New Megastigmane and Tetraketide from the Leaves of *Euscaphis japonica*

Yoshio TAKEDA,*[,a] Yoshihiro OKADA,[a] Toshiya MASUDA,[a] Eiji HIRATA,[b] Takakazu SHINZATO,[c] Anki TAKUSHI,[d] Qian YU,[e] and Hideaki OTSUKA[e]

Faculty of Integrated Arts and Sciences, The University of Tokushima,[a] 1–1 Minamijosanjima-cho, Tokushima 770–8502, Japan, Faculty of Agriculture, University of the Ryukyus,[b] 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 903–0213, Japan, University Forest, Faculty of Agriculture, University of the Ryukyus,[c] 685 Yona, Kunigami-gun, Okinawa 905–1427, Japan, 134 Furugen, Yomitan-son,[d] Nakagami-gun, Okinawa 904–0300, Japan, and Institute of Pharmaceutical Sciences, Hiroshima University Faculty of Medicine,[e] 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan.
Received November 29, 1999; accepted January 19, 2000

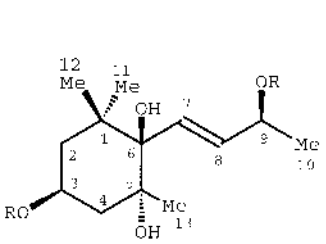
New megastigmane (1) and tetraketide (2) were isolated from the leaves of *Euscaphis japonica* and the structures were elucidated by means of spectroscopic and chemical evidence.

Key words *Euscaphis japonica*; Staphyleaceae; megastigmane; bicyclic tetraketide

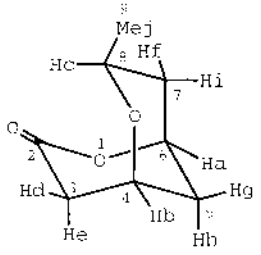
Euscaphis (*E.*) *japonica* KANITZ. (Staphyleaceae) is a tree which is grown in southwestern parts of Japan and in central China.[1)] From the capsule of the plant, flavonol glycosides and anthocyanin,[2)] and compounds[3)] which are positive to Ehrlich's reagent have already been isolated and characterized. In the course of the studies on the constituents of the plants grown in a subtropical climate, we have investigated the constituents of the leaves of *E. japonica* harvested in Okinawa Prefecture, Japan and earlier reported the isolation and structure elucidation of euscapholide and its glucoside.[4)] Further studies on the constituents resulted in the isolation of two new compounds, one is a megastigmane and the other a tetraketide. This report deals with the isolation and structural elucidation of the new compounds.

The *n*-BuOH soluble portion of the MeOH extract of the leaves of *E. japonica* upon repeated column chromatographies over highly porous synthetic resin, Diaion HP-20 and silica gel, and HPLC, afforded two new compounds **1** and **2**.

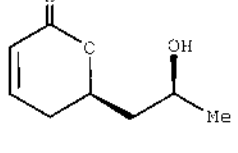
Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{26}$ −25.7° (MeOH) and the molecular formula was determined as $C_{13}H_{24}O_4$ based on its negative ion high resolution (HR)-FAB-MS. The ^{1}H-NMR spectrum displayed a secondary methyl signal at δ 1.27 (H_a), three tertiary methyl signals at δ 0.87 (H_b), 1.10 (H_c) and 1.22 (H_d), two methylene signals at δ 1.45 (H_e), 1.64 (H_f) (each 1H) and 1.76 (H_g) (2H), two secondary carbinyl proton signals at δ 4.06 (H_h) and 4.34 (H_i), and signals due to *trans*-double bond at δ 5.79 (H_j) and 6.07 (H_k). The ^{13}C-NMR spectrum (Table 1), in addition to the signals due to the above functional groups, showed signals due to a quaternary carbon atom at δ 40.6 and two quaternary carbon atoms with an oxygen atom at δ 77.7 and 78.8. In the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum, the cross peaks were followed starting from H_a to $H_i \rightarrow H_j \rightarrow H_k$, successively, establishing the structure of side chain portion (C-7—C-10). Starting from H_e and H_f, the cross peaks were followed to H_h and H_g, successively. Two singlet methyl groups (H_b and H_d) constitute the *gem*-dimethyl system, since the cross peaks due to long-range coupling were observed between these signals. The cross peaks between H_f and H_d were also observed. Thus, the structure around C-1—C-4 was elucidated. A quaternary carbon atom with a hydroxyl group was assigned to C-6 since carbon signals assigned to C-1 and C-6 resonated in almost the same region as those of closely related compounds, dendranthemoside A[5)] (δ 40.5 and 78.3, respectively). Considering the elemental composition and the above functional groups, compound **1** is monocyclic so that the remaining tertiary methyl group and a quaternary carbon atom with hydroxyl group could be assigned to C-13 and C-5. Thus, compound **1** was presumed to have a structure with megastigm-7-ene carbon skeleton to which four hydroxyl groups are introduced on C-3, 5, 6 and 9, and the presumption was supported by the results of the heteronuclear multiple bond correlation spectroscopy (HMBC) spectrum which are shown in Fig. 1. The planar structure thus elucidated is the same as that of the aglycone part of kiwiionoside.[6)] The relative stereochemistry of the secondary hydroxyl group on C-3 was determined to take an equatorial orientation as judged from the coupling pattern of H_2-2 (axial-H, t, J=12.2 Hz, H_f; equatorial-H, ddd, J=12.2, 4.0, 2.0 Hz, H_e). The relative stereochemistry was further examined by phase-sensitive nuclear Overhauser enhancement



