New Diarylheptanoids from *Alpinia pinnanensis*

Phan Minh Giang,[a,b] Phan Tong Son,[a] Katsuyoshi Matsunami,[b] and Hideaki Otsuka*,[b]

[a] *Faculty of Chemistry, College of Natural Science, Vietnam National University; 19 Le Thanh Tong, Hanoi, Vietnam: and* [b] *Graduate School of Biomedical Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan.*
Received April 22, 2005; accepted June 20, 2005

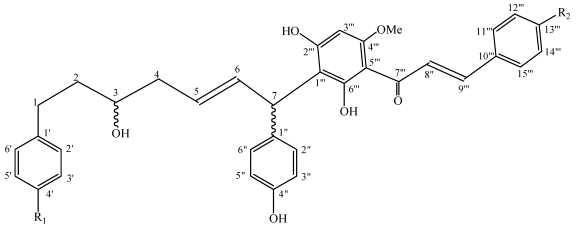
Three new diarylheptanoids, called alpinnanins A—C (1—3), together with two known chalcones, 2′,4′-dihydroxy-6′-methoxychalcone and 4′,6′-dimethylchalconaringenin, two known flavanones, alpinetin and naringenin 5-*O*-methyl ether, a known diarylheptanoid, (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene, stigmasterol and β-sitosterol as a mixture, and β-sitosterol 3-*O*-β-D-glucopyranoside were isolated from the rhizomes of *Alpinia pinnanensis* T. L. Wu *et* Senjen (Zingiberaceae). Their structures were elucidated by spectroscopic analyses.

Key words alpinnanin; *Alpinia pinnanensis*; Zingiberaceae; diarylheptanoid

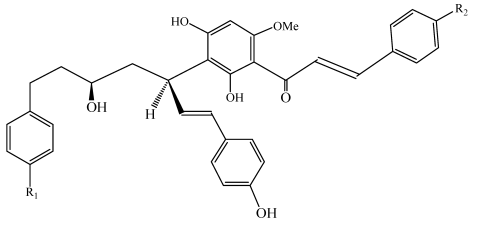
In our continuation of studies on the plants growing in Vietnam,[1,2] we have examined extracts of the rhizomes of *Alpinia pinnanensis* T. L. Wu *et* Senjen (Zingiberaceae), which was recently found in Tam Dao, Vinh Phuc Province, northern Vietnam. This plant is usually distributed on mountainous slopes, under forest shade, at 800—1300 m above sea level.[3] No previous chemical study on this plant has been reported. In this paper, we describe the isolation and structure determination of three new diarylheptanoids (**1**—**3**) (Fig. 1), two known chalcones, 2′,4′-dihydroxy-6′-methoxychalcone[4,5] and 4′,6′-dimethylchalconaringenin,[6] two known flavanones, alpinetin[7] and naringenin 5-*O*-methyl ether,[4] and a known diarylheptanoid, (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene,[8] together with stigmasterol,[9] β-sitosterol,[9] and β-sitosterol 3-*O*-β-D-glucopyranoside,[9] from the rhizomes of *A. pinnanensis* growing in Vietnam. The structures of the new diarylheptanoids (**1**—**3**) were elucidated on the basis of detailed analyses of 1D- and 2D-NMR spectroscopic data, while the known compounds were determined by comparing their physical ($[\alpha]_D$) and spectral properties with literature data.[4—9]

The oven-dried and powdered rhizomes of *A. pinnanensis* were extracted with MeOH by percolation. Sequential solvent partition of the crude MeOH extract gave *n*-hexane- and CH_2Cl_2-soluble fractions, which were subjected to repeated open column chromatography over silica gel and repeated preparative reversed-phase (RP)-HPLC to give the above-mentioned compounds.

