H-14β), 5.20 (1H, *br s*, H-3), 5.93 (1H, *q*, J = 6.2 Hz, H-18), 7.50, 7.87 (each 2H, *d*, J = 8.4 Hz, H-1′, H-2′, H-4′ and H-5′).

Di-p-bromobenzoylation of compound **2**. A soln of **2** (10 mg) in dry pyridine (2 ml) was treated with *p*-bromobenzoylchloride (30 mg) and *N,N*-dimethylaminopyridine (15 mg). The whole mixt. was stirred at 80° for 8 hr. The reaction mixt. was then partitioned into a mixt. of $CHCl_3$ and H_2O, and the organic layer was taken, washed with a satd aq. $NaHCO_3$ soln and brine, dried over $MgSO_4$, and then evapd *in vacuo*. The crude product (25 mg) was purified by MPLC [silica gel CQ-3, CH_2Cl_2–MeOH (50:1), detect 254 nm] to give the 16,18-di-*p*-bromobenzoate **2b** (10 mg). Compound **2b**: amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3420 (br), 1760, 1750, 1285, 1260, 1245, 1150. UV λ_{max}^{MeOH} nm (ε): 244 (41 100). CD (MeOH) $\Delta\varepsilon$: +15.4 at 254 nm, −8.6 at 236 nm. FAB-MS *m/z* 795, 793. ^1H NMR (pyridine-d_5): δ1.20 (3H, *d*, J = 6.6 Hz, H-9), 1.44 (3H, *d*, J = 6.6 Hz, H-10), 1.78 (3H, *d*, J = 6.2 Hz, H-19), 1.97 (3H, *s*, H-7), 2.78 (1H, *sept*, J = 6.6 Hz, H-8), 3.48 (1H, *d*, J = 15.0 Hz, H-14β), 3.43 (1H, *d*, J = 1.0 Hz, H-5), 3.54 (3H, *s*, OMe-2), 3.79 (1H, *br s*, H-2), 4.22 (1H, *d*, J = 2.9 Hz, H-11), 4.32 (1H, *d*, J = 2.9 Hz, H-12), 4.85 (1H, *d*, J = 15.0 Hz, H-14β), 5.10 (1H, *br s*, H-3), 6.25 (1H, *q*, J = 6.2 Hz, H-18), 7.62, 7.65, 8.03 and 8.19 (each 2H, *d*, J = 8.4 Hz, H-2′, H-3′, H-5′, H-6′, H-2″, H-3″, H-5″ and H-6″).

Crystallographic analysis of picrodendrin F 16-p-*bromobenzoate* (**2a**). Crystal data: $C_{27}H_{31}O_{11}Br$; M_r 611.445; orthorhombic, space group $P2_12_12_1$ (No. 19), a = 15.259 (5), b = 24.876 (5), c = 15.029 (4) Å, V = 5704.9 Å^3, Z = 8, D_{calc} = 1.42 g cm^{-3}. A crystal of approximate dimensions 0.3 × 0.2 × 0.1 mm, recrystallized from aq. MeOH. The cell dimension and intensities were measured on an Enraf-Nonius CAD4 diffractometer using monochromated CuK_α radiation with ω-2θ scan mode within $4 < 2\theta < 130°$. A total of 5400 independent reflections were collected, among with 4139 reflections [$I > 3\sigma(I)$] were stored as observed. The structure was solved by the direct method and refined by the full-matrix least-squares method. No hydrogen atoms were included in the structure factor calculation. The final R value was 0.156. The relative molecular structure was determined as shown in Fig. 1.

Picrodendrin-I (**3**). Prisms (MeOH), mp 270–271°, $[\alpha]_D^{27}$ −24.3° (pyridine; *c* 1.3). IR ν_{max}^{KBr} cm^{-1}: 3495, 1760, 1180, 1045, 995, 865. MS *m/z* (rel. int.): 312 $[M]^+$ (17), 294 (12), 247 (8), 221 (21), 193 (11), 155 (35), 91 (100). ^1H NMR and ^{13}C NMR: data are given in Tables 1 and 2, respectively. Anal. calcd $C_{15}H_{20}O_7$: C, 57.69; H, 6.45. Found: C, 57.50; H, 6.47. NOE difference spectra: irradiated proton→observed proton (NOE enhancement %); δ4.58 (H-7)→δ5.14 (H-2, 7) and 5.15 (H-3, 4); δ1.19 (H-10)→δ5.14 (H-2, 3) and 5.15 (H-3, 7), δ1.11 (H-9)→3.21 (H-5, 7); δ4.14 (H-11, 10)→δ3.21 (H-5, 10) and 3.43 (H-6, 6) and 3.62 (H-12, 15); δ3.12 (H-12)→δ3.11 (Hβ-14, 5) and 4.14 (H-11, 7).

Acknowledgements—We are grateful to Prof. Michio Takido and Dr Susumu Kitanaka (Nihon University) for measurement of CD spectrum and P. T. Indonesia Eisai Co., Ltd, for their kind supply of the plant material.

REFERENCES

1. Ohmoto, T., Koike, K., Fukuda, H. Mitsunaga, K., Kagei, K., Kawai, T. and Sato, T. (1989) *Chem. Pharm. Bull.* **37**, 1805.
2. Ohmoto, T., Koike, K., Fukuda, H., Mitsunaga, K., Ogata, K. and Kagei, K. (1989). *Chem. Pharm. Bull.* **37**, 2988.
3. Harada, N. and Nakanishi, K. (1982) *Circular Dichroic Spectroscopy Exciton Coupling Chemistry*. Tokyo Kagaku Dojin, Tokyo.