1 R=H
1a R=(*R*)-MTPA
1b R=(*S*)-MTPA



2



3

* To whom correspondence should be addressed. e-mail: takeda@ias.tokushima-u.ac.jp

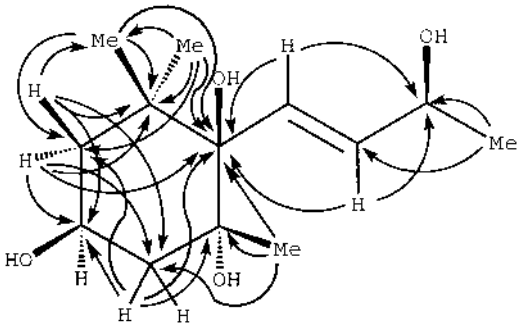


Fig. 1. HMBC Correlations (J=8 Hz, $^1H \rightarrow ^{13}C$) for Compound **1**

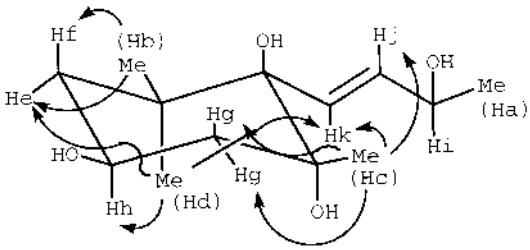


Fig. 2. NOE Correlations for Compound **1**

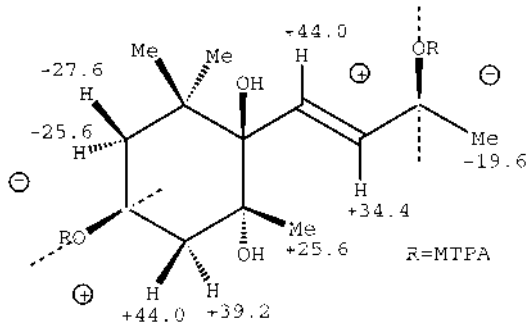


Fig. 3. $\Delta\delta$ Values in Hz ($\delta_S - \delta_R$, 400 MHz)

and exchange spectroscopy (NOESY) and differential nuclear Overhauser enhancement (NOE) experiments. The results shown in Fig. 2 clearly demonstrated that both the methyl group at C-5 and the side chain take equatorial orientations. To determine the absolute stereochemistry, compound **1** was subjected to a modified Mosher's method.[7] Namely, compound **1** was treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) in the presence of dicyclohexyl carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to give the 3,9-di-(*R*)-MTPA ester (**1a**) and the 3,9-di-(*S*)-MTPA ester (**1b**), respectively. As shown in Fig. 3, the signals due to protons at C-4, 7, 8 and 13 in **1a** resonated at a higher field as compared to those in **1b**, while the signals due to protons on C-2 and C-10 resonated at a lower field. Consequently, the absolute configurations at C-3 and C-9 have been elucidated as 3*S* and 9*S*, respectively, and the structure of compound **1** was determined to be as shown.

