J Nat Med (2010) 64:104–108
DOI 10.1007/s11418-009-0370-4

NOTE

Elaeocarpionoside, a megastigmane glucoside from the leaves of *Elaeocarpus japonicus* Sieb. et Zucc.

Junko Shitamoto · Katsuyoshi Matsunami · Hideaki Otsuka · Takakazu Shinzato · Yoshio Takeda

Received: 3 August 2009 / Accepted: 9 October 2009 / Published online: 12 November 2009

Abstract From a 1-BuOH-soluble fraction of an extract of the leaves of *Elaeocarpus japonicus*, six megastigmane glucosides (**1**–**6**) were isolated. Five were known compounds (**2**–**6**), i.e. amelopsisionoside, citroside A, roseoside, alangionoside A and turpinionoside A, respectively. The structure of the new compound, named elaeocarpionoside, was elucidated to be (3*R*,4*R*,5*S*,6*S*,7*E*,9*R*)-megastigman-7-ene-3,4,9-triol 9-*O*-*β*-D-glucopyranoside by spectroscopic analyses and the modified Mosher's method.

Keywords *Elaeocarpus japonicus* · Elaeocarpaceae · Megastigmane glucoside · Elaeocarpionoside · Modified Mosher's method

Introduction

Only three species belonging to the Elaeocarpaceae are known to grow wild in Japan. One of these species, *Elaeocarpus japonicus*, is an evergreen tree of about 7–10 m in height and is found in the temperate zone of Japan, Ryukyu Islands, Taiwan and China [1]. A decoction of dried trunk bark of *E. japonicus* is used as a folk medicine in the Satsunan Islands for diseases of the kidneys and duodenum [2]. Since no systematic investigation on the constituents of *E. japonicus* has so far been reported, we investigated the chemical constituents of the title plant collected in Okinawa.

From a 1-BuOH-soluble fraction of a MeOH extract of the leaves of *E. japonicus*, one new megastigmane glucoside (**1**) was isolated together with five known ones (**2**–**6**). The structures of the known compounds were determined to be amelopsisionoside (**2**) [3], citroside A (**3**) [4], roseoside (**4**) [5], alanagionoside A (**5**) [6] and turpinionoside A (**6**) [7] by comparison of their spectroscopic data with those reported in the literature. This paper deals with structural elucidation of the new megastigmane glucoside.

Results and discussion

Air-dried leaves of *E. japonicus* were extracted with MeOH three times and the concentrated MeOH extract was partitioned between solvents of increasing polarity. The 1-BuOH-soluble fraction was separated by means of various chromatographic procedures including column chromatography (CC) on a highly porous synthetic resin (Diaion HP-20), normal silica gel and reversed-phase octadecyl silica gel (ODS) CC, droplet counter-current chromatography (DCCC) and high-performance liquid chromatography (HPLC) to afford six compounds (**1**–**6**). The details and yields are given in the "Experimental" section. The structure of the new megastigmane glucoside (**1**) was elucidated on the basis of spectroscopic evidence and structures of known compounds were identified by comparison

J. Shitamoto · K. Matsunami · H. Otsuka (✉)
Department of Pharmacognosy,
Graduate School of Biomedical Sciences,
Hiroshima University, 1-2-3 Kasumi, Minami-ku,
Hiroshima 734-8553, Japan
e-mail: hotsuka@hiroshima-u.ac.jp

T. Shinzato
Subtropical Field Science Center, Faculty of Agriculture,
University of the Ryukyus, 1 Senbaru, Nishihara-cho,
Nakagami-gun, Okinawa 903-0213, Japan

Y. Takeda
Faculty of Pharmacy, Yasuda Women's University,
6-13-1 Yasuhigashi, Asaminami-ku,
Hiroshima 731-0153, Japan

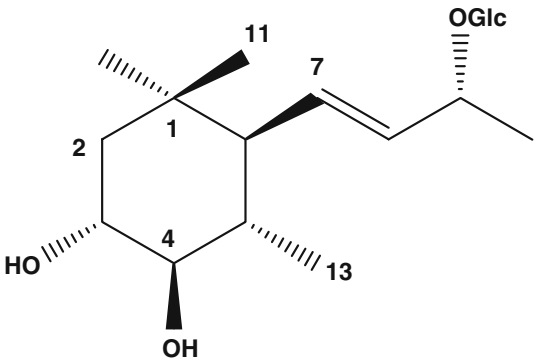

Fig. 1 Structure of elaeocarpionoside (**1**)

