Three New 15,16-Seco-cycloartane Glycosides from Cimicifuga Rhizome

Hitoshi YOSHIMITSU,*[,a] Makiko NISHIDA,[b] and Toshihiro NOHARA[c]

[a] *Faculty of Pharmaceutical Sciences, Sojo University; 4–22–1 Ikeda, Kumamoto 862–0082, Japan:* [b] *Faculty of Engineering, Kyushu Kyoritsu University; 1–8 Jiyugaoka, Yahata-nishi-ku, Kitakyushu 807–8585, Japan: and* [c] *Faculty of Medical and Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.*
Received February 2, 2007; accepted March 10, 2007; published online March 12, 2007

Three new 15,16-seco-cycloartane glycosides, which were constructed by a C–C bond cleavage in the D ring, have been isolated from Cimicifuga Rhizome for the first time. Their structures were determined by the use of 2D NMR techniques and chemical evidence.

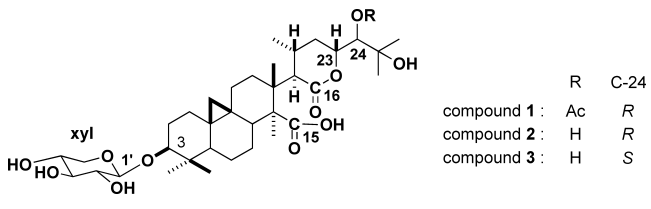
Key words Cimicifuga Rhizome; cycloartane glycoside; *Cimicifuga* sp.; Ranunculaceae

The genus *Cimicifuga* is one of the smallest genera in the family Ranunculaceae. It comprises about 25 species distributed throughout East Asia, Europe, and North America. Cimicifuga Rhizome, originated from a rhizome of the genus *Cimicifuga* plants, has been used as anti-inflammatory, analgesic, and antipyretic remedies in Chinese traditional medicine. Moreover, it has been used in combination with other herbs in the ancient Kampo medicine as anti-inflammatory drugs. Meanwhile, *Cimicifuga racemosa*, commonly known as black cohosh, is a herb indigenous to North America and Europe. Its rhizomes have been used to treat a variety of ailments, including diarrhea, sore throat, and rheumatism by Native Americans.[1)] In the chemical constituents of the genus *Cimicifuga* plants, many kinds of cycloartane triterpene have been reported by several groups.[2—5)] In our extended search for a cycloartane glycoside, we have reported the structural characterization of many cycloartane glycosides, thalictosides D, E, and F from the aerial parts of *Thalictrum thunbergii* D.C.,[6,7)] thalictosides I and II from Thalictri Haba,[8)] aquilegiosides A and B from the aerial parts of *Aquilegia flabellate* SIEB. *et* ZUCC. var. *flabellata*,[9)] aquilegiosides C, D, E, F, G, H, I, and J from the aerial parts of *Aquilegia vulgaris* L.,[10,11)] and two tetranor-cycloartane[12)] and two 15,16-seco-cycloarten[13)] glycosides from Cimicifuga Rhizome. In a continuing study, we have isolated three new 15,16-seco-cycloartane glycosides (**1**—**3**) from Cimicifuga Rhizome. This paper describes their structural elucidation based on 2D NMR spectroscopic analysis and hydrolysis.

Results and Discussion

The methanolic extract of Cimicifuga Rhizome was partitioned into a chloroform–water solvent system. The water-soluble portion was subjected to MCI gel CHP20P, octadecyl silica gel (ODS), and silica gel column chromatographies and finally HPLC to give three glycosides (**1**—**3**).

