Antimicrobial Substances from Rhizomes of the Giant Knotweed *Polygonum sachalinense* against the Fish Pathogen *Photobacterium damselae* subsp. *piscicida*

Hironori Kumagai[a], Yuji Kawai[a,*], Ryo Sawano[b], Hideyuki Kurihara[a], Koji Yamazaki[a], and Norio Inoue[a]

[a] Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan. Fax: +81-138-40-5573. E-mail: kawai@fish.hokudai.ac.jp
[b] Daiichi Pharmaceutical Co., Ltd., Chuo-ku, Tokyo 103-8234, Japan

* Author for correspondence and reprint requests

Z. Naturforsch. **60 c,** 39–44 (2005); received September 17/October 21, 2004

The antimicrobial compounds against the fish pathogen *Photobacterium damselae* subsp. *piscicida* were isolated from *Polygonum sachalinense* rhizomes. The structures of the antimicrobial compounds **1** and **2** were determined by ^{1}H and ^{13}C NMR, 2D-NMR (COSY, HSQC, HMBC and ROESY) and FAB-MS to be phenylpropanoid glycosides, vanicoside A and B, respectively. Both compounds have feruloyl and *p*-coumaroyl groups bonded to a sucrose moiety in their structures. Vanicoside A also has an acetyl group in the sucrose moiety. The MIC values for vanicoside A and B against *Ph. damselae* subsp. *piscicida* DPp-1 were 32 and 64 μg/ml, respectively. The antimicrobial activities of these vanicosides were modest, in contrast to higher activities (MICs at <4 μg/ml) of antibiotics, florphenicol, ampicillin and amoxicillin, which have been generally used for treating pasteurellosis. The activities of the vanicosides, however, were higher than those (MICs at 256 μg/ml) of ferulic acid and *p*-coumaric acid. It was suggested that the structure of phenylpropanoids esterified with sucrose was essential for higher antimicrobial activity of vanicosides and also acetylation of sucrose might affect the activity against the bacterium.

Key words: *Polygonum sachalinense*, Antimicrobial Vanicoside A and B, Phenylpropanoid Glycoside

Introduction

Giant (Sakhalin) knotweed *Polygonum sachalinense* F. Schmidt ex Maxim. (Polygonaceae) is a herbaceous perennial plant. This plant is native to the northern part of Japan and is widely distributed over the North American and European continents. The rhizomes of this plant have been used as a herbal folk medicine for emmenagogue, hydragogue and aperients in China and for analgesic and haemostatic purposes in Japan, as has its closely related species, Japanese knotweed *Polygonum cuspidatum* Siebold & Zucc. (Okuda, 1986; Mizuno and Yoneda, 1995). In general, the *Polygonum* species have many kinds of bioactive components (Nonomura *et al*., 1963; Kudo *et al*., 1966; Arichi *et al*., 1980; Kimura *et al*., 1983; Vastano *et al*., 2000; Yan *et al*., 2001; Xiao *et al*., 2002). Konstantinidou-Doltsinis and Schmitt (1998) reported that extracts from *P. sachalinense* had a fungicidal activity against the fungi that cause powdery mildew in cucumber. Saito *et al*. (1997) also reported that extracts from the leaves and rhizomes of *P. sachalinense* showed antimicrobial activity against several gram-positive and gram-negative bacteria. Furthermore, *P. sachalinense* and *P. cuspidatum* have been used as edible plants, and their younger stems have been eaten as a vegetable (Okuda, 1986).

It also appeared that the extracts from this plant had antimicrobial activity against the fish pathogen *Photobacterium damselae* subsp. *piscicida* (Kawai *et al*., 2004; Kumagai *et al*., unpublished data). This bacterium is a causative agent for pasteurellosis, and a wide variety of marine fish are natural hosts of this pathogen. Pasteurellosis has resulted in great economic loss in worldwide aquaculture (Magariños *et al*., 1996; Romalde, 2002). Some vaccines against pasteurellosis have been developed. Further research, however, has been required to improve the efficacy and suitability of the vaccines for aquaculture.