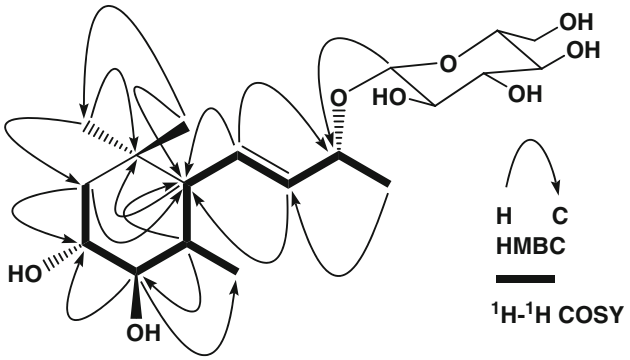

Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of **1**

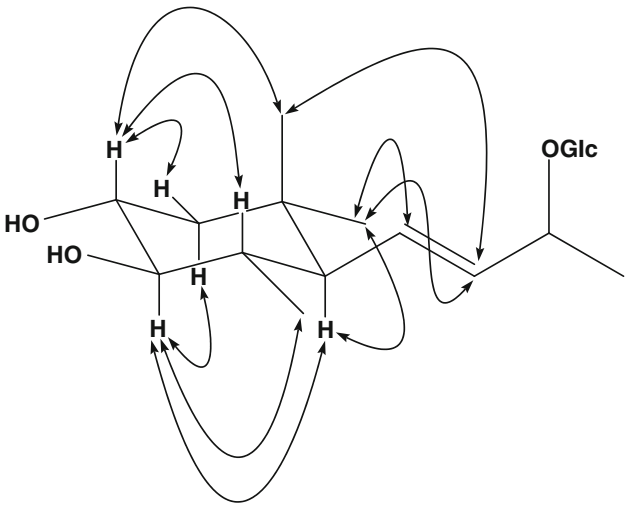

Fig. 3 PS-NOESY correlations of **1**

of spectroscopic data with those reported in the literature (Fig. 1).

Elaeocarpionoside (**1**), $[\alpha]_D^{21}$ −2.9, was obtained as an amorphous powder and its elemental composition was determined to be $C_{19}H_{34}O_8$ by high-resolution (HR)-electrospray ionization (ESI)–MS. The IR spectrum exhibited absorption for glycosidic (3369, 1066 and 1039 cm^{-1}) and aliphatic (2925 and 1455 cm^{-1}) features. In the ^{1}H-NMR spectrum, signals for two singlet (δ_H 0.90 and 0.89) and two doublet (δ_H 1.29 and 0.92) methyls, an anomeric proton (δ_H 4.36, $J = 8$ Hz), and two olefinic protons in the *trans* geometry [δ_H 5.53 (dd, $J = 15$, 7 Hz) and 5.34 (dd, $J = 15$, 9 Hz)] were observed. The ^{13}C-NMR spectrum displayed six signals assignable to a β-D-glucopyranosyl moiety, whose absolute configuration was ascertained by the positive sign of optical rotation value of the glucose, obtained on enzymatic hydrolysis. The remaining 13 signals comprised those of four methyls, one methylene, three methines with oxygen substituents and two without oxygen substituents, and one quaternary sp^3 and two sp^2 carbons. The ^{1}H–^{1}H COSY spectrum showed chains of correlation from H_2-2 through H_3-10 and H-5 to H_3-13. With the aid of the HSQC spectrum, the structure of elaeocarpionoside was assigned as the β-D-glucopyranoside of 3,4,9-trihydroxymegastigman-7-ene. The position of the sugar linkage was determined to be the hydroxyl group at the 9-position, since a significant HMBC correlation peak was observed between H-1′ (δ_H 4.36) and C-9 (δ_C 77.7) (Fig. 2). Judging from the coupling constants in the ^{1}H-NMR spectrum, H-3 and H-4 were in a diaxial relationship, and then H-4 and H-5, and H-5 and H-6 were also in the diaxial relationships. This was substantiated by correlations (H-2ax and H-4ax, H-3ax and H-5ax, and H-4ax and H-6ax) observed in the phase-sensitive (PS)-NEOSY spectrum (Fig. 3). The absolute configuration at the 9-position was expected to be *R*, judging from the ^{13}C-NMR chemical shifts of related compounds [8, 9]. To confirm the absolute configuration of the 9-position and to establish that in the ring system, **1** was enzymatically hydrolyzed, and the modified Mosher's method was applied to the aglycone (**1a**) [10]. The results shown in Fig. 4 clearly demonstrate that **1a** has the 3*R*,4*R*,5*S*,6*S*,9*R* configuration (Fig. 4). Therefore, the structure of elaeocarpionoside (**1**) was elucidated to be (3*R*,4*R*,5*S*,6*S*,7*E*,9*R*)-megastigman-7-ene-3,4,9-triol 9-*O*-β-D-glucopyranoside, as shown in Fig. 1.