The molecular formula of compound **1**, $C_{37}H_{58}O_{12}$, was established by high-resolution (HR)-FAB-MS [*m*/*z* 717.3835, $(M+Na)^+$]. One cyclopropane methylene at δ_H −0.02 (1H, d, J=4.4 Hz) and 0.73 (1H, d, J=4.4 Hz), which appeared at higher fields, six quaternary methyls at δ_H 1.00, 1.25, 1.58, 1.60, 1.65, and 1.98, a secondary methyl at δ_H 1.07 (d, J=6.8 Hz), an acetyl methyl at δ_H 2.11, and an anomeric proton at δ_H 4.89 (d, J=7.8 Hz) on the ^{1}H-NMR spectrum suggested **1** to be a cycloartane glycoside. On acid hydrolysis, **1** afforded D-xylopyranose, the structure of which was confirmed by the ^{1}H-NMR coupling pattern and optical rotation using chiral detection in the HPLC analysis, together with several unidentified artificial sapogenols. Thirty carbon signals due to the aglycon moiety were observed along with a xylopyranose unit (δ_C 107.5, 75.6, 78.6, 71.3, 67.2) and an acetyl unit (δ_C 20.8, 171.0) in the ^{13}C-NMR spectrum. The structural assignment was achieved by ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments. ^{1}H–^{1}H COSY and HMBC led us to the plane structure of **1** as 24-acetoxy-15,16-seco-cycloartane 3-*O*-xylopyranoside (Fig. 1). In particular, the long-range correlations between an acetyl methyl proton (δ_H 2.11) and an acetyl carbon (δ_C 171.0); H-24 (δ_H 5.33) and C-25 (δ_C 71.5), an acetyl carbon (δ_C 171.0); two singlet methyl protons (δ_H 1.58, 1.65) and C-24 (δ_C 79.6), C-25 determined the terminal structure on the side chain. Furthermore, HMBC cross-peaks between H-17 (δ_H 3.05) and C-13 (δ_C 44.3), C-16 (δ_C 172.9), C-18 (δ_C 21.7), C-20 (δ_C 27.6); H_3-18 (δ_H 1.98) and C-12 (δ_C 33.2), C-13, C-14 (δ_C 55.5), C-17 (δ_C 56.5); H_3-28 (δ_H 1.60) and C-8 (δ_C 39.0), C-13, C-14, C-15 (δ_C 178.6); H-23 (δ_H 5.36) and C-16 resulted in the C–C bond cleavage between C-15 and C-16, and the six-membered lactone ring between C-16 and C-23. The glycoside linkage was assigned as β from observation of the anomeric