The ethyl acetate-soluble fraction of the methanol extracts from this knotweed has a potential inhibitory activity against *Ph. damselae* subsp. *pis-*

0939–5075/2005/0100–0039 $ 06.00

cicida. In the present work, two antimicrobial compounds in the *P. sachalinense* rhizomes were determined.

Results and Discussion

Of all the fractions separated with solvents from a methanol extract, the ethyl acetate-soluble fraction was consistently separated by silica gel column chromatography and reversed phase-high performance liquid chromatography (RP-HPLC). Ultimately, compounds **1** and **2**, which had inhibitory activity against *Ph. damselae* subsp. *piscicida* DPp-1, were isolated. From the data of positive and negative FAB-MS, compound **1** showed *m/z* 998. HR-FAB-MS gave a $[M]^-$ ion at *m/z* 998.2899 (calculated *m/z* 998.2844 for $C_{51}H_{50}O_{21}$) and a $[M+Na]^-$ ion at *m/z* 1021.2737 (calculated *m/z* 1021.2742 for $C_{51}H_{50}O_{21}Na$). The UV spectrum of compound **1** had absorption maxima [λ_{max} (ε)] in methanol at 200 (1.16×10^5), 230 sh (1.47×10^4), 298 sh (2.31×10^4) and 316 nm (2.78×10^4).

The ^1H and ^{13}C 1D-NMR and 2D-NMR (COSY, HSQC, HMBC and ROESY) spectra of compound **1** suggested the presence of one feruloyl and three *p*-coumaroyl moieties in its structure. The anomeric proton signals at δ 5.704 and δ 5.561 in the ^1H NMR spectrum (Table I) suggested disaccharide moieties, α-glucose and fructose, respectively. The ^{13}C NMR signals appearing from δ 65 to δ 80 were derived from the sucrose moiety. The aromatic and olefinic signals derived from the *p*-coumaroyl and feruloyl moieties appeared between δ 110 and δ 160. The proton signal at δ 2.098 and the carbon signals at δ 172.46 and δ 21.07 were assigned to the acetyl group. The proton singlet signal at δ 3.850 should indicate a methoxyl moiety of the feruloyl group. Other ^1H and ^{13}C NMR signals were assigned from the results of the 2D-NMR data. According to these analyses, compound **1** was identified as vanicoside A (Fig. 1) (Zimmermann and Sneden, 1994).

The UV spectrum of compound **2** showed signals [λ_{max} (ε)] in methanol at 201 (2.87×10^5), 229 sh (1.82×10^4), 298 sh (2.66×10^4) and 315.5 nm (3.17×10^4). From the data of positive and negative FAB-MS, the molecular weight of compound **2** was determined to be 956. HR-FAB-MS of compound **2** gave a $[M+H]^-$ ion at *m/z* 957.2828 (calculated *m/z* 957.2817 for $C_{49}H_{48}O_{20}$+H) and a $[M+Na]^-$ ion at *m/z* 979.2637 (calculated 979.2636 for $C_{49}H_{48}O_{20}Na$), which indicate a molecular formula of $C_{49}H_{48}O_{20}$. The ^1H NMR (Table I) and COSY spectra of compound **2** were almost the same as those of compound **1**, vanicoside A. However, compound **2** lacked signals of the acetyl group. Consequently compound **2** was identified as vanicoside B (Fig. 1) (Zimmermann and Sneden, 1994). Both compounds have one feruloyl and three *p*-coumaroyl groups bonded to a sucrose moiety in their structures. Vanicoside A also has an acetyl group in the sucrose moiety.