Experimental

General

Optical rotations were measured on a JASCO P-1030 polarimeter. An IR spectrum was measured on a Horiba FT-710 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM α-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HR-MS were measured with an Applied Biosystem QSTAR XL system ESI (Nano Spray)–MS.

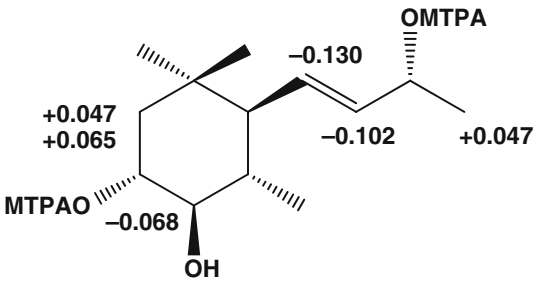

Fig. 4 Results of the modified Mosher's method ($\Delta\delta_S$–δ_R)

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase (ODS gel) open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [$\Phi = 50$ mm, $L = 25$ cm; linear gradient: MeOH–H_2O (1:9, 1 l) → (1:1, 1 l), fractions of 10 g being collected], respectively. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns ($\Phi = 2$ mm, $L = 40$ cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) were used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; $\Phi = 6$ mm, $L = 25$ cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

Emulsin was purchased from Tokyo Chemical Industries Co., Ltd (Tokyo, Japan), and crude hesperidinase was a gift from Tokyo Tanabe Pharmaceutical Co., Ltd (Tokyo, Japan). The (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acids (MTPA) were the products of Wako Pure Chemical Industry Co., Ltd (Tokyo, Japan).

Plant material

Leaves of *E. japonicus* were collected in Okinawa, Japan, in July 2001, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (01-EJ-Okinawa-0705).

Extraction and isolation

Dried leaves of *E. japonicus* (16.4 kg) were extracted three times with MeOH (45 l) at 25°C for 1 week and then concentrated to 6 l in vacuo. The extract was washed with *n*-hexane (6 l, 194 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (6 l) and then extracted with EtOAc (6 l) to give 461 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (6 l) to give a 1-BuOH-soluble fraction (1.54 kg), and the remaining water layer was concentrated to afford 1.66 kg of a water-soluble fraction.

A portion (283 g) of the 1-BuOH-soluble fraction was subjected to CC on a Diaion HP-20 column ($\Phi = 50$ mm, $L = 50$ cm) using H_2O–MeOH [(4:1, 2 l), (2:3, 8 l), (3:2, 8 l) and (1:4, 8 l)] and MeOH (8 l), 1-l fractions being collected. The residue (117 g in fractions 6–9) from the 20–40% MeOH eluent was subjected to silica gel (1.80 kg; $\Phi = 9$ cm, $L = 60$ cm) CC, with $CHCl_3$–MeOH [(9:1, 6 l), (17:3, 8 l) and (4:1, 8 l)] and $CHCl_3$–MeOH–H_2O [(35:15:2, 8 l) and (25:25:4, 8 l)], 400-ml fractions being collected. An aliquot (3.5 g) of the residue (12.8 g) in fractions 59–66 was separated by ODS CC and the residue (134 mg) in fractions 84–90 was further separated by DCCC to give the residue (19.3 mg) in fractions 30–46, which was finally purified by HPLC (MeOH–H_2O, 1.3:2.7) to afford 2.7 mg of **1** from the peak at 12 min. The residue (188 mg) in fractions 91–102 obtained on ODS CC was again separated by silica gel CC (35 g; $\Phi = 2$ cm, $L = 23$ cm) with $CHCl_3$–MeOH–H_2O (15:6:1), 2.5-ml fractions being collected, to give the residue (49.6 mg) in fractions 30–69, which was then purified by HPLC (MeOH–H_2O, 7:13) to yield 2.3 mg of **6** from the peak at 9 min.

