Steroidal Glycosides from the Underground Parts of *Solanum sodomaeum*

Masateru ONO,*[,a] Kazuya NISHIMURA,[a] Keita SUZUKI,[a] Takeshi FUKUSHIMA,[a] Keiji IGOSHI,[a] Hitoshi YOSHIMITSU,[b] Tsuyoshi IKEDA,[c] and Toshihiro NOHARA[c]

[a] *School of Agriculture, Kyushu Tokai University; 5435 Minamiaso, Aso, Kumamoto 869–1404, Japan:* [b] *Faculty of Pharmaceutical Sciences, Sojo University; 4–22–1 Ikeda, Kumamoto 860–0082, Japan: and* [c] *Faculty of Medical and Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.*

Received August 3, 2005; accepted October 18, 2005

A new steroidal glycoside has been isolated from the underground parts of *Solanum sodomaeum* L., along with seven known steroidal glycosides. Their chemical structures were determined on the basis of spectroscopic data and chemical evidence, and the structure of one known pregnane type glycoside was corrected. In addition, their antiproliferative activity against human promyelocytic leukemia (HL-60) cells was investigated, and five compounds exhibited stronger activity than cisplatin.

Key words *Solanum sodomaeum*; steroidal glycoside; solasodoside A; pregnane alkaloid; antiproliferative activity

Solanum sodomaeum L. is a solanaceous plant native to wide areas of the Libyan desert. In a previous paper, the antineoplastic activity of the mixture of glycoalkaloids extracted from the fruits of *S. sodomaeum* L. against Sarcoma 180 in mice was reported.[1)] The glycoalkaloids extracted from the fruits, leaves and stems of this plant were investigated using MS and HPLC analyses, and solamargine and solasonine were identified in all the plant materials.[2)] Furthermore, it was reported that a cream formulation containing purified glycoalkaloids from the fruits of the title plant was effective in the treatment of malignant human skin tumours; basal cell carcinomas, squamous cell carcinomas and benign tumours; keratoses and keratoacanthomas.[3)] Regarding another constituents of the fruits of *S. sodomaeum* L., two new pyrrole alkaloids were studied.[4)] Here we describe the isolation and structural elucidation of a new steroidal glycoside from the underground parts of *S. sodomaeum* L., along with seven known steroidal glycosides, one of whose structures was corrected. Additionally, we report their antiproliferative activity against human promyelocytic leukemia (HL-60) cells.

The MeOH extract of the underground parts of *S. sodomaeum* L. was successively subjected to Diaion HP20, silica gel, Sephadex LH20 and Chromatorex ODS column chromatographies, as well as HPLC on ODS, to afford eight steroidal glycosides (**1**—**8**).

Compounds **3**—**8** were identified as solamargine (**3**),[5)] pregna-5,16-dien-3β-ol-20-one 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**4**),[6)] diosgenin 3-*O*-β-solatrioside (**5**),[7)] dioscin (**6**),[8)] indioside D (**7**)[7)] and protodioscin (**8**),[9)] respectively, based on their physical and spectral data.

Compound **1** was obtained as an amorphous powder, and its positive FAB-MS showed $[M+Na]^+$ ion peak at *m*/*z* 1053. The molecular formula of **1** was determined to be $C_{51}H_{82}O_{21}$ by high-resolution (HR) positive FAB-MS. The ^{1}H-NMR spectrum of **1** showed signals due to two tertiary methyl groups (δ 1.06, 0.83), four secondary methyl groups [δ 1.76 (d, J=6.0 Hz), 1.56 (d, J=6.0 Hz), 1.14 (d, J=7.5 Hz), 0.96 (d, J=6.0 Hz)], one methoxyl group (δ 3.52), one olefinic proton [δ 5.34 (d like, J=5.0 Hz)], and four monosaccharide groups, two of which were considered to be rhamnosyl groups. The ^{13}C-NMR spectrum of the aglycone (Ag) moiety of **1** gave signals similar to those of **6**, apart from the appearance of the signals due to one methoxyl carbon (δ 55.6) and one hemiacetal carbon (δ 103.1) and the lack of the signal due to one oxygenated methylene carbon. These NMR signals were assigned in detail with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser and exchange spectroscopy (NOESY) spectra. The HMBC correlations and the coupling constant value for H-26 of Ag (d, J=8 Hz) indicated **1** to be a tetraglycoside of 26β-methoxy diosgenin as shown in Fig. 1. On acidic hydrolysis, **1** afforded D-glucose, L-rhamnose and D-xylose. Furthermore, the ^{13}C-NMR data of the Ag moiety and the sugar moiety were quite similar to those of (25*R*,26*R*)-26-methoxyspirost-5-en-3β-ol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}[10)] and xylosyl-β-solamarine,[11)] respectively. Based on these data, **1** was defined as (25*R*,26*R*)-26-methoxyspirost-5-en-3β-ol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside}, which is tentatively named solasodoside A.

