Constituents of the Capsules of *Euscaphis japonica* (THUNB.) KANTIZ

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Two compounds related to amino acid amides, named euscamines A and B, have been isolated from the capsule of *Euscaphis japonica* (THUNB.) KANTIZ together with three known triterpenoic acids. Euscamines A and B were established as *N*-(3′-hydroxymethyl-2′,5′-dihydro-2′-furyl)-succinamic acid and N^5-(3′-hydroxymethyl-2′,5′-dihydro-2′-furyl)-N^5-methyl-glutamine methyl ester, respectively.

Key words *Euscaphis japonica*; Staphyleaceae; euscamine A; euscamine B; Ehrlich's reagent; amino acid amide

Euscaphis japonica (THUNB.) KANTIZ is a deciduous broad-leaved tree generally found in the fields and mountains of western Japan. In the autumn, the capsule is deeply red colored on both sides of the pericarps. In 1971, Ishikura[1] reported on the isolation and structures of three flavonol glycosides and an anthocyanin from the capsules. In addition, four triterpenoic acids were isolated from the ether extract of the pericarps by Takahashi *et al.*[2] In the course of our investigation with Ehrlich's reagent for detection of the constituents on thin-layer chromatography, we have found some amino acid amides with oxypinnatanine (**3**)[3] from *Hemerocallis fluva* var. *kwanso* (Liliaceae).[4] This paper deals with the isolation and establishment of the structures of two new compounds related to amino acid amides, named euscamine A (**1**) and B (**2**), along with three known triterpenoic acids from the capsules of *E. japonica*.

A methanol extract from the fresh capsules of *E. japonica* was suspended in water, and the suspended solution was filtered to separate the precipitate from the solution. The precipitate was subjected to column chromatography to produce three triterpenoic acids, oleanolic acid, ursolic acid and pomolic acid, which were identified by comparison with authentic samples and/or reference data.[2,5]

The filtrate was chromatographed on a Diaion HP-20 column to give a positive fraction for Ehrlich's reagent. The obtained fraction was subjected to column chromatographies of Sephadex G-10, Toyopearl HW-40s, and silica gel in order to give euscamine A (**1**) and B (**2**).

Euscamine A (**1**), a colorless amorphous solid, showed a molecular ion peak at *m/z* 215 in the FAB-MS, and the molecular formula was determined to be $C_9H_{13}NO_5$ by high-resolution MS (HR-MS). It showed absorption bands at 3450 (OH), 1686 (COOH) and 1625 (amide) cm^{-1} in the IR spectrum. The ^{1}H-NMR spectrum showed a coupling system similar to the protons of 3′-hydroxymethyl-2′-furyl moiety in oxypinnatanine, (**3**).[3]

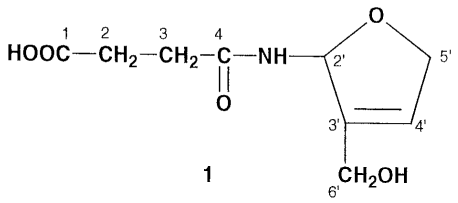
This was also supported by comparisons of the ^{13}C-NMR spectra between **1** and **3**. In addition, the four unequivalent protons of two sets of carbonyl methylene, shifted in the A_2B_2 system, appeared in the low-field at δ 2.05, 2.45, 2.59 and 2.61, respectively, suggesting a hydrogen bond between –COOH and –CO. The ^{13}C-NMR spectrum showed two carbonyl carbons at δ 180.6 and 182.6 with two signals of the ethylene group at δ 33.6 and 35.6, indicating the presence of a succinamic acid moiety.

Consequently, the structure of **1** was determined to be *N*-(3′-hydroxymethyl-2′,5′-dihydro-2′-furyl)-succinamic acid.

