Phytochemistry, Vol. 31, No. 7, pp. 2439–2443, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

STEROIDAL SAPONINS FROM *SMILAX RIPARIA* AND *S. CHINA*

YUTAKA SASHIDA,* SATOSHI KUBO, YOSHIHIRO MIMAKI, TAMOTSU NIKAIDO† and TAICHI OHMOTO†

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan; †Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan

(*Received* 24 *September* 1991)

Key Word Index– *Smilax riparia*; *Smilax china*; Liliaceae; steroidal saponins; neotigogenin glycosides; isonarthogenin glycoside; cAMP phosphodiesterase.

Abstract—Two new neotigogenin glycosides were isolated from the rhizomes and roots of *Smilax riparia* and a new isonarthogenin glycoside from those of *S. china*. The structures were elucidated by a combination of spectroscopic analysis and hydrolysis followed by spectral and chromatographic analysis. Several known saponins were also isolated and identified. The inhibitory activity of the saponins on cAMP phosphodiesterase was examined.

INTRODUCTION

Smilax plants (Liliaceae), with about 350 species, are widely distributed in the tropical and temperate zones throughout the world, and especially in the tropical regions of East Asia and North America [1]. As a continuation of our studies on the steroidal saponins of the Liliaceae plants [2–12], we have investigated those of the *Smilax* plants. A survey of the literature showed that several *Smilax* species are used in medicine [13, 14], from which several steroidal saponins have been reported [15–17]. *Smilax riparia* and *S. china* are indigenous to Japan, and the rhizomes and roots of *S. riparia* and those of *S. china* have been reputed in the traditional Chinese medicine to be effective as diuretic and anti-inflammatory agents [13, 14]. This paper reports the structural assignments and inhibitory activity on cyclic adenosine monophosphate (cAMP) phosphodiesterase of the steroidal saponins isolated from the plants.

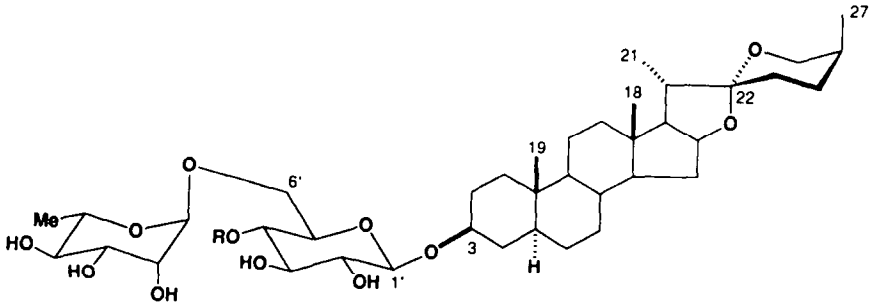
RESULTS AND DISCUSSION

The rhizomes and roots of *S. riparia* were extracted with hot methanol. Chromatographic fractionation of the *n*-butanol-soluble phase of the methanolic extract gave compounds **1** and **2**.