	R	C-24
compound **1** :	Ac	*R*
compound **2** :	H	*R*
compound **3** :	H	*S*

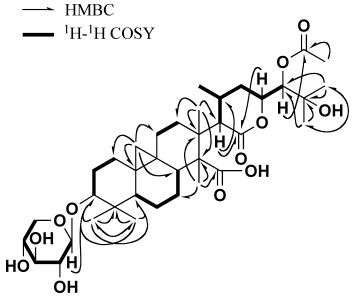


Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**

* To whom correspondence should be addressed. e-mail: hyoshimi@ph.sojo-u.ac.jp

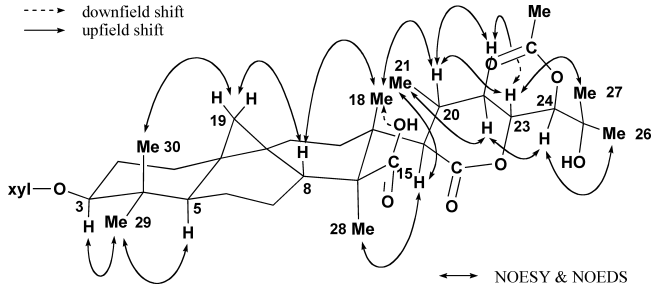


Fig. 2. NOE Correlations of **1** in Pyridine-d_5

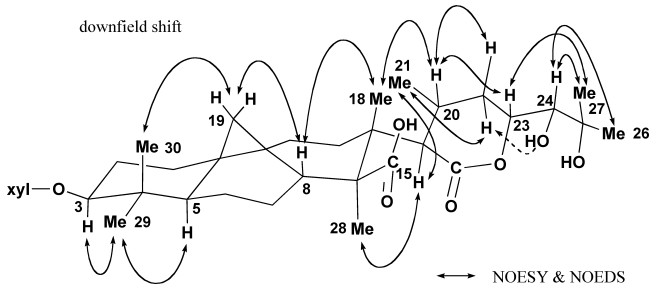


Fig. 3. NOE Correlations of **3** in Pyridine-d_5

proton signal at δ_H 4.89 (d, J=7.8 Hz). As a result, the anomeric center was determined to be *R* configuration. Meanwhile, the H-3 [δ_H 3.48 (1H, dd, J=4.2, 11.7 Hz)] signal was superimposable on that of 26-deoxyactein,[14] the absolute configuration of which was elucidated on the basis of the spectroscopic data and single-crystal X-ray data analysis. Furthermore, a comparative study of the ^{13}C-NMR spectrum of **1** with that of 26-deoxyactein[14] revealed identical signals due to the xylopyranose moiety and C-2 (β-C), C-3 (α-C), and C-4 (β'-C) in the A ring of the aglycon moiety. In consideration of glycosylation shifts,[15,16] the foregoing evidence indicated that C-3 of the aglycon moiety was assigned as *S* configuration. Nuclear Overhauser and exchange spectroscopy (NOESY) cross-peaks were observed between the following protons: H_3-18 and H-8, H-20; H-20 and H-22β, H-23; H_3-21 and H-17, H-22α; H-22α and H-24; H_3-26 and H-24; H_3-27 and H-23; H_3-28 and H-17; H_3-29 and H-3, H-5; H_3-30 and H_2-19 (Fig. 2). Consequently, this NOESY experiment decided the 3*S*, 13*R*, 14*R*, 17*R*, 20*R*, and 23*R* configurations. Meanwhile, *R* configuration at C-14 was supported by the following observation: The signal due to H_3-18 (δ 1.98) was shifted to a lower field, which must be caused by the hydroxyl group of a carboxyl group at C-14 (Fig. 2). Therefore, the structure of **1** was elucidated except for the stereo configuration at C-24.

The HR-FAB-MS of compound **2** showed a peak at *m*/*z* 675.3729 corresponding to the molecular formula $[C_{35}H_{56}O_{11}Na]^+$ (Calcd for 675.3720). The ^{1}H-NMR spectrums of **2** and **1** were almost identical, with a remarkable difference being the H-24 (δ_H 3.70) signal, which was shifted to a higher field by 1.63 ppm, and the disappearance of the acetyl methyl signal. The ^{13}C-NMR spectrum showed 35 carbon signals due to the aglycon moiety (30 carbons) and the sugar moiety (5 carbons), indicating that **2** was a cycloartane glycoside related to **1**. Hydrolysis of **2** afforded D-xylopyranose together with several unidentified artificial sapogenols. HMBC correlations of H-24 (δ_H 3.70) to C-25 (δ_C 72.6) and H_3-26 (δ_H 1.64) to C-24 (δ_C 79.8) and C-25 and H_3-27 (δ_H 1.69) to C-24 and C-25 implied the disappearance of an acetyl group at C-24 in **1**. The identical NOESY cross-peak pattern with **1** decided that **2** had the same stereochemistry as **1** had. Meanwhile, the identical stereo configuration at C-24 with **1** was suggested that **2** revealed the same NOE correlations of H_3-26/H-24 and H_3-27/H-23 and the coupling pattern of H-24 (br s) as **1** revealed. In a comparative study of the ^{1}H-NMR spectrum of **1** with that of **2**, *R* configuration at C-24 in **1** determined that H-22β (δ_H 1.53) was shifted to a higher field by 0.44 ppm and H-23 (δ_H 5.36) was shifted to a lower field by 0.11 ppm to the latter compound. These shifts were caused by the carbonyl group of an acetyl group at C-24 in **1** (Fig. 2). A similar shift pattern was observed in the 24-*epi*-24-*O*-acetyl-7,8-didehydrohydro-shengmanol 3-*O*-β-D-xylopyranoside (24*R*-type) and not in the C-24 epimer (24*S*-type).[17] Furthermore, *R* configuration at C-24 in **2** was supported by the following observation: The signal due to H_3-27 (δ_H 1.69) in **2** was shifted to a lower field by 0.11 ppm as compared with that in **1**, which was caused by the hydroxyl group at C-24.[18,19]