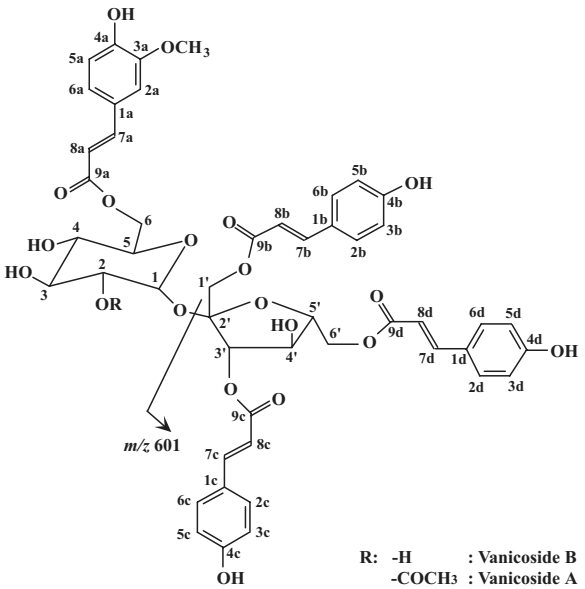

Fig. 1. Chemical structures of vanicoside A and B isolated from *P. sachalinense* rhizomes. The specific fragment with *m/z* 601 in FAB-MS is shown by an arrow.

A fragmentation ion at *m/z* 601 was clearly observed in the FAB-MS for compounds **1** and **2**. This fragmentation is assigned to the tris-*p*-coumaroylfructose moiety (Fig. 1). Both compounds are pale brown powders and are insoluble in water and hexane, moderately soluble in methanol and ethyl acetate, and easily soluble in dimethylsulfoxide (DMSO).

The MIC values for vanicoside A and B were 32 μg/ml and 64 μg/ml against *Ph. damselae* subsp. *piscicida* DPp-1, respectively. The antibacterial activities of these vanicosides are modest, in contrast to higher activities (MICs at <4 μg/ml) of the typical antibiotics florphenicol, ampicillin and amoxicillin which have been generally used for treating pasteurellosis. The activities of vanicoside A and B, however, were greater than those of feru-

Table I. Assignments of ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data (multiplicity, *J* in Hz) of compounds **1** and **2** in CD_3OD.

Position	Compound **1**		Compound **2**	
	δ_H	δ_C	δ_H	δ_C
C				
1′	4.286 (m)	66.36	4.527 (m)	66.34
	4.180 (m)	–	4.527 (m)	–
2′	–	103.60	–	103.43
3′	5.561 (d, 8.30)	79.44	5.640 (d, 8.30)	79.16
4′	4.609 (m)	74.29	4.729 (t, 8.30)	74.07
5′	4.185 (m)	81.05	4.220 (m)	81.03
6′	4.523 (m)	65.48	4.566 (m)	65.51
	4.523 (m)	–	4.566 (m)	–
2-OCO**C**H_3	2.098 (s)	21.07	–	–
2-O**C**OCH_3	–	172.46	–	–
1	5.704 (d, 3.42)	90.64	5.562 (d, 3.42)	92.95
2	4.674 (m)	74.29	3.466 (dd, 9.77, 3.91)	72.95
3	3.889 (d, 9.77)	72.25	3.657 (t, 9.28)	75.01
4	3.398 (t, 9.28, 8.79)	72.11	3.309 (m)	72.29
5	4.237 (m)	72.36	4.527 (m)	72.45
6	4.680 (m)	65.40	4.718 (d, 9.28)	65.80
	4.237 (m)	–	4.220 (m)	–
1a	–	127.11	–	127.79
2a	7.207 (s)	111.64	7.205 (d, 1.46)	111.59
3a	–	149.30	–	149.35
4a	–	150.58	–	150.61
5a	6.759 (m)	116.80	6.755 (d, 8.30)	116.35
6a	7.026 (dd, 1.46, 0.98)	124.51	7.007 (dd, 8.30, 1.46)	124.56
7a	7.619 (d, 16.11)	147.22	7.618 (d, 15.63)	147.21
8a	6.461 (d, 16.11)	115.31	6.481 (d, 15.63)	115.42
9a	–	169.17	–	169.31
OCH_3	3.850 (s)	56.48	3.840 (s)	56.50
1b	–	127.02	–	127.12
2b, 6b	7.417 (d, 8.79)	131.29	7.422 (d, 8.30)	131.32
3b, 5b	6.759 (m)	116.35	6.755 (d, 8.30)	116.84
4b	–	161.35 or 161.23	–	161.35
7b	7.643 (d, 16.11)	147.22	7.658 (d, 16.11)	147.21
8b	6.340 (d, 15.63)	114.58	6.351 (d, 15.21)	114.78
9b	–	168.33	–	168.53
1c	–	127.02	–	127.12
2c, 6c	7.499 (d, 8.30)	131.57	7.486 (d, 8.30)	131.32
3c, 5c	6.806 (d, 8.79)	116.85	6.795 (d, 8.30)	116.84
4c	–	161.53	–	161.35
7c	7.723 (d, 15.63)	148.03	7.716 (d, 16.11)	147.21
8c	6.473 (d, 16.11)	114.22	6.422 (d, 15.63)	114.41
9c	–	168.33	–	168.53
1d	–	127.14	–	127.12
2d, 6d	7.354 (d, 8.79)	131.22	7.340 (d, 8.79)	131.24
3d, 5d	6.759 (m)	116.83	6.764 (d, 8.30)	116.84
4d	–	161.35 or 161.23	–	161.30
7d	7.558 (d, 16.11)	146.91	7.580 (d, 15.14)	146.86
8d	6.281 (d, 15.63)	114.83	6.268 (d, 16.11)	114.89
9d	–	168.87	–	168.93