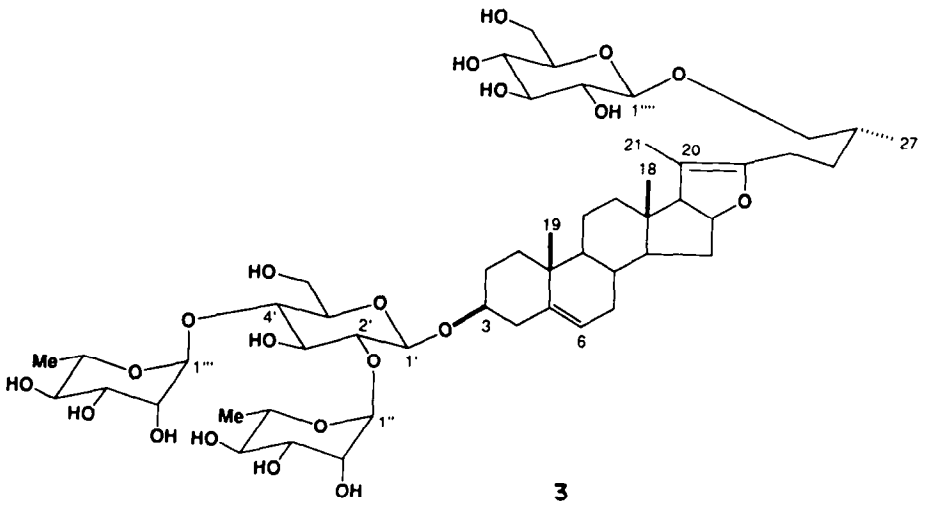
Compound **1** was obtained as a white amorphous powder, $[\alpha]_D$ −53.4° (EtOH). The molecular formula $C_{39}H_{64}O_{12}$ was confirmed by the secondary ion (SI) mass spectrum (*m/z* 725 $[M+H]^+$) and elemental analysis. Compound **1** was predicated to be a glycoside of a (25*S*)-spirostanol steroid based on the characteristic absorption bands at 990, 920, 900 and 850 cm^{-1}, with the absorption at 920 cm^{-1} being of greater intensity than at 900 cm^{-1} in the IR spectrum [18–20]. The ^1H NMR spectrum exhibited three secondary methyl proton signals at δ1.64 (J = 6.2 Hz), 1.15 (J = 6.9 Hz) and 1.08 (J = 7.1 Hz), two tertiary methyl proton signals at δ0.81 and 0.67, and two anomeric proton signals at δ5.53 (*br s*) and 5.00 (*d*, J = 7.6 Hz). The signal at δ1.64 was due to 6-deoxyhexose. The ^{13}C NMR spectrum showed 39 carbon resonances. The number of the attached hydrogens to each individual carbon atom was determined by the DEPT spectrum, which indicated the presence of Me × 5, CH_2 × 12, CH × 19 and C × 3. The above data suggested that **1** was a (25*S*)-spirostanol disaccharide. Acid hydrolysis of **1** with 1 M hydrochloric acid (dioxane–H_2O, 1:1) gave D-glucose and L-rhamnose as the carbohydrate compounds, and a sapogenin, which was identified as (25*S*)-5α-spirostan-3β-ol (neotigogenin) by the IR and ^1H NMR spectra. The assignments of carbon signals due to the monosaccharides of **1** was carried out by comparison with the chemical shifts of methyl glycosides [21], and by considering the glycosylation shift. The ^{13}C NMR chemical shifts of a terminal α-L-rhamnopyranosyl unit could be attributed. The C-6 signal of the inner β-D-glucopyranosyl unit was observed at δ68.6, indicating the involvement in glycosidic linkage formation. Thus, the structure of **1** was determined to be neotigogenin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **2**, $[\alpha]_D$ −53.7° (MeOH), was a more polar constituent than **1**. The SI mass spectrum showed a quasimolecular ion peak at *m/z* 887 $[M+H]^+$, shifted 162 mass unit with respect to **1**. On comparison of the whole ^{13}C NMR spectrum of **2** with that of **1**, a set of additional signals, corresponding to a terminal β-D-glucopyranosyl unit appeared, and the signals due to the inner glucose moiety varied, while all other signals remained almost unaffected, confirming the identity of the aglycone of **2** as neotigogenin. It was observed that the signal of the C-4 of the inner glucose was markedly displaced downfield at δ82.5 as compared with that of **1**, suggesting that the C-4 position of the glucose was the glycosylated position to which the additional D-glucose was linked. Compound **2** was treated with 0.2 M hydrochloric acid to yield **1** as one of the hydrolysates. Thus, the structure of **2** was established as neotigogenin 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

*Author to whom correspondence should be addressed.



R
1 H
2 Glc



3

The rhizomes and roots of *S. china* were extracted with methanol. The *n*-butanol-soluble phase on the repeated chromatographic separations gave **3**-**6**. Compound **4** was also isolated from the residual water phase.