HR-FAB-MS data [*m*/*z* 675.3741, $(M+Na)^+$] of compound **3** established the molecular formula, $C_{35}H_{56}O_{11}$, which showed the identical molecular formula with **2**. The ^{1}H-NMR spectrum of **3** was similar to that of **2** with a remarkable difference being the H-24 [δ_H 4.01 (d, J=4.0 Hz)] signal. In the ^{13}C-NMR spectrum, chemical shifts of the aglycon moiety, except for signals due to the side chain and the sugar moiety showed coincidence with those of **2**. Hydrolysis of **3** afforded D-xylopyranose together with several unidentified artificial sapogenols. HMBC correlations of H-24 (δ_H 4.01) to C-25 (δ_C 72.0) and H_3-26 (δ_H 1.63) to C-24 (δ_C 79.0) and C-25 and H_3-27 (δ_H 1.59) to C-24 and C-25 led us to the same side chain structure as that of **2**. The foregoing evidence indicated that **3** was an epimer at C-24 in **2**. NOESY cross-peaks were observed between the following protons: H_3-18 and H-8, H-20; H-20 and H-22β, H-23; H_3-21 and H-17, H-22α; H_3-26 and H-24; H_3-27 and H-23, H-24; H_3-28 and H-17; H_3-29 and H-3, H-5; H_3-30 and H_2-19 (Fig. 3). In a comparative study of the NMR data of **3** with those of **2**, *S* configuration at C-24 in **3** was elucidated by the following NMR data. The NOE correlation of **3** revealed the appearance of H_3-27/H-24 and the disappearance of H-22α/H-24. The ^{1}H-NMR spectrum of **3** showed the coupling constants of H-24 (d, J=4.0 Hz), which was observed br s in **2**, and a higher field shift of H_3-27 (δ_H 1.59), which was observed at δ_H 1.69 in **2**. Furthermore, the H-22α (δ_H 2.59) signal, which was shifted to a lower field by 0.48 ppm as compared with that in **2**, indicated *S* configuration at C-24 in **3** (Fig. 3). The shift to a lower field was caused by the hydroxyl group at C-24.[18,19]

These new cycloartane glycosides have structural peculiarities, namely, C–C bond cleavage between C-15 and C-16. Meanwhile, we recently isolated one more 3,16-bisdesmoside of a 15,16-seco-cycloartane glycoside, in which the xylose and glucose linked to the hydroxyl group at C-3 and the carboxyl group at C-16, respectively. Accordingly, C-C bond cleavage between C-15 and C-16 in a cycloartane skeleton might be biosynthetically caused.

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of **1**—**3** (Pyridine-d_5)

	δ_H			δ_C		
	1	**2**	**3**	**1**	**2**	**3**
1	1.39 m, 1.67[a)]	1.40 m, 1.68[a)]	1.39 m, 1.66 m	30.3	30.3	30.3
2	1.92 m, 2.33 m	1.93 m, 2.33 m	1.93 m, 2.33 m	29.5	29.5	29.5
3	3.48 dd (4.2, 11.7)	3.49 dd (4.1, 11.7)	3.50 dd (4.0, 12.0)	88.0	88.0	88.0
4				41.3	41.3	41.3
5	1.55[a)]	1.55[a)]	1.55[a)]	44.1	44.1	44.1
6	1.03[a)], 1.47[a)]	1.03[a)], 1.48[a)]	1.04[a)], 1.49[a)]	18.4	18.4	18.4
7	1.47[a)], 18.2[a)]	1.48[a)], 1.82[a)]	1.49[a)], 1.79[a)]	23.0	23.0	23.0
8	2.88 dd (3.2, 7.6)	2.90 dd (3.4, 7.8)	2.91 dd (4.0, 8.0)	39.0	38.7	38.7
9				20.7	20.8	20.7
10				26.0	26.0	25.9
11	1.51[a)], 1.77[a)]	1.52[a)], 1.77[a)]	1.52[a)], 1.83 m	27.5	27.6	27.5
12	1.51[a)], 2.13[a)]	1.52[a)], 2.14[a)]	1.60[a)], 2.13[a)]	33.2	33.0	33.0
13				44.3	43.8	44.0
14				55.5	55.9	55.7
15				178.6	178.8	178.6
16				172.9	174.0	173.8
17	3.05 d (4.4)	3.14 d (3.4)	3.14 d (3.5)	56.5	56.3	56.6
18	1.98 s	1.98 s	1.81 s	21.7	21.7	21.7
19a	−0.02 d (4.4)	−0.02 d (4.4)	−0.02 d (4.6)	21.8	21.7	21.7
19b	0.73 d (4.4)	0.73 d (4.4)	0.73 d (4.6)			
20	2.45 m	2.50 m	2.49 m	27.6	27.0	27.7
21	1.07 d (6.8)	1.10 d (6.8)	1.12 d (6.3)	25.2	25.2	25.5
22a	1.53 dd (11.7, 13.7)	1.97 dd (11.7, 13.7)	1.87 dd (11.5, 14.3)	35.7	35.4	33.6
22b	2.15 dd (7.3, 13.7)	2.11 dd (7.4, 13.7)	2.59 dd (7.6, 14.3)			
23	5.36 br d (11.2)	5.25 br d (11.2)	5.17 dd (4.0, 11.5)	75.5	77.0	79.1
24	5.33 br s	3.70 br s	4.01 d (4.0)	79.6	79.8	79.0
25				71.5	72.6	72.0
26	1.65 s	1.64 s	1.63 s	26.5	26.1	27.5
27	1.58 s	1.69 s	1.59 s	28.4	29.0	27.3
28	1.60 s	1.58 s	1.61 s	16.2	16.2	16.2
29	1.25 s	1.25 s	1.25 s	25.4	25.4	25.4
30	1.00 s	1.00 s	0.99 s	14.7	14.7	14.7
OAc	2.11 s			20.8		
OAc				171.0		
1′	4.89 d (7.8)	4.88 d (7.8)	4.89 d (7.5)	107.5	107.5	107.5
2′	4.03 dd (7.8, 8.5)	4.02 dd (7.8, 8.5)	4.03 dd (7.5, 8.5)	75.6	75.6	75.6
3′	4.17 dd (8.5, 8.5)	4.15 dd (8.5, 8.5)	4.17 dd (8.5, 8.5)	78.6	78.6	78.6
4′	4.24 m	4.22 m	4.24 m	71.3	71.3	71.3
5′a	3.82 dd (10.2, 11.2)	3.80 dd (10.2, 11.2)	3.82 dd (10.3, 11.5)	67.2	67.2	67.1
5′b	4.41 dd (4.9, 11.2)	4.40 dd (4.9, 11.2)	4.41 dd (5.2, 11.5)			