lic acid and *p*-coumaric acid comprised in the vanicoside structure. Ferulic acid and *p*-coumaric acid had MIC values at 256 μg/ml, and their mixture (1:1 w/w) did not show higher inhibition (Table II). This suggests that the esterified structure of phenylpropanoids with sucrose is essential for higher antimicrobial activity of vanicosides. Furthermore, considering the difference between the MICs of vanicoside A and B, the acetyl group in the sucrose moiety seems to affect the antimicrobial

Table II. MICs of vanicosides, their related phenylpropanoic acids and typical antibiotics against *Ph. damselae* subsp. *piscicida* DPp-1.

Compound	MIC [μg/ml (mM)]
Vanicoside A	32 (0.032)
Vanicoside B	64 (0.067)
Ferulic acid	256 (1.32)
p-Coumaric acid	256 (1.56)
Ferulic acid + *p*-coumaric acid (1:1 w/w)	256 (0.66 + 0.78)*
Florphenicol	<4 (<0.011)
Ampicillin	<4 (<0.011)
Amoxicillin	<4 (<0.011)

* The concentrations (mM) of ferulic acid and *p*-coumaric acid are expressed individually.

activity against this bacterium. We have confirmed that the ethyl acetate-soluble fraction from rhizome extracts of *P. sachalinense* dissipated the membrane potential of *Ph. damselae* subsp. *piscicida* cells (Kumagai *et al*., unpublished data). Further research is required to elucidate the mode of antimicrobial action of vanicoside A and B.

In plant metabolism, it has been known that phenylpropanoid compounds and their glycosides are produced by the shikimate pathway from phenylalanine as a starting substance (Waterman and Mole, 1994). Vanicoside A and B are known compounds isolated from *Polygonum pensylvanicum* (Zimmermann and Sneden, 1994). The phenylpropanoid glycosides such as vanicosides have been found in many plants belonging to the families Polygonaceae (*P. pensylvanicum, P. perfoliatum, P. lapathifolium*) (Zimmermann and Sneden, 1994; Brown *et al*., 1998; Sun *et al*., 2000), Rosaceae, Brassicaceae, Bignoniaceae (Gafner *et al*., 1997), Ballotaceae (Seidel *et al*., 1997) and Liliaceae (Shimomura *et al*., 1986; Shoyama *et al*., 1987). It has been reported that vanicoside A and B showed cytotoxicity against MCF cell-line and protein kinase C inhibitory activity (Zimmermann and Sneden, 1994; Sneden *et al*., 1995). Vanicoside B and lapathoside A, a vanicoside-related substance, from *Polygonum lapathifolium* showed significant anti-tumor-promoting effects on mouse two-stage skin carcinogenesis induced by 7,12-dimethylbenzanthracene (Takasaki *et al*., 2001). However, there have been no reports on the antimicrobial activity of vanicosides. The present report is the first on vanicoside A and B from giant knotweed *P. sachalinense* and their inhibitory activity against *Ph. damselae* subsp. *piscicida*.