The residue (56.7 g) in fractions 10–16 obtained on Diaion HP-20 CC was subjected to silica gel CC (1.3 kg; $\Phi = 8$ cm, $L = 22$ cm) eluting with $CHCl_3$ (4 l) and $CHCl_3$–MeOH [(99:1, 8 l), (97:3, 8 l), (19:1, 8 l), (37:3, 8 l), (9:1, 8 l), (35:5, 8 l), (17:3, 8 l), (33:7, 8 l), (4:1, 8 l), (3:1, 8 l) and (7:3, 8 l)], 500-ml fractions being collected. The residue (2.12 g) in fractions 81–100 was separated by ODS CC and 45.5 mg of **4** was isolated from the residue (234 mg) in fractions 68–78, followed by DCCC (in fractions 43–51). The residue (2.15 g) in fractions 101–105 was separated by ODS and the residue (430 mg) in fractions 66–78 was again separated by DCCC to give two residues (79.8 and 112 mg) in fractions 40–45 and 55–65, respectively. The former was then purified by HPLC (MeOH–H_2O, 9:31) to give 8.9 mg of **5** from the peak at 38 min. The latter was found to be a pure compound, **3**. The residue (243 mg) in fractions 79–87 was purified by DCCC to give 43.4 mg of **2** in fractions 40–49.

*Elaeocarpionoside (**1**)*

Amorphous powder, $[\alpha]_D^{21}$ −2.9 (*c* 0.17, MeOH). IR ν_{max} (film) cm^{-1}: 3369, 2925, 1650, 1455, 1370, 1066, 1039. ^1H NMR (CD_3OD, 400 MHz) δ: 5.53 (1H, dd, $J = 15$, 7 Hz, H-8), 5.34 (1H, dd, $J = 15$, 9 Hz, H-7), 4.36 (1H, d, $J = 8$ Hz, H-1′), 4.36 (1H, dq, $J = 7$, 6 Hz, H-9), 3.80 (1H, dd, $J = 12$, 2 Hz, H-6′a), 3.68 (1H, dd, $J = 12$, 5 Hz, H-6′b), 3.54 (1H, ddd, $J = 12$, 9, 5 Hz, H-3), 3.34 (2H, m,

H-3′ and 4′), 3.20 (1H, ddd, $J = 10$, 5, 2 Hz, H-5′), 3.18 (1H, dd, $J = 9$, 8 Hz, H-2′), 2.82 (1H, dd, $J = 9$, 9 Hz, H-4), 1.67 (1H, dd, $J = 12$, 5 Hz, H-2a), 1.50 (1H, dd, $J = 9$, 9 Hz, H-6), 1.45 (1H, m, H-5), 1.29 (3H, d, $J = 6$ Hz, H_3-10), 1.23 (1H, dd, $J = 12$, 12 Hz, H-2b), 0.92 (3H, s, H_3-13), 0.90 (3H, s, H_3-11), 0.89 (3H, s, H_3-12). ^{13}C NMR (CD_3OD, 100 MHz) δ: 136.7 (C-8), 132.6 (C-7), 102.4 (C-1′), 82.2 (C-4), 78.2 (C-3′), 77.9 (C-5′), 77.7 (C-9), 75.5 (C-2′), 72.4 (C-3), 71.5 (C-4′), 62.7 (C-6′), 57.4 (C-6), 48.6 (C-2), 38.8 (C-5), 35.4 (C-1), 31.8 (C-12), 21.8 (C-11), 21.4 (C-10), 17.4 (C-13). HR-ESI–MS (positive-ion mode) *m/z*: 413.2150 $[M + Na]^+$ (calcd for $C_{19}H_{34}O_8Na$: 413.2145).