Compound **2** was obtained as colorless needles, mp 86—87 °C, $[\alpha]_D^{26}$ −13.9° (MeOH) and the molecular formula was determined as $C_8H_{12}O_3$ based on its high resolution electron impact MS (HR-EI-MS). Its IR spectrum showed the presence of a δ-lactone (ν_{max} 1720 cm^{-1}). The ^{1}H-NMR spectrum showed a clearly resolved spectrum when measured in $CDCl_3$ and C_6D_6 (1 : 1): it showed signals due to a secondary methyl group (δ 0.99) (H_j), three methylene groups [δ 2.32 (H_e) and 2.60 (H_d), 1.33 (H_g) and 1.48 (H_h), and 1.08 (H_f) and 1.12 (H_i)], one (H_d, H_e) of which located vicinal at a carbonyl group and three methine protons [δ 3.68 (H_c), 3.89 (H_b) and 4.40 (H_a)] geminal at an oxygen atom. The ^{13}C-NMR spectrum (Table 1) showed the presence of a lactonic carbon atom at δ 169.7 in addition to the signals arising from the above functional groups. These facts together with the elemental composition demonstrated that compound **2** has a bicyclic ring system. The planar structure was deduced as shown by interpretation of the ^{1}H–^{1}H COSY spectrum. Thus, the cross peaks, $H_j \rightarrow H_c \rightarrow H_i$ (H_f) $\rightarrow H_a \rightarrow H_h$ (H_g)$\rightarrow H_b \rightarrow H_e$ (H_d) were followed successively. Several long-range couplings *via* a W-letter interaction were observed between H_b and H_a, H_d and H_g, and H_f and H_h. In addition, H_i was observed as a doublet of double doublets (J=12.0, 11.6, 2.0 Hz) indicating that Hi takes an axial orientation and the methyl group (H_j) an equatorial orientation. The observed long-range coupling *via* W-letter interaction clearly supported the proposed bicyclic structure, so that the relative stereostructure of compound **2** was elucidated as shown. The absolute stereochemistry was established as shown by the chemical correlation of euscapholide (**3**)[4,8] with known absolute stereochemistry. Euscapholide (**3**) was treated with sodium hydride in CH_2Cl_2[9] to give compound **2**.

Table 1. ^{13}C-NMR Data for Compounds **1** and **2**[a]

Carbon	**1**[b]	**2**[c]
1	40.6	—
2	46.3	169.7
3	65.1	36.4
4	45.5	65.8
5	77.7	29.5
6	78.8	73.0
7	130.9	38.4
8	135.9	61.9
9	69.4	21.3
10	24.0	
11	27.5	
12	26.1	
13	27.1	

a) The assignments were based on noise decoupling, distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{13}C COSY and HMBC spectra. *b*) Taken in CD_3OD. *c*) Taken in $CDCl_3$.

Experimental

^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a JEOL JNM EX-400 or α-400 spectrometer, using tetramethylsilane as internal standard. Two dimensional spectra and NOE were measured by the usual pulse sequences with which the spectrometer was equipped; mass spectra were obtained on a JEOL JMS SX-102 spectrometer. FAB-MS were recorded using PEG-400, PEG-600 or *m*-nitrobenzyl alcohol as a matrix. IR spectra were taken on Shimadzu IR-400 or Perkin-Elmer 1720 infrared FT spectrophotometer. Specific optical rotations were determined using a JASCO DIP-360 digital polarimeter. For compound purification, the following were used: the highly porous synthetic resin, Diaion HP-20 (Mitsubishi Chemical Co., Ltd., Tokyo), silica gel 60 (Merck, 230—400 mesh), packed column for HPLC (Cosmosil 10 C18, 20×250 mm) and silica gel 60 F_{254} TLC plates (Merck, 0.25 and 0.5 mm in thickness).

Plant Material The leaves of *E. japonica* KANITZ. were collected in August, 1994, at Kunigami-son, Okinawa Prefecture, Japan. A specimen was authenticated by one of the authors (A.T.) and a voucher herbarium speci-

men (EJ-Okinawa 9407) is deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University Faculty of Medicine.

Extraction and Isolation Dried leaves (2.9 kg) of *E. japonica* were extracted with MeOH (45 l) at room temperature for 3 weeks. Concentration of MeOH extract *in vacuo* gave a residue which was dissolved in 90% MeOH (2.1 l). After washing with *n*-hexane (1 l×3), the 90% MeOH solution was concentrated *in vacuo*. The residue was suspended in H_2O (1 l) and the suspension was extracted successively with EtOAc (1 l×3) and *n*-BuOH (1 l ×3). The *n*-BuOH extract was concentrated *in vacuo* to give a residue (77 g) which was chromatographed on Diaion HP-20 (i.d.=78 mm, L=536 mm) with stepwise increase of MeOH content in H_2O [0 (4 l), 10 (5 l), 30 (6 l), 40 (6.5 l), 50 (6.5 l), 70 (6.5 l) and 100 (6 l)]; fractions of 500 ml wre collected.