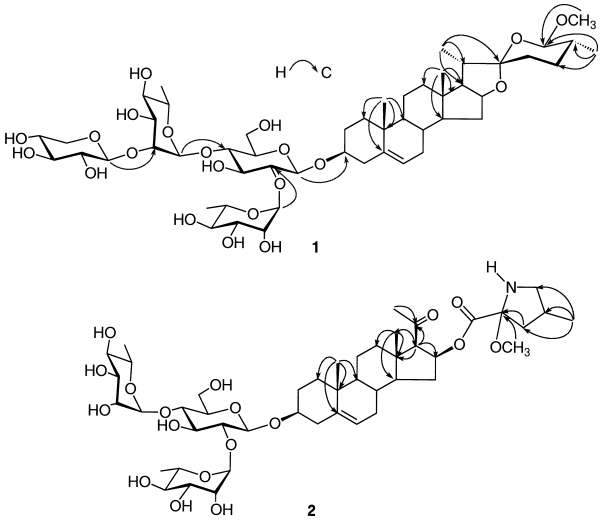

Fig. 1. ^{1}H–^{13}C Long-Range Correlations Observed for **1** and **2** in the HMBC Spectra (in Pyridine-d_5, 500 MHz)

* To whom correspondence should be addressed. e-mail: mono@ktmail.ktokai-u.ac.jp

Compound **2** indicated an $[M-H]^-$ ion peak at *m/z* 926, along with a fragment ion peak at *m/z* 780 [926−146 (6-deoxyhexose unit)] in the negative FAB-MS and an $[M+Na]^+$ ion peak at *m/z* 950 in the positive FAB-MS. The HR positive FAB-MS indicated the molecular formula of **2** to be $C_{46}H_{73}NO_{18}$. The ^{1}H-NMR spectrum of **2** showed signals due to two tertiary methyl groups (δ 1.40, 1.05), three secondary methyl groups [δ 1.76 (d, J=6.0 Hz), 1.63 (d, J=6.0 Hz), 0.76 (d, J=6.5 Hz)], one acetyl group (δ 2.30), one methoxyl group (δ 3.42), one olefinic proton [δ 5.32 (d, J=3.5 Hz)], one amino proton (δ 8.45), and three monosaccharide groups. The ^{13}C-NMR spectrum of **2** showed 45 carbon signals, including two carbonyl carbons (δ 206.5, 167.5), two olefinic carbons (δ 140.9, 121.8), one nitrogen- and oxygen-bearing carbon (δ 97.2), and three anomeric carbons (δ 103.0, 102.0, 100.3), suggesting **2** to be a triglycoside of steroidal alkaloid. These ^{1}H- and ^{13}C-NMR signals were assigned in detail by the techniques similar to those of **1**. In the HMBC spectrum, key correlations of the Ag moiety were observed as illustrated in Fig. 1. From these findings, **2** was established as a pregnane glycoside, possessing an alkaloid ester group and triglycosyl group. The ^{1}H- and ^{13}C-NMR data of the sugar moiety and C-1—C-11 of the Ag moiety of **2** were superimposable on those of **3**, indicating the same sugar chain as **3** was attached to C-3 of Ag. The stereochemistry of C-16 of Ag and C-17 of Ag was determined by analysis of the coupling constant values of the signals due to H-16 of Ag [δ 5.69 (ddd, J=4.5, 8.0, 8.0 Hz)] and H-17 of Ag [δ 2.44 (d, J=8.0 Hz)], which were similar to those of 16β-[[4*S*]-5-(β-D-glucopyranosyloxy)-4-methyl-1-oxopentyl]oxy]-3β-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranosyl)oxy]pregn-5-en-20-one,[12] in the ^{1}H-NMR spectrum. However, the configurations at C-23 of Ag and C-25 of Ag have not been confirmed. Consequently, **2** was elucidated to be 3β,16β-dihydroxy-pregn-5-en-20-one 16-*O*-(2,5-epimino-2-methoxy-4-pentanoic acid)-ester 3-*O*-β-chacotrioside. In a previous paper, two of the present authors reported the structure of abutiloside O to be 3β,16β-dihydroxy-pregn-5-en-20-one 16-*O*-(2,5-epoxy-2-methoxy-4-pentanoic acid)-ester 3-*O*-β-chacotrioside.[13] However, the ^{1}H- and ^{13}C-NMR data of **2** were quite similar to those of abutiloide O. Therefore, **2** was con-