a) Overlapped.

Experimental

General Procedure Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were measured with a JEOL alpha 500 NMR spectrometer. The NMR samples of compounds **1**—**3** were prepared by pyridine-d_5. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The HR-FAB-MS was recorded with a JEOL HX-110 spectrometer. HPLC was carried out using a TSK gel-120A (7.8 mm i.d.×300 mm) column with a Tosoh CCPM pump, Tosoh RI-8010 detector, and JASCO OR-2090 detector. TLC was performed on pre-coated Kieselgel 60 F_{254} (Merck), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck), octadecyl silica gel (PrePAK-500/C_{18}, Waters) and MCI gel CHP20P (Mitsubishi Chemical Ind.).

Plant Material Cimicifuga Rhizome was purchased from Uchida Wakanyaku Co., Ltd. This dried rhizome originated from Heilungkiang Province in China.

Extraction and Isolation Cimicifuga Rhizome (20 kg) was extracted with MeOH at room temperature for six months. The MeOH extract (2 kg) was partitioned between chloroform-soluble (1046 g), water-soluble (941 g) and insoluble (12 g) portions. The water-soluble portion was subjected to MCI gel CHP20P column chromatography (MeOH/H_2O, 1 : 1→9 : 1) to afford eight fractions [Fractions 1 (1.6 g), 2 (819 mg), 3 (1.4 g), 4 (1.6 g), 5 (1.5 g), 6 (919 mg), 7 (1.5 g), and 8 (802 mg)]. Fraction 4 (1.6 g) was further separated by octadecyl silica gel column chromatography (MeOH/H_2O, 1 : 1→9 : 1) to afford five fractions [Fr. 4-1, Fr. 4-2, Fr. 4-3, Fr. 4-4, and Fr. 4-5]. Fraction 4-3 was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 40 : 10 : 1), followed by HPLC (MeOH/H_2O, 13 : 7), to furnish compound **2** (12 mg). Fraction 4-4 was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 40 : 10 : 1), followed by HPLC (MeOH/H_2O, 13 : 7), to furnish compound **3** (13 mg). Fraction 5 (1.5 g) was further separated by octadecyl silica gel column chromatography (MeOH/H_2O, 1 : 1→9 : 1) to afford six fractions [Fr. 5-1, Fr. 5-2, Fr. 5-3, Fr. 5-4, Fr. 5-5, and Fr. 5-6]. Fraction 5-3 was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O, 40 : 10 : 1), followed by HPLC (MeOH/H_2O, 13 : 7), to furnish compound **1** (13 mg).

Compound **1**: A white powder; $[\alpha]_D$ −29.9° (c=0.63, MeOH); HR-FAB-MS m/z 717.3835 (M+Na; Calcd for $C_{37}H_{58}O_{12}Na$, 717.3826); ^{1}H- and ^{13}C-NMR data (Table 1).