Compounds **3**, $C_{51}H_{82}O_{21}$, $[\alpha]_D$ −72.0° (MeOH), **4**, $C_{52}H_{86}O_{22}$, $[\alpha]_D$ −86.0° (MeOH) and **5**, $C_{45}H_{72}O_{16}$, $[\alpha]_D$ −110.0° (MeOH) were identified as 26-*O*-β-D-glucopyranosyl-(25*R*)-furosa-5,20-dien-3β,26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (pseudoprotodioscin) [22, 23], 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-furosa-5-en-3β,22,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (methylprotodioscin) [24-26] and (25*R*)-spirost-5-en-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (dioscin) [24, 25], respectively.

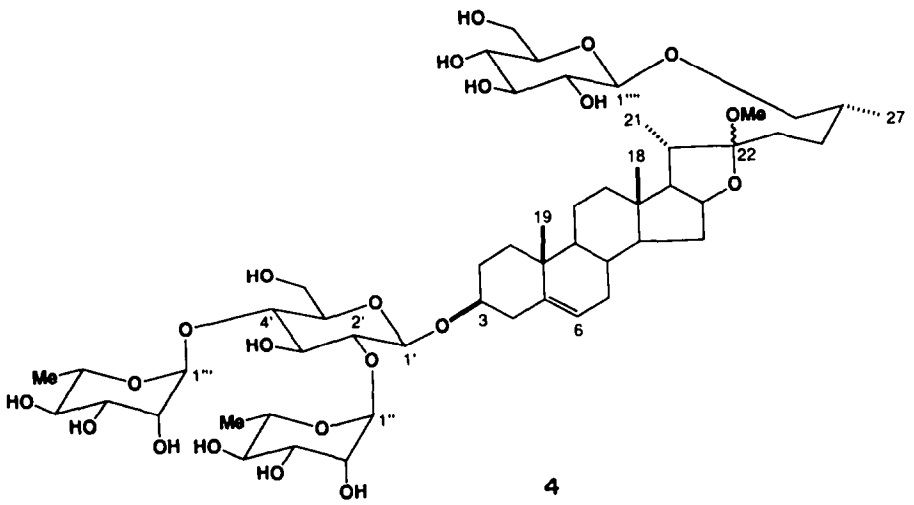
Compound **6** is a minor constituent, $[\alpha]_D$ −76.0° (MeOH). The SI mass spectrum showed quasimolecular ion peaks at *m/z* 908 $[M+Na+H]^+$ and 885 $[M+H]^+$, consistent with the molecular formula, $C_{45}H_{72}O_{17}$. The spectral features of **6** were similar to those of **5**. The ^1H and ^{13}C NMR spectra of **6** indicated that the C-27 which was present as a methyl in **5** was modified as hydroxymethyl in **6**. Acid hydrolysis of **6** as for **1** gave D-glucose, L-rhamnose and (25*S*)-spirost-5-en-3β,27-diol (isonarthogenin) [27, 28]. Thus, the structure of **6** was characterized as isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside. Compounds **1**, **2** and **6** are new steroidal saponins.

The inhibitory activity of the saponins on cAMP phosphodiesterase was examined [29, 30]. The IC_{50} inhibitory concentrations are shown in Table 2. Compound **3** had almost equal inhibitory activity to that of papaverine (IC_{50} 3.0×10^{-5} M).