Table 1. ^{13}C-NMR Data for **1** and **2** (in Pyridine-d_5, 125 MHz)

C	**1**	**2**	C	**1**	**2**
Ag-1	37.5	37.5	Glc-1	100.3	100.3
Ag-2	30.1	30.2	Glc-2	77.9	77.9
Ag-3	78.2	78.2	Glc-3	77.1	76.9
Ag-4	39.0	39.0	Glc-4	78.1*a)*	78.9
Ag-5	140.9	140.9	Glc-5	77.8	78.0
Ag-6	121.7	121.8	Glc-6	61.4	61.4
Ag-7	32.3	32.1	Rha-1	102.0	102.0
Ag-8	31.7	31.4	Rha-2	72.4	72.5
Ag-9	50.4	50.6	Rha-3	72.8*b)*	72.8
Ag-10	37.1	37.1	Rha-4	74.1	74.2
Ag-11	21.1	20.8	Rha-5	70.0	69.5
Ag-12	39.8	38.3	Rha-6	18.3	18.6
Ag-13	40.5	42.5	Rha′-1	101.4	103.0
Ag-14	56.7	54.3	Rha′-2	81.7	72.7
Ag-15	32.2	37.7	Rha′-3	72.7*b)*	72.5
Ag-16	81.4	74.6	Rha′-4	74.2	73.9
Ag-17	62.9	67.8	Rha′-5	69.4	70.5
Ag-18	16.3	14.8	Rha′-6	18.6	18.6
Ag-19	19.4	19.4	Xyl-1	107.5	
Ag-20	42.0	206.5	Xyl-2	75.5	
Ag-21	15.0	31.2	Xyl-3	78.3*a)*	
Ag-22	111.8	167.5	Xyl-4	70.9	
Ag-23	31.4	97.2	Xyl-5	67.3	
Ag-24	28.4	40.7			
Ag-25	35.5	26.0			
Ag-26	103.1	49.0			
Ag-27	16.7	18.0			
OCH_3	55.6	50.0			

δ in ppm from TMS. *a*, *b*) Assignments may be interchanged. Glc, glucopyranosyl; Rha, rhamnopyranosyl; Xyl, xylopyranosyl.