Compound **1** was isolated as a pale yellow amorphous powder, $[\alpha]_D^{25}$ −33.3° (c=0.21, MeOH). The molecular formula was determined as $C_{35}H_{34}O_6$ by negative-ion HR-FAB-MS, which exhibited a pseudo-molecular ion-peak at m/z 549.2313 $[M-H]^-$ (Calcd for $C_{35}H_{33}O_6$: 549.2277). The IR spectrum indicated the presence of hydroxyl (3300 cm^{-1}) and ketone (1611 cm^{-1}) functional groups and aromatic rings (1560, 1511, 1450 cm^{-1}). The ^{1}H- and ^{13}C-NMR (Tables 1, 2) spectroscopic data of **1** showed the presence of three methylenes (δ_C 41.3, 39.2, 32.5), one hydroxy-bearing methine [δ_C 71.6, δ_H 3.61, assigned from a cross peak observed in the heteronuclear single quantum correlation (HSQC) spectrum], one methine (δ_C 43.2, δ_H 5.14), two double bonds (δ_C 135.6, δ_H 6.33; δ_C 127.5, δ_H 5.55 and δ_C 142.1, δ_H 7.66; δ_C 129.19, δ_H 7.92), both with *trans*-geometries as indicated by their large coupling constants (J=15.1 Hz and J=15.6 Hz, respectively), one carbonyl (δ_C 193.5), and one methoxyl (δ_C 56.0, δ_H 3.89), and 24 sp^2 aromatic carbons. Thus **1** was found to have a planar structure of a diarylheptanoid containing a chalcone moiety, closely related to calyxins B (**4**) and H (**6**), and epicalyxins B (**5**) and H (**7**),[10,11] isolated from *Alpinia blepharocalyx*. By analysis of the ^{1}H–^{1}H correlated spectroscopy (COSY) (Fig. 2), the hydroxyl group was affixed at C-3 (δ_C 71.6), while the chalcone part was attached to C-7 (δ_C 43.2), causing the downfield shift of H-7. This assignment was confirmed by heteronuclear multiple bond correlation (HMBC) (Fig. 2) cross peaks from H-7 (δ_H 5.14) to C-1″ (δ_C 136.3), C-1‴ (δ_C 112.2), C-2‴ (δ_C 165.1), C-6‴ (δ_C 166.1), C-5 (δ_C 127.5), and C-6 (δ_C 135.6), from H-5 (δ_H 5.55) to C-3 (δ_C 71.6) and C-7 (δ_C 43.2), and from H-2″ and H-6″ (δ_C 7.05) to C-7, as well as from H_2-1 (δ_H 2.71/2.59) to C-3. The HMBC spectrum allowed us to assign two monosubstituted benzene units at C-1 and C-9‴, and a 1,4-disub-



	R_1	R_2		
1	H	H	3*R*	7*S*
2	H	H	3*S*	7*S*
4	OH	OH	3*S*	7*S*
5	OH	OH	3*S*	7*R*
6	H	OH	3*S*	7*S*
7	H	OH	3*S*	7*R*



	R_1	R_2
3	H	H
8	OH	OH

Fig. 1. Structures of Compounds **1**—**8**

* To whom correspondence should be addressed. e-mail: hotsuka@hiroshima-u.ac.jp

Table 1. ^{1}H-NMR Spectroscopic Data of **1**—**3** (δ in ppm, J in Hz, 400 MHz, CD_3OD)

H	1	2	3
1	2.71 m[a)]	2.71 m[a)]	2.66 m[a)]
	2.59 m[a)]	2.59 m[a)]	2.58 m[a)]
2	1.75 m[a)]	1.79 m[a)]	1.75 m[a)]
	1.64 m[a)]	1.66 m[a)]	1.68 m[a)]
3	3.61 m[a)]	3.63 m[a)]	3.42 m[a)]
4	2.25 q-like (7.1)	2.29 t (6.4)	1.89 m[a)]
			2.27 m[a)]
5	5.55 dt (15.1, 7.1)	5.58 dt (15.2, 6.4)	4.22 q (8.5)
6	6.33 dd (15.1, 8.5)	6.34 dd (15.2, 8.4)	6.58 dd (16.1, 8.5)
7	5.14 d (8.5)	5.15 d (8.4)	6.28 d (16.1)
2′,6′	7.09 br d (7.3)	7.11 m[a)]	7.13 m[a)]
3′,5′	7.18 br t (7.3)	7.19 br t (7.3)	7.13 m[a)]
4′	7.11 m[a)]	7.11 m[a)]	7.13 m[a)]
2″,6″	7.05 d (8.2)	7.05 d (8.6)	7.14 d (9.0)
3″,5″	6.62 d (8.2)	6.62 d (8.6)	6.68 d (9.0)
3‴	6.02 s	6.05 s	6.04 s
8‴	7.92 d (15.6)	7.92 d (15.6)	7.92 d (15.6)
9‴	7.66 d (15.6)	7.68 d (15.6)	7.69 d (15.6)
11‴,15‴	7.61 br d (7.3)	7.62 br d (6.4)	7.62 br d (6.7)
12‴,14‴	7.36 m[a)]	7.40 m[a)]	7.39 m[a)]
13‴	7.36 m[a)]	7.42 m[a)]	7.40 m[a)]
OMe	3.89 s	3.92 s	3.89 s