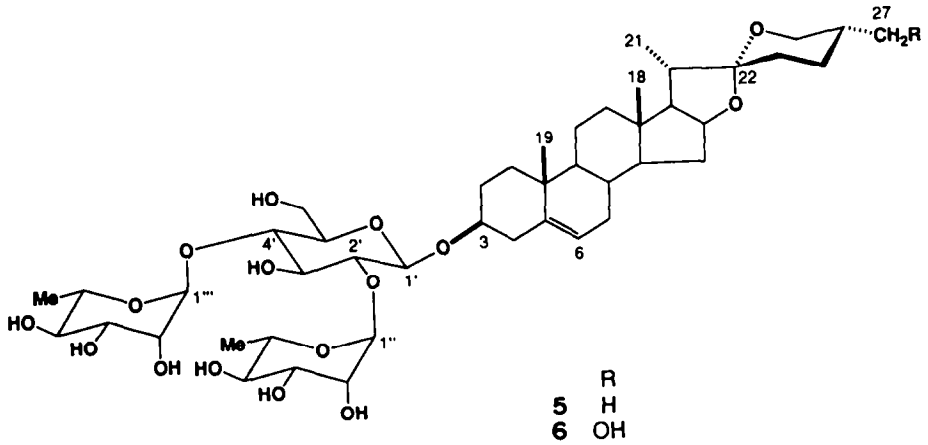
EXPERIMENTAL

General. ^1H NMR (400 MHz) and ^{13}C NMR (100.6 MHz): TMS as int. standard. CC: silica gel (Fuji Davison), ODS (Nacalai Tesque), Sephadex LH-20 (Pharmacia) and Diaion HP-20 (Mitsubishi-kasei). TLC: precoatd Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 $F_{254}S$ (Merck). HPLC: Tosoh HPLC system (Tosoh: pump, Tosoh CCPM; detector, Tosoh RI-8010; controller, CCP controller PX-8010) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-kasei, 4.6 i.d. × 250 mm or 10 i.d. × 250 mm, ODS 5 μm).

Plant materials. The rhizomes and roots of *S. riparia* were collected at Minamitsuru, Yamanashi prefecture, Japan and



4



	R
5	H
6	OH

those of *S. china* at Kakegawa, Shizuoka prefecture, Japan. Voucher specimens are on file at our laboratory.

Extraction and isolation. The dried rhizomes and roots of *S. riparia* (1.8 kg) were extracted with MeOH under reflux. The MeOH extract was concd. under red. pres. and partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract was chromatographed on silica gel with a gradient mixt. of $CHCl_3$–MeOH to give seven fractions. Frs. 5, 6 and 7 were combined and chromatographed on Diaion HP-20 with an increasing amount of MeOH in H_2O. The 80% MeOH and MeOH eluate frs were combined and subjected to Sephadex LH-20 CC with MeOH and ODS CC with MeOH–H_2O (3:1) to provide **1** and **2** with a few impurities, which were further purified by HPLC with MeOH–H_2O (3:1) to furnish **1** (50.8 mg) and **2** (11.0 mg).

The dried rhizomes and roots of *S. china* (2.6 kg) were treated as in the case of those of *S. riparia*. The *n*-BuOH-sol. phase was chromatographed on silica gel with a gradient mixt. of $CHCl_3$–MeOH to give seven fractions. Fr. 4 was separated by Diaion HP-20 CC with H_2O–MeOH system and Sephadex LH-20 CC with MeOH into **3** (97.2 mg). Frs 5 and 6 were combined and separated by Diaion HP-20 CC with H_2O–MeOH system and HPLC with MeOH–H_2O (3:1) into products **5** (35.7 mg) and **6** (5.8 mg). Fr. 7 was chromatographed on Diaion HP-20 with H_2O–MeOH system and on Sephadex LH-20 with MeOH, and finally purified by HPLC with MeOH–H_2O (2:1) to yield **4** (1.00 g). Compound **4** (660 mg) was also isolated from the H_2O-sol. phase after the similar chromatographic separations as for the *n*-BuOH-sol. phase.

Compound **1**. Amorphous powder, $[\alpha]_D^{24}$ −53.4° (EtOH; *c* 0.16). (Found: C, 63.79; H, 8.73. Calc. for $C_{39}H_{64}O_{12}\cdot 1/2H_2O$: C, 63.82; H, 8.93%.) SI MS *m/z* 725 $[M+H]^+$; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950 (CH), 1470, 1455, 1385, 1340, 1275, 1265, 1220, 1155, 1135, 1100, 1060, 1045, 990, 975, 920, 900, 850, 840, 810, ((25*S*)-spiroacetal, intensity 920 > 900); ^1H NMR (pyridine-d_5): δ 5.53 (1H, *br s*, H-1''), 5.00 (1H, *d*, J = 7.6 Hz, H-1'), 1.64 (3H, *d*, J = 6.2 Hz, H-6''), 1.15 (3H, *d*, J = 6.9 Hz, H-21), 1.08 (3H, *d*, J = 7.1 Hz, H-27), 0.81 (3H, *s*, H-19), 0.67 (3H, *s*, H-18).