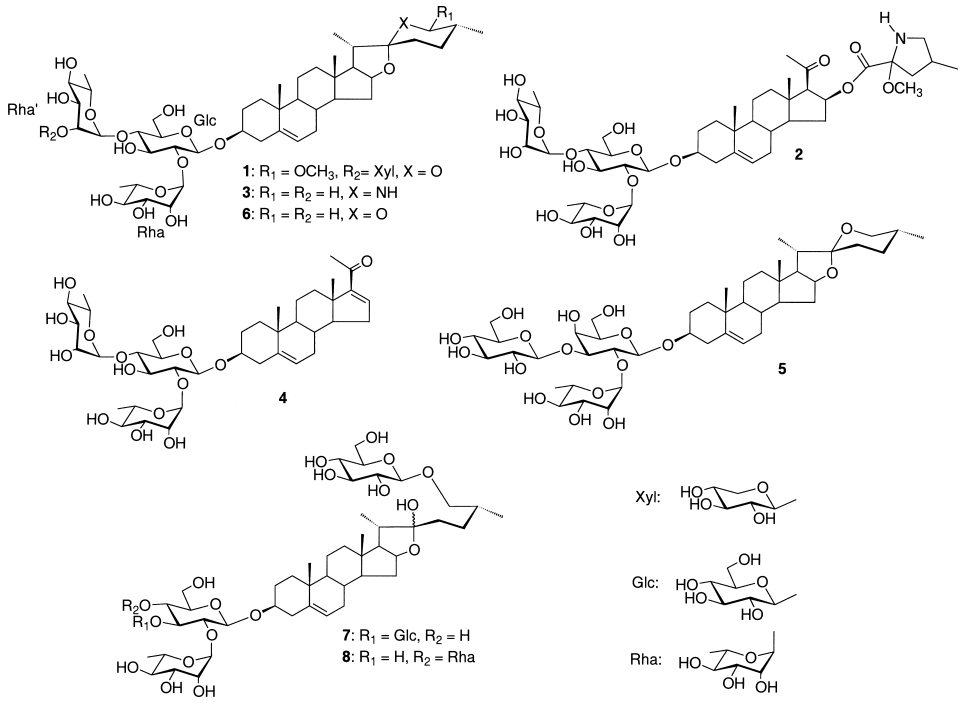

Fig. 2. Structures of **1**—**8**

Table 2. GI_{50} Values of **1**—**8** and Cisplatin

Compound	GI_{50} (μM)
1	80.0<
2	80.0<
3	7.5
4	80.0<
5	4.2
6	4.2
7	4.9
8	4.2
Cisplatin	8.5

sidered to be identical with abutiloide O, which was finally confirmed by direct comparison with the ^{1}H-NMR spectrum of an authentic sample. Based on this evidence, the structure of abutiloside O is corrected as shown in Fig. 2.

The *in vitro* antiproliferative activity of **1**—**8** against HL-60 cells was examined. Compounds **3**, **5**—**8** showed stronger activity than cisplatin (Table 2). Recently, the cytotoxic activity of **5**, **6** and **8** against HL-60 cells using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide assay was reported by Mimaki *et al.*,[14] and the present results were similar to theirs.

To the best of our knowledge, **1** is a new compound, and the isolation of **2** and **4**—**8** from *S. sodomaeum* L. is described here for the first time.

Experimental

All instruments and materials used were the same as those cited in a previous report,[15] unless otherwise specified.

Plant Material The underground parts of *Solanum sodomaeum* L. were collected in the Medical Plant Garden of Kumamoto University, Kumamoto prefecture, Japan, in October 2002, and identified by Professor Toshihiro Nohara, Faculty of Pharmaceutical Sciences, Kumamoto University.

Extraction and Isolation The cut fresh underground parts of *Solanum sodomaeum* L. (1200 g) were extracted with MeOH (1300 ml) at room temperature, and the solvent was removed under reduced pressure to give a syrup (27.7 g). The MeOH extract was chromatographed over Diaion HP20 (H_2O, MeOH, acetone) to give fractions (frs.) 1—3. Fraction 2 (10.3 g) was subjected to silica gel column chromatography [Merck. Art. 7734, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to afford frs. 4—6. Fraction 5 (2701 mg) was partitioned between hexane (100 ml) and MeOH (40 ml). Chromatography of the MeOH layer (2266 mg) over Sephadex LH20 (MeOH) furnished frs. 7—9. Fraction 8 (1214 mg) was chromatographed over silica gel [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to give frs. 10—20. Fraction 15 (38 mg) was subjected to HPLC (column, COSMOSIL 5C18 AR-II, Nacalai Tesque, Inc., 250 mm×20 mm i.d.; solvent, 80% MeOH) to give **6** (9 mg). Fraction 16 (107 mg) was successively subjected to Chromatorex ODS (60% MeOH, MeOH) and HPLC (70% MeOH) under the similar conditions to fr. 15 to afford **2** (7 mg). Similar HPLC (85% MeOH) of fr. 17 (312 mg) to fr. 15 gave **3** (40 mg), **1** (32 mg) and **5** (56 mg). Fraction 19 (103 mg) was subjected to HPLC under the same conditions as for fr. 16 to afford **8** (23 mg). Chromatography of fr. 6 (6.10 g) over silica gel [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] furnished frs. 21—32. Fraction 23 (50 mg) and fr. 24 (82 mg) were each subjected to HPLC (fr. 23, 70% MeOH; fr. 24, 85% MeOH) under the similar conditions to fr. 15 to afford **4** (4 mg) from fr. 23, and **1** (11 mg) from fr. 24. Fraction 28 (2538 mg) was successively subjected to Chromatorex ODS (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) and HPLC under the same conditions as for fr. 23 to give **8** (30 mg) and **7** (52 mg).