a) Average values for unresolved signals, which were determined as multiplets from HSQC experiments.

Table 2. ^{13}C-NMR Spectroscopic Data of **1**—**3** (δ in ppm, 100 MHz, CD_3OD)

C	1	2	3
1	32.5	32.8	32.9
2	39.2	39.4	40.3
3	71.6	71.9	70.7
4	41.3	41.4	41.8
5	127.5	127.8	36.7
6	135.6	135.6	131.4
7	43.2	43.4	129.7
1′	143.5	143.8	143.7
2′,6′	129.0	129.24	129.20
3′,5′	129.17	129.4	129.4
4′	126.3	126.6	126.5
1″	136.3	136.6	131.39
2″,6″	129.3	129.5	128.2
3″,5″	115.3	115.5	116.2
4″	155.7	155.9	157.4
1‴	112.2	112.4	111.2
2‴	165.1	164.2	164.5
3‴	92.4	92.2	92.4
4‴	162.7	162.9	162.8
5‴	106.3	106.7	106.2
6‴	166.1	166.3	166.5
7‴	193.5	194.5	194.2
8‴	129.19	129.3	129.4
9‴	142.1	142.5	142.6
10‴	136.9	137.1	137.0
11‴,15‴	129.1	129.28	129.25
12‴,14‴	129.8	130.0	130.0
13‴	130.8	131.1	131.1
OMe	56.0	56.3	56.3

stituted benzene unit at C-7. Long-range correlations which were detected in the HMBC spectrum between the singlet aromatic proton at C-3‴ (δ_H 6.02) and C-1‴, C-2‴, C-4‴ (δ_C 162.7), C-5‴ (δ_C 106.3), and between the methoxyl group (δ_H 3.89) and C-4‴, and similarity of the ^{13}C chemical shifts of the aromatic carbons [δ_C 112.2 (C-1‴), 165.1 (C-2‴), 92.4 (C-3‴), 162.7 (C-4‴), 106.3 (C-5‴), and 166.1 (C-6‴)] and methoxyl group (δ_C 56.0) in the A-ring of the chalcone moiety to those reported for **4**—**7**,[10,11] established the planar structure of **1** as shown (Fig. 1). The absolute stereochemistry at C-3 and C-7 was determined by an established comparative method[11] based on comparison of proton splitting patterns at C-4 and optical activity with structurally related calyxins B (**4**) and H (**6**), and epicalyxins B (**5**) and H (**7**). The splitting patterns of protons at C-4 in the ^{1}H-NMR spectrum are triplet (J=7.5 Hz) in the levorotatory calyxins B and H (3*S*,7*S*) and quartet-like (J=7.5 Hz) in the dextrorotatory epicalyxins B and H (3*S*,7*R*). The differences in the splitting patterns of H_2-4 indicate the relative stereochemistry of the hydroxyl group at C-3 and the aromatic ring at C-7 as *anti* (triplet splitting pattern) or *syn* (quartet-like splitting pattern), and the negative and positive optical rotations determine the 7*S* and 7*R* configurations, respectively.[10,11] Compound **1** was levorotatory and showed a quartet-like signal of H_2-4 (δ_H 2.25, 2H, q-like, J=7.1 Hz) in the ^{1}H-NMR spectrum, therefore the absolute stereochemistry at C-3 and C-7 of **1**, called alpinnanin A, was determined to be 3*R*,7*S*.