Recently, it has been reported that the antibiotic-resistant *Ph. damselae* subsp. *piscicida* has often appeared in many countries. In fact, pasteurellosis has been a severe problem in aquaculture in the Mediterranean area and Japan (Thyssen and Ollevier, 2001; Bakopoulos *et al*., 1995; Romalde, 2002). Giant knotweed has been recognized as noxious weeds spread to several countries because it can propagate so vigorously that it can wipe out native plant species (USDA, NRCS, 2004). Giant knotweed *P. sachalinense* might have the potential to be useful for restricting pasteurellosis and limiting large economic losses in aquaculture, as the extract has a considerable inhibitory activity against *Ph. damselae* subsp. *piscicida*.

Experimental

General

UV spectra of constituents were measured in methanol using an UV-VIS spectrophotometer (U-2000, Hitachi, Tokyo, Japan). ^{1}H and ^{13}C and 2D-NMR (COSY, HMBC, HSQC, NOESY, ROESY) spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) in CD_3OD using a NMR spectrometer (ECP-400, JEOL, Tokyo, Japan) with tetramethylsilane (TMS) as an internal standard. Positive and negative FAB-MS spectra were obtained using a mass spectrometer (JMS-HX110, JEOL). Wako Gel C-100 (Wako Pure Chemical Industries, Osaka, Japan) and a glass column (23×2.1 cm i.d.) were used for silica gel column chromatography with a hexane/ethyl acetate solvent system. The HPLC system included a HPLC pump (880-PU, JASCO, Tokyo, Japan) equipped with an automated gradient controller (880-02, JASCO), an UV/VIS detector (UV-970, JASCO) and a LiChrosorb RP-18 (7 μm) column (250×25 mm i.d.; Merck, Darmstadt, Germany). Elution was carried out by a linear gradient system with methanol/water containing 5% acetic acid. The methanol/water composition was initially 20% methanol/5% acetic acid and changed to 95% methanol/5% acetic acid for 40 min at 9.0 ml/min of flow rate at room temperature. Elution was detected by absorbance at 280 nm.

Plant material

The rhizomes of fresh giant knotweed *P. sachalinense* were collected in a suburb of Hakodate, Hokkaido, Japan in June 1999, washed with tap water, dried at room temperature and cut into

small pieces. This plant is very common in Japan and could be surely identified using a weed manual (Numata *et al.,* 1978).

Extraction and isolation

Briefly, the dried rhizome pieces (1.0 kg) were soaked with a 20-fold volume of methanol for one month to create an extract. The methanol extracts were filtrated through a filter paper (No. 5A; Toyo Roshi, Tokyo, Japan), and the solvent was removed *in vacuo* using a rotary evaporator to yield methanol extracts (34 g). The obtained methanol extracts were resuspended in water and partitioned in turn with an equal volume of four kinds of organic solvents, *i.e.* hexane, chloroform, ethyl acetate and 1-butanol. The separated fractions were filtrated through a filter paper and evaporated *in vacuo* to remove the solvent. In this work, the ethyl acetate-soluble fraction was used to isolate antimicrobial substances because it showed greater antimicrobial activity than the other fractions (Kawai *et al*., 2004; Kumagai *et al*., unpublished data).

The ethyl acetate-soluble fraction (3.4 g) was further separated by silica gel column chromatography eluted with hexane/ethyl acetate mixtures. Fraction No. 2 (826 mg) eluted with hexane/ethyl acetate (15:85 v/v), which followed fraction No. 1 eluted with hexane/ethyl acetate (20:80 v/v), was subjected to preparative RP-HPLC to obtain compounds **1** (25 mg) and **2** (54 mg).