Acid hydrolysis of **1**. Compound **1** (20.0 mg) was hydrolysed with 1 M HCl in dioxane–H_2O (1:1) on a boiling water bath for 1.5 hr under an N_2 atmosphere. The reaction mixt. was neutralized by passing through an Amberlite IRA-93ZU (OH^- form) column and the crude product was chromatographed on silica gel with gradients of MeOH in $CHCl_3$ [initial solvent, $CHCl_3$–MeOH (6:1)] and on ODS with MeOH–H_2O (19:1) to

Table 1. ^{13}C NMR spectral data for compounds **1**, **2** and **6**

C	1	2	6
1	37.2	37.2	37.5
2	30.1	30.1	30.2
3	77.7	77.8	78.2
4	35.0	35.0	39.0
5	44.6	44.6	140.9
6	29.0	29.0	121.8
7	32.4*	32.4*	32.4*
8	35.3	35.3	31.7
9	54.4	54.4	50.4
10	35.9	35.8	37.2
11	21.3	21.3	21.2
12	40.2	40.2	39.9
13	40.8	40.8	40.5
14	56.4	56.4	56.7
15	32.1*	32.1*	32.3*
16	81.2	81.2	81.2
17	62.9	62.9	63.0
18	16.6	16.6	16.4
19	12.3	12.3	19.4
20	42.5	42.5	42.1
21	14.9	14.9	15.1
22	109.7	109.7	109.7
23	26.2†	26.2†	31.6
24	26.4†	26.4†	24.1
25	27.6	27.6	39.3
26	65.1	65.1	64.1†
27	16.3	16.3	64.5†
1′	102.4	102.1	100.4
2′	75.3	75.2‡	78.8
3′	78.7	76.8	76.9
4′	72.1	82.5	78.0‡
5′	77.1	74.8	77.9‡
6′	68.6	67.8	61.4
1″	102.7	105.3	102.0
2″	72.4	75.0‡	72.5§
3″	72.8	78.5§	72.8‖
4″	74.1	71.8	74.2
5″	69.8	78.4§	69.5
6″	18.7	62.7	18.7
1‴		102.6	103.0
2‴		72.4	72.6§
3‴		72.8	72.9‖
4‴		74.1	74.0
5‴		69.8	70.5
6‴		18.7	18.5

Spectra were measured in pyridine-d_5.
*†‡§‖Assignments may be interchanged.

yield D-glucose (1.0 mg), L-rhamnose (1.1 mg) and neotigogenin (3.2 mg). D-Glucose: $[\alpha]_D^{26}$ +55.0° (H_2O; *c* 0.04); TLC, R_f 0.40 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). L-Rhamnose: $[\alpha]_D^{26}$ +11.3° (H_2O; *c* 0.04); TLC, R_f 0.65 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). Neotigogenin: $[\alpha]_D^{26}$ −62.3° ($CHCl_3$; *c* 0.13). IR ν_{max}^{Film} cm^{-1}: 3382 (OH), 2927 and 2847 (CH), 1470, 1450, 1378, 1350, 1340, 1303, 1261, 1224, 1174, 1131, 1094, 1051, 1010, 988, 955, 921, 897, 852, 801, 755, [(25*S*)-spiroketal, intensity 921 > 897]; ^1H NMR (pyridine-d_5): δ4.53 (1H, *q*-like, *J* = 6.7 Hz, H-16), 4.07 (1H, *dd*, *J* = 11.0, 2.7 Hz, H-26a), 3.86 (1H, *m*, H-3), 3.37 (1H, *br d*, *J* = 11.0 Hz, H-26b), 1.16 (3H, *d*, *J* = 6.9 Hz, H-21), 1.08 (3H, *d*, *J* = 7.1 Hz, H-27), 0.85 (3H, *s*, H-19), 0.82 (3H, *s*, H-18).