Compound **2** was isolated as a pale yellow amorphous powder, $[\alpha]_D^{25}$ −39.3° (c=0.28, MeOH). The IR spectrum indicated the presence of hydroxyl (3330 cm^{-1}) and ketone (1609 cm^{-1}) functional groups and aromatic rings (1559, 1510, 1451 cm^{-1}). The ^{1}H- and ^{13}C-NMR (Tables 1, 2) spectroscopic data of **2** resembled those of **1**, and the molecular formula was analyzed for $C_{35}H_{34}O_6$, and found to be the same as that of **1**, by negative-ion HR-FAB-MS. Taken together, all the data suggested that **2** was a stereoisomer of **1** either at C-3 or C-7. The triplet splitting pattern of H-4 protons [δ_H 2.29 (2H, t, J=6.4 Hz)] in the ^{1}H-NMR spectrum of **2** was the same as those of calyxins B (**4**) and H (**6**),[10,11] instead of the quartet-like pattern in the ^{1}H-NMR spectrum of **1**. Based on the comparison with the model compounds ca-

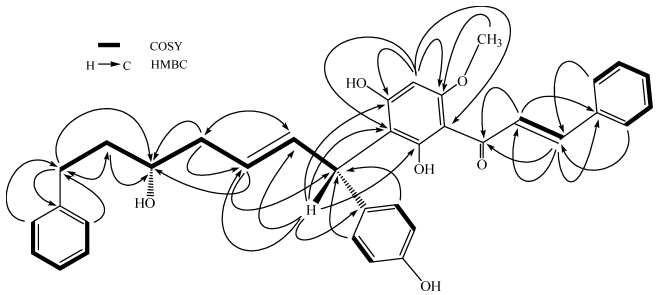


Fig. 2. ^{1}H–^{1}H COSY and HMBC Correlations Observed for Compound **1**

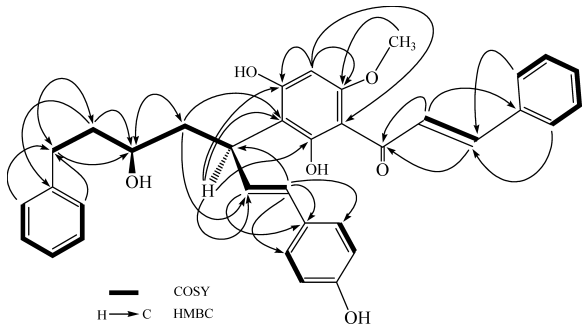


Fig. 3. ^{1}H–^{1}H COSY and HMBC Correlations Observed for Compound **3**

lyxins B and H,[10,11] **2** was assigned to possess a 3*S*,7*S* configuration. Thus the absolute structure of **2**, called alpinnanin B, was determined as shown in Fig. 1.

Compound **3**, a pale yellow amorphous powder, $[\alpha]_D^{25}$ +42.9° (c=0.14, MeOH), was assigned the molecular formula $C_{35}H_{34}O_6$ on the basis of negative-ion HR-FAB-MS. The presence of hydroxyl (3300 cm^{-1}) and ketone (1610 cm^{-1}) functional groups and aromatic rings (1562, 1512, 1450 cm^{-1}) was inferred from the IR spectrum. In the ^{13}C-NMR spectrum of **3**, 35 carbon signals were observed, including three methylenes (δ_C 41.8, 40.3, 32.9), one hydroxy-bearing methine (δ_C 70.7), one methine (δ_C 36.7), one methoxyl (δ_C 56.3), one carbonyl (δ_C 194.2), and 28 sp^2 carbons, four of which were attributable to two disubstituted double bonds (δ_C 131.4, 129.7; δ_C 142.6, 129.4). The large coupling constants of the olefinic protons (δ_H 6.58, dd, J=16.1, 8.5 Hz; 6.28, d, J=16.1 Hz and δ_H 7.92, d, J=15.6 Hz; 7.69, d, J=15.6 Hz) were consistent with *trans*-geometries of two double bonds. Consistent with the molecular weight, a diarylheptanoid chain and a chalcone moiety were constructed by detailed analyses of the ^{1}H- and ^{13}C-NMR (Tables 1, 2), ^{1}H–^{1}H COSY, HSQC, and HMBC spectra of **3** (Fig. 3). In the heptanoid chain the chalcone moiety was assigned at C-5, since H-5 [δ_H 4.22 (q, J=8.5 Hz)] was coupled with the terminal *trans*-double bond [δ_H 6.58 (dd, J=16.1, 8.5 Hz) (H-6), δ_H 6.28 (d, J=16.1 Hz) (H-7)], while the hydroxyl group was located at C-3. This was confirmed by HMBC correlations between H-7 (δ_H 6.28) and C-5 (δ_C 36.7), between H-5 (δ_H 4.22) and C-6 (δ_C 131.4), C-1‴ (δ_C 111.2), C-2‴ (δ_C 164.5), C-6‴ (δ_C 166.5), and between H_2-4 (δ_H 2.27/1.89) and C-1‴, C-6, as well as between H_2-1 (δ_H 2.66/2.58) and C-3 (δ_C 70.7), between H_2-2 (δ_H 1.75/1.68) and C-3, and between H_2-4 and C-3. The distribution of the benzene units was determined by cross peaks observed in the HMBC spectrum. Further comparison of NMR data of **3** with those of **1** and **2** suggested **3** had the same chalcone part as **1** and **2**. Considering the splitting pattern of H-5 [δ_H 4.22 (q, J=8.5 Hz)] and dextrorotatory specific optical rotation of **3**, the same as those of model compound deoxycalyxin A (**8**),[12] possessing close structural similarity, the absolute configurations at C-3 and C-7 of **3**, called alpinnanin C, were deduced to be 3*S*,7*R* (Fig. 1).