Antimicrobial assay of fractions separated by chromatography

The fish pathogenic bacterium *Ph. damselae* subsp. *piscicida* strain DPp-1, which had been isolated from the kidneys of diseased fish as a virulent strain, was used for the antimicrobial assay. The strain was maintained on Brain Heart Infusion (BHI) agar (Difco Laboratories, Detroit, MI, USA) containing 2% NaCl at 25 °C and freshly cultured in BHI broth (Difco) containing 2% NaCl at 25 °C for 24 h before use. Bacterial cells in the stationary phase were suspended in 2% NaCl/BHI broth to a cell density of 1×10^4 cells/ml and added to a 96-well microplate. The fractions separated from the rhizomes were dissolved in DMSO or methanol and added to the cell suspension in the well. After incubation for 48 h at 25 °C, the absorbance at 655 nm was measured using a microplate reader (model 550, Bio-Rad Laboratories, Hercules, CA, USA). The antimicrobial activity was judged based on the inhibition of growth.

Minimum inhibitory concentration (MIC) of isolated substances

The determination procedure for MIC was based on the broth microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). Briefly, the isolated substances dissolved in methanol were added to cation-adjusted Mueller-Hinton broth (Difco) containing 2% NaCl (CAMHB-2) at final concentrations of 4 μg/ml to 256 μg/ml to prepare the test broth. Stationary cells of *Ph. damselae* subsp. *piscicida* precultured in Mueller-Hinton broth (Difco) containing 2% NaCl (MH-2) were suspended in CAMHB-2 to a cell density of 1×10^7 cells/ml. The prepared test broth (100 μl) and the cell suspension (5 μl) were mixed in each well of a 96-well microplate. After 16 h-incubation at 25 °C, the MIC values were determined to be the minimum concentrations that could inhibit bacterial growth. It was confirmed that the methanol and DMSO concentrations used in each experiment did not affect bacterial growth. For comparison of the antimicrobial activity, MICs of florphenicol, ampicillin and amoxicillin (Sigma Chemical, St. Louis, MO, USA), as effective antibiotics, and *p*-coumaric acid (ICN Biochemicals, Aurora, OH, USA) and ferulic acid (Sigma), as phenylpropanoic acids were determined.

Acknowledgements

The NMR and mass spectrometry were performed at the Center for Instrumental Analysis, Hokkaido University, Japan. The authors would like to thank Ms. Hiroko Matsumoto at the Center for operating the instruments.

Arichi H., Kimura Y., Okuda H., Baba K., Kozawa M., and Arichi S. (1980), Effects of stilbene components of the roots of *Polygonum cuspidatum* Sieb. et Zucc. on lipid metabolism. Chem. Pharm. Bull. **30**, 1766–1770.

Bakopoulos V., Adams A., and Richards R. H. (1995), Some biochemical properties and antibiotic sensitivities of *Pasteurella piscicida* isolated in Greece and comparison with strains from Japan, France, Italy. J. Fish Dis. **18**, 1–7.

Brown L. L., Larson S. R., and Sneden A. T. (1998), Vanicosides C–F, new phenylpropanoid glycosides from *Polygonum pensylvanicum*. J. Nat. Prod. **61**, 762–766.

Gafner S., Wolfender J.-L., Nianga M., and Hostettmann K. (1997), Phenylpropanoid glycosides from *Newbouldia laevis* roots. Phytochemistry **44**, 687–690.

Kawai Y., Sawano R., Kumagai H., Yamazaki K., Suzuki H., Tedzuka M., and Inoue N. (2004), Antimicrobial activity of extracts from giant knotweed *Polygonum sachalinense* against animal pathogenic bacteria. Bull. Fish. Sci. Hokkaido Univ. **55**, 139–144.

Kimura Y., Kozawa M., Baba K., and Hata K. (1983), New constituents of roots of *Polygonum cuspidatum*. Planta Med. **48**, 164–168.

Konstantinidou-Doltsinis S. and Schmitt A. (1998), Impact of treatment with plants extracts from *Reynoutria sachalinensis* (F. Schmidt) Nakai on intensity of powdery mildew severity and yield in cucumber under high disease pressure. Crop Prot. **17**, 649–656.

Kudo Y., Yasuhuku H., and Kido S. (1966), Studies on the components of edible plants (part 4). Chemical components of *Polygonum cuspidatum* Sieb. et Zucc. J. Food Sci. **18**, 35–38.

Magariños B., Toranzo A. E., and Romalde J. L. (1996), Phenotypic and pathobiological characteristics of *Pasteurella piscicida*. Ann. Rev. Fish Dis. **6**, 41–64.