Table 2. Inhibitory activity on cAMP phosphodiesterase of compounds **1**–**6**

Compounds	IC_{50} (× 10^{-5} M)
1	10.2
2	5.5
3	4.7
4	29.4
5	33.3
6	9.3

Compound **2**. Amorphous powder, $[\alpha]_D^{24}$ −53.7° (MeOH; *c* 0.16). (Found: C, 58.83; H, 8.52. Calc for $C_{45}H_{74}O_{17} \cdot 2H_2O$: C, 58.55; H, 8.52%.) SI MS *m/z* 887 $[M+H]^+$; IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 2950 (CH), 1455, 1380, 1340, 1265, 1230, 1220, 1045, 990, 955, 925, 900, 855, 840, 810, 760 (25*S*)-spiroacetal, intensity 925 > 900); ^1H NMR (pyridine-d_5): δ5.64 (1H, *br s*, H-1‴), 5.03 (1H, *d*, *J* = 7.7 Hz, H-1″), 4.96 (1H, *d*, *J* = 7.7 Hz, H-1′), 3.96 (1H, *m*, H-3), 1.65 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.15 (3H, *d*, *J* = 6.8 Hz, H-21), 1.08 (3H, *d*, *J* = 7.0 Hz, H-27), 0.81 (3H, *s*, H-19), 0.66 (3H, *s*, H-18).

Partial hydrolysis of **2**. Compound **2** (2.1 mg) was treated with 0.2 M HCl (dioxane–H_2O, 1:1) for 30 min at 100°. The reaction mixt. was neutralized with 1 M NaOH and passed through a Sep-Pak C_{18} cartridge (Waters) successively with H_2O–MeOH (4:1) and H_2O–MeOH (1:9). Compound **1** was detected in the H_2O–MeOH (1:9) eluate by HPLC. HPLC: R_t 16.5 min (column: Kaseisorb LC ODS-120-5, 4.6 i.d. × 250 mm, ODS 5 μm; solvent: MeOH–H_2O (19:1); flow rate: 0.40 ml min^{-1}; detection: RI).

Compound **6**. Amorphous powder, $[\alpha]_D^{30}$ −76.0° (MeOH; *c* 0.08). SI MS *m/z* 908 $[M+Na+H]^+$, 885 $[M+H]^+$; IR ν_{max}^{Film} cm^{-1}: 3400 (OH), 2923 and 2857 (CH), 1458, 1250, 1094, 1055, 985, 840; ^1H NMR (pyridine-d_5): δ6.38 (1H, *br s*, H-1‴), 5.84 (1H, *br s*, H-1″), 5.33 (1H, *br d*, *J* = 4.4 Hz, H-6), 4.94 (1H, *d*, *J* = 7.4 Hz, H-1′), 1.77 (1H, *d*, *J* = 6.2 Hz, H-6‴), 1.63 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.16 (3H, *d*, *J* = 6.9 Hz, H-21), 1.06 (3H, *s*, H-19), 0.85 (3H, *s*, H-18).

Acid hydrolysis of **6**. Compound **6** (2.4 mg) was subjected to acid hydrolysis as for **1**. D-Glucose, L-rhamnose and isonarthogenin were detected in the reaction mixture by TLC. Isonarthogenin: TLC, R_f 0.50 ($CHCl_3$–MeOH, 15:1); R_f 0.40 ($CHCl_3$–Me_2CO, 4:1).

Assay of cAMP phosphodiesterase. cAMP phosphodiesterase activity was assayed by the method as described in the previous paper [29].

Acknowledgements –We are grateful to Dr Y. Shida and Mrs Y. Katoh, Tokyo College of Pharmacy, for the measurements of the SI mass spectra.

REFERENCES

1. Tsukamoto, Y. (ed.) (1988) in *The Grand Dictionary of Horticulture*, Vol. 2, p. 507. Shogakukan, Tokyo.
2. Shimomura, H., Sashida, Y. and Mimaki, Y. (1988) *Chem. Pharm. Bull.* **36**, 3226.
3. Shimomura, H., Sashida, Y. and Mimaki, Y. (1989) *Phytochemistry* **28**, 3163.
4. Mimaki, Y. and Sashida, Y. (1990) *Phytochemistry* **29**, 2267.