Alpinnanins A—C (**1**—**3**) are classified as new members of acyclic diarylheptanoids having a chalcone moiety. So far, this type of diarylheptanoid has been isolated from *A. blepharocalyx*[10—12]; therefore, the presence of alpinnanins A—C in *A. pinnanensis* may be significant from a chemotaxonomic point of view.

Experimental

General Procedure Optical rotations were measured on a Union Giken PM-101 digital polarimeter at 25 °C. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a JEOL JNM α-400 NMR spectrometer. Negative-ion HR-FAB-MS were measured on a JEOL SX-102 mass spectrometer with PEG-400 as a calibration matrix. HPLC was carried out with a JASCO PU-1580 pump and an UV-2075 Plus detector (set at 254 nm). Silica gel 60 (0.063—0.200 mm, Merck) and reversed-phase octadecyl silica (ODS, YMC) gel were used for open column chromatography. TLC was carried out on Merck precoated TLC plates (silica gel 60 F_{254}), and detected by spraying with 10% H_2SO_4 in 50% EtOH, followed by heating on a hot plate at 200 °C.

Plant Material The fresh rhizomes of *A. pinnanensis* were collected in Tam Dao, Vinh Phuc Province, Vietnam, and identified by Taxonomist Nguyen Quoc Binh (Institute of Ecology and Biological Resources, National Center for Science and Technology, Hanoi, Vietnam), in August 2003. A voucher specimen (No. STTN 2003-9) is deposited in the Herbarium of the same Institute.

Extraction and Isolation The fresh rhizomes (4.0 kg) were sliced, oven-dried at 40 °C, and then powdered to give 1.0 kg of a dry material which was extracted with MeOH by percolation at room temperature. The obtained MeOH extract (123.9 g) was partitioned between H_2O and solvents of increasing polarity (*n*-hexane, CH_2Cl_2, EtOAc, and 1-BuOH, successively). The *n*-hexane-soluble fraction (5.8 g) was subjected to silica gel open column chromatography (*n*-hexane–EtOAc solvent systems, 5 : 1 and 2 : 1) to give six fractions, collected on the basis of their TLC patterns. A mixture of stigmasterol and β-sitosterol (82.6 mg) was obtained from fraction 3 by silica gel open column chromatography (*n*-hexane–EtOAc, 5 : 1), 2′,4′-dihydroxy-6′-methoxychalcone (20.0 mg) was precipitated from fraction 5 and from the CH_2Cl_2-soluble fraction after treatment with MeOH, (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (31.1 mg) was purified from fraction 6 by silica gel open column chromatography (*n*-hexane–EtOAc, 2 : 1), and β-sitosterol 3-*O*-β-D-glucopyranoside (5.0 mg) was precipitated from fraction 6 with MeOH. After precipitation of 2′,4′-dihydroxy-6′-methoxychalcone, the residue of the CH_2Cl_2-soluble fraction (3.9 g) was subjected to silica gel open column chromatography (*n*-hexane–EtOAc solvent systems, 4 : 1, 2 : 1, 1 : 1, and 2 : 3) to give seven fractions. Repeated preparative HPLC of fractions 6 and 7 on a YMC ODS column (150×20 mm i.d.) at a flow rate of 5 ml/min using MeOH–H_2O (4 : 1) as a solvent system gave alpinetin (24.6 mg), naringenin 5-*O*-methyl ether (27.9 mg), (3*S*,5*S*)-*trans*-3,5-dihydroxy-1,7-diphenyl-1-heptene (12.1 mg), **1** (2.7 mg), **2** (3.2 mg), and **3** (3.0 mg). The structures of the known compounds were determined by comparing their $[\alpha]_D$, ^{1}H- and ^{13}C-NMR data with literature values.[4—9]