1: Amorphous powder. $[\alpha]_D^{17}$ −97.8° (c=3.6, MeOH). Positive FAB-MS m/z: 1053 $[M+Na]^+$. HR positive FAB-MS m/z: 1053.5393 $[M+Na]^+$ (Calcd for $C_{51}H_{82}O_{21}Na$: 1053.5246). ^{1}H-NMR (in pyridine-d_5, 500 MHz) δ: 6.38 (1H, s, H-1 of Rha), 5.96 (1H, s, H-1 of Rha′), 5.34 (1H, d, J=5.0 Hz, H-6 of Ag), 5.21 (1H, d, J=8.0 Hz, H-1 of Xyl), 4.82 (1H, d, J=3.5 Hz, H-2 of Rha), 4.68 (1H, ddd, J=7.5, 7.5, 7.5 Hz, H-16 of Ag), 4.64 (1H, d, J=3.5 Hz, H-2 of Rha′), 4.62 (1H, dd, J=3.5, 9.5 Hz, H-3 of Rha), 4.53 (1H, dd, J=3.5, 9.5 Hz, H-3 of Rha′), 4.49 (1H, d, J=8.0 Hz, H-26 of Ag), 4.29 (1H, dd, J=5.0, 11.0 Hz, Ha-5 of Xyl), 4.06 (1H, dd, J=8.0, 8.5 Hz, H-2 of Xyl), 3.90 (1H, m, H-3 of Ag), 3.73 (1H, ddd, J=2.5, 2.5, 9.0 Hz, H-5 of Glc), 3.66 (1H, dd, J=10.0, 11.0 Hz, Hb-5 of Xyl), 3.52 (3H, s, OCH_3), 1.76 (3H, d, J=6.0 Hz, H_3-6 of Rha), 1.56 (3H, d, J=6.0 Hz, H_3-6 of Rha′), 1.14 (3H, d, J=7.5 Hz, H_3-21 of Ag), 1.06 (3H, s, H_3-19 of Ag), 0.96 (3H, d, J=6.0 Hz, H_3-27 of Ag), 0.83 (3H, s, H_3-18 of Ag). ^{13}C-NMR data: see Table 1.

2: Amorphous powder. $[\alpha]_D^{17}$ −45.5° (c=0.6, MeOH). Negative FAB-MS m/z: 926 $[M-H]^-$, 780 $[M-146$ (6-deoxyhexose unit)$]^-$. Positive FAB-MS m/z: 950 $[M+Na]^+$. HR positive FAB-MS m/z: 950.4739 $[M+Na]^+$ (Calcd for $C_{46}H_{73}O_{18}NNa$: 950.4725). ^{1}H-NMR (in pyridine-d_5, 500 MHz) δ: 8.45 (1H, s, NH), 6.39 (1H, s, H-1 of Rha), 5.84 (1H, s, H-1 of Rha′), 5.69 (1H, ddd, J=4.5, 8.0, 8.0 Hz, H-16 of Ag), 5.32 (1H, d, J=3.5 Hz, H-6 of Ag), 4.83 (1H, s, H-2 of Rha), 4.68 (1H, s, H-2 of Rha′), 4.62 (1H, d, J=9.5 Hz, H-3 of Rha), 4.54 (1H, d, J=9.5 Hz, H-3 of Rha′), 4.10 (1H, d, J=11.5 Hz, Hb-6 of Glc), 3.88 (1H, m, H-3 of Ag), 3.66 (1H, d, J=9.5 Hz, H-5 of Glc), 3.42 (3H, s, OCH_3), 3.25 (1H, m, Ha-26 of Ag), 2.44 (1H, d, J=8.0 Hz, H-17 of Ag), 2.30 (3H, s, H_3-21 of Ag), 1.76 (3H, d, J=6.0 Hz, H_3-6 of Rha), 1.63 (3H, d, J=6.0 Hz, H_3-6 of Rha′), 1.40 (3H, s, H_3-18 of Ag), 1.05 (3H, s, H_3-19 of Ag), 0.76 (3H, d, J=6.5 Hz, H_3-27 of Ag). ^{13}C-NMR data: see Table 1.