*Enzymatic hydrolysis of elaeocarpionoside (**1**)*

Elaeocarpionoside (**1**) (2.7 mg) in 2 ml of H_2O was hydrolyzed with emulsin (4.0 mg) and crude hesperidinase (4.0 mg) for 18 h at 37°C. The reaction mixture was evaporated to dryness, and then the methanolic solution was absorbed on silica gel and subjected to CC (6 g, $\Phi = 10$ mm, $L = 17$ cm) with $CHCl_3$ (50 ml) and $CHCl_3$–MeOH [(19:1, 50 ml), (9:1, 50 ml) and (7:3, 150 ml)], 5-ml fractions being collected. The aglycone (**1a**) (1.2 mg, 76%) and D-glucose (1.1 mg, 85%) were recovered in fractions 23–28 and 43–52, respectively. Aglycone (**1a**): amorphous powder, $[\alpha]_D^{24}$ +10.0° (*c* 0.08, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.45 (1H, dd, $J = 15$, 6 Hz, H-8), 5.29 (1H, dd, $J = 15$, 9 Hz, H-7), 4.23 (1H, qd, $J = 6$, 1 Hz, H-9), 3.53 (1H, ddd, $J = 12$, 9, 5 Hz, H-3), 2.82 (1H, dd, $J = 9$, 9 Hz, H-4), 1.67 (1H, dd, $J = 12$, 5 Hz, H-2a), 1.46 (1H, dd, $J = 9$, 9 Hz, H-6), 1.42 (1H, m, H-5), 1.23 (3H, d, $J = 6$ Hz, H_3-10), 1.21 (1H, dd, $J = 12$, 12 Hz, H-2b), 0.94 (3H, d, $J = 6$ Hz, H_3-13), 0.90 (3H, s, H_3-11), 0.89 (3H, s, H_3-12); ^{13}C NMR (CD_3OD, 100 MHz) δ: 138.8 (C-8), 130.7 (C-7), 82.3 (C-4), 72.4 (C-3), 69.3 (C-9), 57.3 (C-6), 48.6 (C-2), 38.8 (C-5), 35.1 (C-1), 31.5 (C-12), 24.1 (C-10), 21.8 (C-11), 17.3 (C-13). HR-ESI–MS (positive-ion mode) *m/z*: 251.1620 $[M + Na]^+$ (calcd for $C_{13}H_{24}O_3Na$: 251.1617). D-Glucose: $[\alpha]_D^{25}$ +28.1° ($c = 0.07$, H_2O, 24 h after being dissolved in the solvent).

Preparation of (R)- and (S)-MPTA esters

A solution of **1a** (0.4 mg) in 1 ml of anhydrous CH_2Cl_2 was reacted with (*R*)-MTPA (25 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (15 mg) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (12 mg), and then the mixture was occasionally stirred at 25°C for 3 h. After the addition of 1 ml of CH_2Cl_2, the solution was washed successively with H_2O (1 ml), 4 N HCl (1 ml), saturated aqueous $NaHCO_3$ and then brine (1 ml). The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25-mm thickness), being applied for 18 cm and developed with $CHCl_3$–$(CH_3)_2CO$ (19:1) for 9 cm; the band at R_f 0.6 was scraped off and eluted with $CHCl_3$–MeOH (9:1)] to afford an ester, **1b** (0.3 mg, 26%). Through a similar procedure, **1c** (0.3 mg, 26%) was prepared from **1a** (0.4 mg) using (*S*)-MTPA (24 mg), EDC (16 mg) and 4-DMAP (10 mg).

(3*R*,4*R*,5*S*,6*S*,7*E*,9*R*)-Megastigman-7-ene-3,4,9-triol 3,9-di-*O*-(*R*)-MTPA ester (**1b**): an amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.55–7.51 (4H, m, aromatic protons), 7.41–7.36 (6H, m, aromatic protons), 5.49 (1H, m, H-7), 5.47 (1H, m, H-8), 5.06 (1H, ddd, $J = 12$, 9, 5 Hz, H-3), 3.55 (3H, br s, –OCH_3), 3.52 (3H, br s, –OCH_3), 3.20 (1H, dtt, $J = 9$, 5, 5 Hz, H-4), 1.84 (1H, dd, $J = 12$, 5 Hz, H-2a), 1.90 (1H, m, H-5), 1.50 (1H, dd, $J = 9$, 9 Hz, H-6), 1.37 (3H, d, $J = 6$ Hz, H_3-10), 1.33 (1H, dd, $J = 12$, 12 Hz, H-2b), 0.96 (3H, s, H_3-11), 0.94 (3H, d, $J = 6$ Hz, H_3-13), 0.82 (3H, s, H_3-12); HR-ESI–MS (positive-ion mode) *m/z*: 683.2415 $[M + Na]^+$ (calcd for $C_{33}H_{38}O_7F_6Na$: 683.2413).