Alpinnanin A (**1**): Pale yellow amorphous powder. $[\alpha]_D^{25}$ −33.3° (c=0.21, MeOH). IR ν_{max} (film) cm^{-1}: 3300, 1611, 1560, 1511, 1450, 1335, 1227, 1141, 1107. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 549.2313 $[M-H]^-$ (Calcd for $C_{35}H_{33}O_6$: 549.2277).

Alpinnanin B (**2**): Pale yellow amorphous powder. $[\alpha]_D^{25}$ −39.3° (c=0.28, MeOH). IR ν_{max} (film) cm^{-1}: 3330, 1609, 1559, 1510, 1451, 1334, 1227, 1140, 1105. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 549.2285 $[M-H]^-$ (Calcd for $C_{35}H_{33}O_6$: 549.2277).

Alpinnanin C (**3**): Pale yellow amorphous powder. $[\alpha]_D^{25}$ +42.9° (c=0.14, MeOH). IR ν_{max} (film) cm^{-1}: 3300, 1610, 1562, 1512, 1450, 1335, 1228, 1139. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 549.2245 $[M-H]^-$ (Calcd for $C_{35}H_{33}O_6$: 549.2277).

Acknowledgments This work was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS). One of the authors (P.M.G.) is grateful to JSPS for a Postdoctoral Research Fellowship at Hiroshima University and to the International Foundation for Science (Stockholm, Sweden) for a Research Grant. The authors thank the Research Center of Molecular Medicine of the Hiroshima University Faculty of Medicine, for the use of its 400 MHz NMR instrument.

References

1) Phan M. G., Phan T. S., Lee J. J., Otsuka H., *Chem. Pharm. Bull.*, **52**, 879—882 (2004).
2) Phan M. G., Phan T. S., Otsuka H., *Chem. Pharm. Bull.*, **53**, 232—234 (2005).
3) Nguyen Q. B., "Selection of Research Works on Ecology and Biological Resources (1996—2000)," Agriculture, Hanoi, 2001, pp. 36—38.
4) Wollenweber E., Jay M., Favre-Bonvin J., *Phytochemistry*, **13**, 2618—2619 (1974).
5) "The Flavonoids: Advances in Research," ed. by Harborne J. B., Mabry T. J., Chapman and Hall, London, 1982.
6) Stevens J. F., Taylor A. W., Nickerson G. B., Ivancic M., Henning J., Haunold A., Deinzer M. L., *Phytochemistry*, **53**, 759—775 (2000).
7) Itokawa H., Morita M., Mihashi S., *Phytochemistry*, **20**, 2503—2506 (1981).
8) Kuroyanagi M., Noro T., Fukushima S., Aiyama R., Ikuta A., Itokawa H., Morita M., *Chem. Pharm. Bull.*, **31**, 1544—1550 (1983).
9) Brooks C. J. W., "Rodd's Chemistry of Carbon Compounds," 2nd ed., Vol. IID, Elsevier, Amsterdam, 1970.
10) Prasain J. K., Tezuka Y., Li J. X., Tanaka K., Basnet P., Dong H., Namba T., Kadota S., *Tetrahedron*, **53**, 7833—7842 (1997).
11) Prasain J. K., Li J. X., Tezuka Y., Tanaka K., Basnet P., Dong H., Namba T., Kadota S., *J. Nat. Prod.*, **61**, 212—216 (1998).
12) Tezuka Y., Gewali M. B., Ali M. S., Banskota A. H., Kadota S., *J. Nat. Prod.*, **64**, 208—213 (2001).