5. Mimaki, Y. and Sashida, Y. (1990) *Chem. Pharm. Bull.* **38**, 3055.
6. Mimaki, Y. and Sashida, Y. (1991) *Phytochemistry* **30**, 937.
7. Sashida, Y., Ori, K. and Mimaki, Y. (1991) *Chem. Pharm. Bull.* **39**, 2362.
8. Mimaki, Y., Ishibashi, N., Ori, K. and Sashida, Y. (1992) *Phytochemistry* (in press).
9. Sashida, Y., Kawashima, K. and Mimaki, Y. (1991) *Chem. Pharm. Bull.* **39**, 698.
10. Kawashima, K., Mimaki, Y. and Sashida, Y. (1991) *Phytochemistry* **30**, 3063.
11. Mimaki, Y., Sashida, Y. and Kawashima, K. (1991) *Phytochemistry* **30**, 3721.
12. Mimaki, Y., Sashida, Y. and Kawashima, K. (1992) *Chem. Pharm. Bull.* **40**, 148.
13. Hotta, M. (ed.) (1989) in *Useful Plants of the World*, p. 989. Heibonsha, Tokyo.
14. Xiao, P. G. (ed.) (1988–1990) in *Zhong guo bei cao tu lu*, Vol. 2, p. 224; Vol. 5, pp. 209–210; Vol. 6, p. 201; Vol. 7, p. 187; Vol. 9, pp. 184–185; and Vol. 10, pp. 190–191. Commercial Press, Hong Kong.
15. Mahato, S. B., Ganguly, A. N. and Sahu, N. P. (1982) *Phytochemistry* **21**, 959.
16. Kawasaki, T., Nishioka, I., Tsukamoto, T. and Mihashi, K. (1966) *Yakugaku Zasshi* **86**, 673.
17. Kim, S. W., Chung, K. C., Son, K. H. and Kang, S. S. (1989) *Saengyak Hakhoechi* **20**, 76 [(1989) *Chem. Abstr.* **111**, 208611*t*)].
18. Wall, M. E., Eddy, C. R., McClennan, M. L. and Klumpp, M. E. (1952) *Analyt. Chem.* **24**, 1337.
19. Eddy, C. R., Wall, M. E. and Scott, M. K. (1953) *Analyt. Chem.* **25**, 266.
20. Jones, R. N., Katzenellenbogen, K. and Dobriner, K. (1953) *J. Am. Chem. Soc.* **75**, 158.
21. Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S. (1985) *Phytochemistry* **24**, 2479.
22. Liang, Z. Z., Aquino, R., Simone, F. D., Dini, A., Schettino, O. and Pizza, C. (1988) *Planta Med.* **54**, 344.
23. Hirai, Y., Sanada, S., Ida, Y. and Shoji, J. (1986) *Chem. Pharm. Bull.* **34**, 82.
24. Hirai, Y., Sanada, S., Ida, Y. and Shoji, J. (1984) *Chem. Pharm. Bull.* **32**, 4003.
25. Hirai, Y., Sanada, S., Ida, Y. and Shoji, J. (1984) *Chem. Pharm. Bull.* **32**, 295.
26. Teshima, S., Kajimoto, T., Nakano, K., Tomimatsu, T., Yamasaki, M. and Nohara, T. (1986) *Chem. Pharm. Bull.* **34**, 3925.
27. Minato, H. and Shimaoka, A. (1963) *Chem. Pharm. Bull.* **11**, 876.
28. Blunden, G. and Patel, A. V. (1986) *J. Nat. Prod.* **49**, 687.
29. Nikaido, T., Ohmoto, T., Noguchi, H., Kinoshita, T., Saitoh, H. and Sankawa, U. (1981) *Planta Med.* **43**, 18.
30. Nikaido, T., Ohmoto, T., Kinoshita, T., Sankawa, U., Nishibe, S. and Hisada, S. (1981) *Chem. Pharm. Bull.* **29**, 3586.