Compound **2**: A white powder; $[\alpha]_D$ −49.6° (c=0.60, MeOH); HR-FAB-MS m/z 657.3729 (M+Na; Calcd for $C_{35}H_{56}O_{11}Na$, 675.3720); ^{1}H- and ^{13}C-NMR data (Table 1).

Compound **3**: A white powder; $[\alpha]_D$ −61.3° (c=1.08, MeOH); HR-FAB-MS m/z 657.3741 (M+Na; Calcd for $C_{35}H_{56}O_{11}Na$, 675.3720); ^{1}H- and ^{13}C-NMR data (Table 1).

Sugar Analysis A solution of each compound (**1**, **2**, or **3**) (1 mg) in 2 N HCl/dioxane (1 : 1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was

neutralized with Amberlite MB-3 and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH_3CN/H_2O (3 : 1, 250 μl). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d.×150 mm, Showa Denko, Tokyo, Japan); solvent, CH_3CN/H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 70 °C; detection, optical rotation (OR). The t_R (min) of the sugars was as follows. **1**: D-xylose 5.5 (+), **2**: D-xylose 5.5 (+), **3**: D-xylose 5.5 (+). [reference: D-xylose 5.5 (positive optical rotation: +)].

Acknowledgements We thank Mr. K. Takeda and Mr. T. Iriguchi of the Center for Instrumental Analysis, Kumamoto University for, the NMR measurements, and Prof. H. Okabe and Dr. J. Kinjo of Fukuoka University for the HR FAB MS measurements.

References

1) Liberman S., *J. Womens Health*, **7**, 525—529 (1998).
2) Sakurai N., Koeda M., Aoki Y., Nagai M., *Chem. Pharm. Bull.*, **43**, 1475—1482 (1995).
3) Kusano A., Shibano M., Tsukamoto D., Kusano G., *Chem. Pharm. Bull.*, **49**, 437—441 (2001).
4) Kadota S., Li J. X., Tanaka K., Namba T., *Tetrahedron*, **51**, 1143—1166 (1995).
5) Radics L., Kajtar-peredy M., Corsano S., Standioli L., *Tetrahedron Lett.*, **1975**, 4287—4290 (1975).
6) Yoshimitsu H., Nishida M., Yahara S., Nohara T., *Tetrahedron Lett.*, **39**, 6919—6920 (1998).
7) Yoshimitsu H., Nishida M., Nohara T., *Tetrahedron*, **57**, 10247—10252 (2001).
8) Yoshimitsu H., Hayashi K., Kumabe M., Nohara T., *Chem. Pharm. Bull.*, **41**, 786—788 (1993).
9) Yoshimitsu H., Nishida M., Hashimoto F., Nohara T., *Phytochemistry*, **51**, 449—452 (1999).
10) Nishida M., Yoshimitsu H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **51**, 683—687 (2003).
11) Nishida M., Yoshimitsu H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **51**, 956—959 (2003).
12) Nishida M., Yoshimitsu H., Nohara T., *Chem. Pharm. Bull.*, **51**, 1117—1118 (2003).
13) Nishida M., Yoshimitsu H., Okawa M., Ikeda T., Nohara T., *Chem. Pharm. Bull.*, **51**, 1215—1216 (2003).
14) Chen S. N., Li W., Fabricant D. S., Santarsiero B. D., Mesecar A., Fitzloff J. F., Fong H. H. S., Farnsworth N. R., *J. Nat. Prod.*, **65**, 601—605 (2002).
15) Kasai R., Suzuo M., Asakawa J., Tanaka O., *Tetrahedron Lett.*, **18**, 175—178 (1977).
16) Seo S., Tomita Y., Tori K., Yoshimura Y., *J. Am. Chem. Soc.*, **100**, 3331—3339 (1978).
17) Kusano A., Takahira M., Shibano M., Miyase T., Kusano G., *Chem. Pharm. Bull.*, **47**, 511—516 (1999).
18) Demarco P. V., Farkas E., Doddrell D., Mylari B. L., Wenkert E., *J. Am. Chem. Soc.*, **25**, 5480—5486 (1968).
19) Mimaki Y., Kubo S., Kinoshita Y., Sahida Y., Song L.-G., Nikaido T., Ohmoto T., *Phytochemistry*, **34**, 791—797 (1993).