(3*R*,4*R*,5*R*,6*S*,7*E*,9*R*)-Megastigman-7-ene-3,4,9-triol 3,9-di-*O*-(*S*)-MTPA ester (**1c**): an amorphous powder, ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.55–7.50 (4H, m, aromatic protons), 7.42–7.36 (6H, m, aromatic protons), 5.37 (1H, m, H-8), 5.36 (1H, m, H-7), 5.50 (1H, ddd, $J = 12$, 9, 5 Hz, H-3), 3.57 (3H, q, $J = 1$ Hz, –OCH_3), 3.56 (3H, q, $J = 1$ Hz, –OCH_3), 3.18 (1H, ddd, $J = 9$, 5, 5 Hz, H-4), 1.90 (1H, dd, $J = 12$, 5 Hz, H-2a), 1.78 (1H, m, H-5), 1.47 (1H, dd, $J = 9$, 9 Hz, H-6), 1.42 (3H, d, $J = 6$ Hz, H_3-10), 1.37 (1H, dd, $J = 12$, 12 Hz, H-2b), 0.93 (3H, s, H_3-11), 0.89 (3H, d, $J = 6$ Hz, H_3-13), 0.81 (3H, s, H_3-12); HR-ESI–MS (positive-ion mode) *m/z*: 683.2421 $[M + Na]^+$ (calcd for $C_{33}H_{38}O_7F_6Na$: 683.2413).

Acknowledgments The authors are grateful for access to the superconducting NMR instrument and the HR-ESI–MS at the Analytical Center of Molecular Medicine and the Analysis Center of Life Science, respectively, of the Graduate School of Biomedical Sciences, Hiroshima University. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, Culture and Technology of Japan, and the Japan Society for the Promotion of Science. Thanks are also due to the Astellas Foundation for Research on Medicinal Resources and the Takeda Science Foundation for the financial support.

References

1. Hatusima S (1975) Flora of Ryukyus, added and corrected. The Biological Society of Okinawa, Okinawa, p 397
2. Higashi S, Abe M, Ogata S, Tobita H, Yokota K (1976) Traditional medicinal and poisonous plants in the Satsunana Islands. Part II. Amamioshima, Kakeromajima, Ukejima, Yoronjima, Tokunoshima, Okierabujima and Kikaijima. Rep Fac Sci Kagoshima Univ Earth Sci Biol 9:129–150

3. Inada A, Nakamura Y, Konishi M, Murata H, Kitamura F, Toya H, Natanishi T (1991) A new ionone glucoside and a new phenylpropanoid rhamnoside from stems of *Ampelopsis brevipeduncula* (Maxim.) Trautv. Chem Parm Bull 39:2437–2439
4. Umehara K, Hattori I, Miyase T, Ueno A, Hara S, Kageyama C (1988) Studies on the constituents of leaves of *Citrus unshiu* Marcov. Chem Pharm Bull 36:5004–5008
5. Otsuka H, Yao M, Kamada K, Takeda Y (1995) Alangionoside G–M: glycosides of megastigmane derivatives from the leaves of *Alangium premnifolium*. Chem Pharm Bull 43:754–759
6. Otsuka H, Kamada K, Ogimi C, Hirata E, Takushi A, Takeda Y (1994) Alangionosides A and B: ionol glycosides from leaves of *Alangium premnifolium*. Phytochemistry 35:1331–1334
7. Qian Y, Otsuka H, Hirata E, Shinzato T, Takeda Y (2002) Turpinionosides A–E: megastigmane glucosides from leaves of *Turpinia ternata* Nakai. Chem Pharm Bull 50:640–644
8. Yamamoto M, Akita T, Koyama Y, Sueyoshi E, Matsunami K, Otsuka H, Shinzato T, Takashima A, Aramoto M, Takeda Y (2008) Eudionosides A–G: megastigmane glucosides from leaves of *Euodia meliaefolia*. Phytochemistry 69:1586–1596
9. Qian Y, Matsunami K, Otsuka H, Takeda Y (2005) Staphylionosides A–K: megastigmane glucosides from the leaves of *Staphylea bumalda* DC. Chem Pharm Bull 53:800–807
10. Ohtani I, Kusumi T, Kashman Y, Kakisawa H (1991) High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. J Am Chem Soc 113:4092–4096