Fractions 14—17 were combined and concentrated *in vacuo* to give a residue (10.7 g) which was separated by silica gel (400 g) column chromatography with $CHCl_3$–MeOH as eluent with an increasing amount of MeOH. The fractions which contained a spot (*Rf* 0.41) on TLC (solvent: $CHCl_3$–MeOH–H_2O, 15 : 6 : 1) were combined and the solvent was removed *in vacuo* to give a residue (180.4 mg) which was further purified by preparative HPLC (MeOH–H_2O, 1 : 4, 6 ml/min, detection 210 nm) to give compound **1** (t_R 37 min) (106.7 mg) as an amorphous powder.

Fractions 18—23 were combined and concentrated *in vacuo* to give a residue (12.2 g) which was subjected to silica gel (400 g) column chromatography with $CHCl_3$–MeOH as eluent with an increasing amount of MeOH content. The fraction which contained a spot (*Rf* 0.44) on TLC (solvent: $CHCl_3$–Me_2CO, 4 : 1) was purified by repeated silica gel chromatography (solvent: Et_2O, $CHCl_3$–MeOH) to give compound **2** (335 mg) as colorless needles.

Compound 1 An amorphous powder, $[\alpha]_D^{26}$ −25.7° (*c*=1.52, MeOH). IR (dry film): 3691 (br, OH) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.87 (3H, s, H-12), 1.10 (3H, s, H-13), 1.22 (3H, s, H-11), 1.27 (3H, d, *J*=6.4 Hz, H-10), 1.45 (1H, ddd, *J*=2.0, 4.0, 12.2 Hz, H-2_{eq}), 1.64 (1H, t, *J*=12.2 Hz, H-2_{ax}), 1.76 (2H, m, H-4), 4.06 (1H, m, H-3), 4.34 (1H, qd, *J*=6.4, 1.2 Hz,H-9), 5.79 (1H, dd, *J*=6.4, 16.0 Hz, H-8), 6.07 (1H, dd, *J*=1.2, 16.0 Hz, H-7). ^{13}C-NMR: given in Table 1. Negative HR-FAB-MS *m/z*: 243.1584 $[M-H]^-$ (Calcd for $C_{13}H_{23}O_4$: 243.1596).

Compound 2 Colorless needles, mp 86—87 °C, $[\alpha]_D^{26}$−13.9° (*c*=2.18, MeOH). IR ($CHCl_3$): 1720 (lactone) cm^{-1}. ^{1}H-NMR (C_6D_6–$CDCl_3$, 1 : 1) δ: 0.99 (3H, d, *J*=5.6 Hz, H-8), 1.12 (1H, ddd, *J*=2.0, 11.6, 12.0 Hz, H-7_{ax}), 1.33 (1H, br dt, *J*=13.6, 2.0 Hz, H-5_{eq}), 1.48 (1H, ddt, *J*=13.6, 4.0, 2.0 Hz, H-5_{ax}), 1.68 (1H, br d, *J*=13.6 Hz, H-7_{eq}), 2.32 (1H, dd, *J*=5.2, 18.8 Hz, H-3_{ax}), 2.60 (1H, br d, *J*=18.8 Hz, H-3_{eq}), 3.68 (1H, ddq, *J*=11.2, 2.4, 5.6 Hz, H-8), 3.89 (1H, br s, *W*/2=10 Hz, H-4), 4.40 (1H, br s, *W*/2=9 Hz, H-6). ^{13}C-NMR given in Table 1. HR-EI-MS *m/z*: 156.0801 $[M]^+$ (Calcd for $C_8H_{12}O_3$: 156.0786).

***(R)*-MTPA Ester (1a) of Compound 1** Compound **1** (15.3 mg) was dissolved in CH_2Cl_2 (10 ml). (*R*)-MTPA (purchased from Merck) (65.7 mg), DCC (57.0 mg) and DMAP (19.1 mg) were added to the solution and the solution was stirred at room temperature for 10 min. After dilution with EtOAc, the solution was washed with 5% HCl, saturated $NaHCO_3$ aqueous solution and saturated NaCl aqueous solution, successively, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by silica gel (5 g) column chromatography ($CHCl_3$) and then preparative TLC (solvent: $CHCl_3$–Me_2CO, 19 : 1) to give the di-(*R*)-MTPA ester (**1a**) (9.4 mg) as an amorphous powder. IR ($CHCl_3$): 1715 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, H-12), 1.09 (3H, s, H-13), 1.25 (3H, s, H-11), 1.46 (3H, d, *J*=6.4 Hz, H-10), 1.68 (1H, ddd, *J*=12.2, 4.0, 2.2 Hz, H-2_{eq}), 1.81 (1H, t, *J*=12.2 Hz, H-2_{ax}), 1.86 (1H, ddd, *J*=12.7, 4.9, 2.0 Hz, H-4_{eq}), 1.91 (1H, t, *J*=12.7 Hz, H-4_{ax}), 3.56, 3.57 (each 3H, s, 2×OMe), 5.47 (1H, dddd, *J*=12.7, 12.2, 4.9, 4.0 Hz, H-3), 5.69 (1H, quintet, *J*=6.4 Hz, H-9), 5.70 (1H, dd, *J*=14.7, 6.4 Hz, H-8), 6.18 (1H, d, *J*=14.7 Hz, H-7), 7.39 (6H, m), 7.53 (4H, m). HR-FAB-MS *m/z*: 699.2322 $[M+Na]^+$ (Calcd for $C_{33}H_{38}F_6NaO_8$: 699.2369).