Mizuno M. and Yoneda K. (1995), Herbal Folk Medicines in Japan. Shinnippon-Hoki Publishing, Tokyo, pp. 128–129 (in Japanese).

NCCLS (2002), Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard, 2nd Ed. NCCLS document M31-A2, NCCLS, Wayne.

Nonomura S., Kanagawa S., and Makimoto A. (1963), Constituents of polygonaceous plants. I. Studies on the components of Ko-jo-kon (*Polygonum cuspidatum* Sieb.). J. Pharm. Soc. **83**, 988–990.

Numata M., Yoshizawa N., Asano S., Kuwabara Y., Okuda S., and Iwase T. (1978), Weed Flora of Japan – Illustrated by Colour (Numata M. and Yoshizawa N., eds.). Zenkoku Noson Kyoiku Kyokai, Tokyo (in Japanese).

Okuda T. (1986), Encyclopedia of Natural Medicine. Hirokawa Publishing, Tokyo (in Japanese).

Romalde J. L. (2002), *Photobacterium damselae* subsp. *piscicida*: an integrated view of a bacterial fish pathogen. Int. Microbiol. **5**, 3–9.

Saito M., Kawai Y., Yamazaki K., Inoue N., and Shinano H. (1997), Antimicrobial activities of extracts from Sachaline giant knotweed *Polygonum sachalinense*. Food Sci. Technol. Int. Tokyo **3**, 290–293.

Seidel V., Bailleul F., Libot F., and Tillequin F. (1997), A phenylpropanoid glycoside from *Ballota nigra*. Phytochemistry **44**, 691–693.

Shimomura H., Sashida Y., and Mimaki Y. (1986), Bitter phenylpropanoid glycosides from *Lilium speciosum* var. *rubrum*. Phytochemistry **25**, 2897–2899.

Shoyama Y., Hatano K., Nishioka I., and Yamagishi T. (1987), Phenolic glycosides from *Lilium longiforum.* Phytochemistry **26**, 2965–2968.

Sneden A. T., Zimmermann M. I., and Sumper T. L. (1995), Negative-ion fast atom bombardment and tandem mass spectrometric investigation of phenylpropanoid glycosides isolated from *Polygonum pensylvanicum* L. J. Mass Spec. **30**, 1628–1632.

Sun X., Zimmermann M. L., Campagne J.-M., and Sneden A. T. (2000), New sucrose phenylpropanoid esters from *Polygonum perfoliatum.* J. Nat. Prod. **63**, 1094–1097.

Takasaki M., Konoshima T., Kuroki S., Tokuda H., and Nishino H. (2001), Cancer chemopreventive activity of phenylpropanoid esters of sucrose, vanicoside B and lapathoside A from *Polygonum lapathifolium*. Cancer Lett. **173**, 133–138.

Thyssen A. and Ollevier F. (2001), *In vitro* antimicrobial susceptibility of *Photobacterium damselae* subsp. *piscicida* to 15 different antimicrobial agents. Aquaculture **200**, 259–269.

USDA, NRCS (2004), The Plants Database, Version 3.5 (http://plants.usda.gov). National Plant Data Center, Baton Rouge.

Vastano B. C., Chen Y., Zhu N., Ho C. T., Zhou Z., and Rosen R. T. (2000), Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. J. Agric. Food Chem. **48**, 253–256.

Waterman P. G. and Mole S. (1994), Analysis of Phenolic Plant Metabolites. Blackwell Scientific Publications, Oxford.

Xiao K., Xuan L., Bai D., and Zhong D. (2002), Constituents from *Polygonum cuspidatum*. Chem. Pharm. Bull. **50**, 605–608.

Yan H., Zhou J. Y., Ni W., and Cheng C. (2001), Studies on the constituents of *Reynoutria japonica* Houtt. Nat. Prod. Res. Develop. **13**, 16–18.

Zimmermann M. L. and Sneden A. T. (1994), Vanicosides A and B, protein kinase C inhibitors from *Polygonum pensylvanicum*. J. Nat. Prod. **57**, 236–242.