Acidic Hydrolysis of 1 Compound **1** (6 mg) in 2 N HCl–dioxane (1 : 1, 2 ml) was heated at 95 °C for 1 h. The reaction mixture was neutralized with 2 N NaOH and then evaporated under reduced pressure to give a residue. The residue was extracted with MeOH and the MeOH extract was chromatographed over silica gel [Merck, Art. 9385, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1)] to give a sugar fr. This fr. was analyzed by HPLC under the following conditions: column, YMC pack Polyamine II (YMC Co., Ltd., 4.6 mm i.d.×250 mm); solvent, 80% CH_3CN; flow rate, 0.8 ml/min; column temperature, 30 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; column oven, JASCO CO-2060. The retention time and optical activity of the sugars were identical with those of L-rhamnose [t_R (min): 8.2; optical activity: negative], D-xylose [t_R (min): 11.2; optical activity: positive] and of D-glucose [t_R (min): 17.3; optical activity: positive]. However, the Ag of **1** could not be obtained.

Cell Culture and Assay of Antiproliferative Activity HL-60 cells were obtained from the Japanese Cancer Research Resources Bank (HCRB), Tokyo. The cells were routinely cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 mg/ml streptomycin, and 100 U/ml penicillin at 37 °C in a humidified atmosphere containing 5% CO_2. The samples were dissolved in DMSO, sterilized by filtration through filter paper (0.45 μm), and 100 μl of each was added to 2 ml of culture media containing 10^5 cells. As a control, 100 μl of DMSO was used instead of sample. At 72 h after the start of incubation under 5% CO_2 at 37 °C, viable cells were counted by the trypan blue exclusion method. The GI_{50} value (μM) was defined as the concentration of sample necessary to inhibit the growth to 50% of the control. Cisplatin (Nippon Kayaku Co., Tokyo) was used as a standard sample.

Acknowledgments We express our appreciation to Mr. K. Takeda and Mr. T. Iriguchi of Kumamoto University for their measurement of the NMR and MS spectra.

References

1) Cham B. E., Gilliver M., Wilson L., *Planta Med.*, **53**, 34—36 (1987).
2) Cham B. E., Wilson L., *Planta Med.*, **53**, 59—62 (1987).
3) Cham B. E., Meares H. M., *Cancer Lett.*, **36**, 111—118 (1987).
4) Sayed K. A. E., Hamann M. T., El-Rahaman H. A. A., Zaghloul A. M., *J. Nat. Prod.*, **61**, 848—850 (1998).
5) Mahato S. R., Saha N. P., Ganguly A. N., Kasai R., Tanaka O., *Phytochemistry*, **19**, 2017—1323 (1980).
6) Hu K., Yao X. S., Dong A. J., Kobayashi H., Iwasaki S., Jing Y. K., *J. Nat. Prod.*, **62**, 299—301 (1999).
7) Yahara S., Nakamura T., Someya Y., Matsumoto T., Yamashita T., Nohara T., *Phytochemistry*, **43**, 1319—1323 (1996).
8) Nakano K., Murakami K., Takaishi Y., Tomimatsu T., Nohara T., *Chem. Pharm. Bull.*, **37**, 116—118 (1989).
9) Ikeda T., Tsumagari H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **52**, 142—145 (2004).
10) Mimaki Y., Satou T., Kuroda M., Sashida Y., Hatakeyama Y., *Chem.*

Pharm. Bull., **46**, 1829—1832 (1998).
11) Lorey S., Porzel A., Ripperger H., *Phytochemistry*, **41**, 1633—1635 (1996).
12) Yokosuka A., Mimaki Y., Sashida Y., *Nat. Med.*, **56**, 208—211 (2002).
13) Yoshimitsu H., Nishida M., Nohara T., *Phytochemistry*, **64**, 1361—1366 (2003).
14) Mimaki Y., Yokosuka A., Kuroda M., Sashida Y., *Biol. Pharm. Bull.*, **24**, 1286—1289 (2001).
15) Ono M., Yamamoto M., Yanaka T., Ito Y., Nohara T., *Chem. Pharm. Bull.*, **49**, 82—86 (2001).