***(S)*-MTPA Ester (1b) of Compound 1** Compound **1** (15.5 mg) was treated as above to give the di-(*S*)-MTPA ester (**1b**) (14.4 mg) as an amorphous powder. IR ($CHCl_3$): 1715 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, s, H-12), 1.15 (3H, s, H-13), 1.28 (3H, s, H-11), 1.41 (1H, d, *J*=6.4 Hz, H-10), 1.62 (1H, ddd, *J*=12.2, 4.4, 2.2 Hz, H-2_{eq}), 1.74 (1H, t, *J*=12.2 Hz, H-2_{ax}), 1.96 (1H, ddd, *J*=12.7, 4.9, 2.0 Hz, H-4_{eq}), 2.02 (1H, t, *J*=12.7 Hz, H-4_{ax}), 3.54, 3.56 (each 3H, s, 2×OMe), 5.49 (1H, dddd, *J*=12.7, 12.2, 4.9, 4.4 Hz, H-3), 5.66 (1H, dd, *J*=15.6, 7.1 Hz, H-9), 5.79 (1H, dd, *J*=6.8, 15.6 Hz, H-8), 6.29 (1H, d, *J*=15.6 Hz, H-7), 7.41 (6H, m), 7.53 (4H, m). HR-FAB-MS *m/z* : 699.2413 $[M+Na]^+$ (Calcd for $C_{33}H_{38}F_6NaO_8$: 699.2369).

Conversion of Euscapohlide (3) to Compound 2 Euscapholide (**3**) (85.5 mg) was dissolved in CH_2Cl_2 (5 ml) and 60% NaH in oil (400 mg) was added to the solution after washing with *n*-hexane. After standing for 10 min at room temperature, the reaction was quenched with EtOAc (35 ml) and the precipitates were removed by filtration. The filtrate was concentrated *in vacuo* to give a residue which was purified by silica gel (10 g) column chromatography with $CHCl_3$ as eluant to give compound **2** (70.2 mg), mp 88—89 °C, $[\alpha]_D^{25}$ −17.8° (*c*=1.12, MeOH). HR-EI-MS *m/z*: 156.0792 $[M]^+$ (Calcd for $C_8H_{12}O_3$: 156.0786). This compound was identified with natural **2** by mixed melting point determination and comparisons of the ^{1}H- and ^{13}C-NMR spectra.

Acknowledgement We thank the Cooperative Center of the University of Tokushima for permitting us to access to the NMR instrument.

References and Notes

1) Makino T., "Makino's New Illustrated Flora of Japan," Hokuryu-kan Publishing Co., Ltd., Tokyo, 1970, p. 364.
2) Ishikura N., *Bot. Mag*. (Tokyo), **84**, 1—7 (1971).
3) Konishi T., Ohtani Y., Kiyosawa S., *Chem. Pharm. Bull.*, **44**, 863—864 (1996).
4) Takeda Y., Okada Y., Masuda T., Hirata E., Takushi A., Otsuka H., *Phytochemistry*, **49**, 2565—2568 (1998).
5) Otsuka H., Takeda Ya., Yamasaki K., Takeda Yo., *Planta Med.*, **58**, 373—375 (1992).
6) Murai F., Tagawa M., Ohishi H., *Planta Med*., **58**, 112—113 (1992).
7) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc*., **113**, 4092—4096 (1991).
8) Murakami N., Wang W., Aoki M., Tstsui Y., Higuchi K., Aoki S., Kobayashi M., *Tetrahedron Lett.*, **38**, 5533—5536 (1997).
9) Drewes S. E., Horn M. M., Shaw R. S., *Phytochemistry*, **40**, 321—323 (1995).