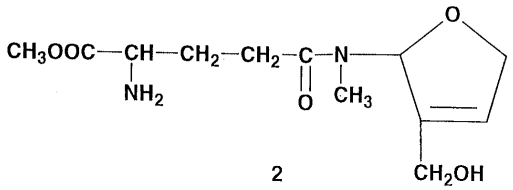
Table 1. ^{13}C-NMR Data of Compounds **1**, **2** and **3**

Carbon	**1** (D_2O)	**2** (CD_3OD)	**3** (D_2O)
1	182.6[a]	175.4[a] (174.9)[a]	175.9
2	33.6	63.4 (55.3)	55.2
3	35.6	29.1[b] (27.3)	36.0
4	180.6[a]	33.1[b] (31.9)	71.7
5		175.7[a] (178.5)[a]	178.6
2′	92.0	86.7	87.5
3′	140.2	140.5	138.6
4′	129.3	126.2	129.1
5′	79.2	74.7	76.6
6′	60.7	58.2	58.9
N-CH_3		34.4	
O-CH_3		52.4	

The chemical shifts of glutamine are given in parentheses.[6] *a, b*) In each vertical column may be interchanged.



1



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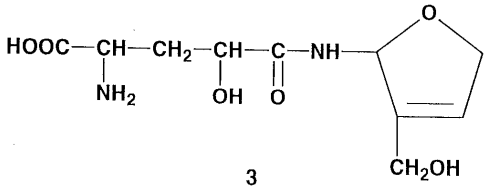

3

Chart 1

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Euscamine B (**2**), a colorless amorphous solid, gave a positive ninhydrin and Ehrlich's test, and it was presumed that its molecular formula is $C_{12}H_{20}N_2O_5$ from the molecular ion peaks, *m/z* 272.3365, in the HR-MS.

The IR spectrum revealed remarkable absorptions, which were attributed to hydroxy, ester and tertiary amide groups at 3400, 1736 and 1655 cm^{-1}, respectively. The ^{1}H-NMR spectrum also exhibited the proton signals of a 3′-hydroxymethyl-2′-furyl moiety, as shown in the spectra of **1** and **3**.

Furthermore, the spin–spin coupling systems between the methine and methylene groups, equivalently coupled with *J* values at 7.0 Hz, showed characteristic signals of the two triplets (t) and a double triplet (dt) at δ 3.23 (t), 2.26 (t) and 1.95 (dt), based on the alkane, –CH–CH_2–CH_2–, with two signals, N- and O-methyl, at δ 3.30 and 3.73, respectively. In the ^{13}C-NMR spectrum, **2** showed a striking resemblance to the signal pattern of compound **3** in the furyl moiety, and indicated five signals assigned to the respective carbons of a glutamine moiety with two methyl signals at δ 52.4 and 34.4, which individually formed methyl ester and tertiary amide functions. It was considered that the low-field shift of 2-C, about 8 ppm, compared with that of glutamine and **3**, resulted in the disappearance of a mutual electronic interaction between the two functional groups, vicinal –COOH and $-NH_2$, under the formation of a carboxylic acid methyl ester, $-COOCH_3$.

Therefore, compound **2** was concluded to be N^5-(3′-hydroxymethyl-2′,5′-dihydro-2′-furyl)-N^5-methyl-glutamine methyl ester.

The absolute configuration was characterized as *R* for the asymmetric 2′-C in **3** by direct methods from 1314 X-ray intensities.[7] However, it is difficult to determine the configuration of compounds **1** and **2** at 2′-C based on the results, as the stereochemistry of the interacting protons is not clearly understood in the dihydrofuryl ring. The stereochemistries of **1** and **2** are under investigation.

The new compounds, euscamine A and B, relate to novel N-substituted amino acids. In view of chemotaxonomy, it is interesting that oxypinnatanine had first been isolated from the seeds of the *Staphylea* plant, closely related to *Euscaphis* SIEB, *et* ZUCC.

Experimental

The melting points were measured with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were taken on a Horiba digital polarimeter. IR spectra were measured with a Shimadzu FTIR8100 spectrometer. NMR spectra were recorded on Varian XL-300 spectrometer with tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TPS) as internal standards. MS spectra were obtained with a JEOL JMS-SX 102AQQ mass spectrometer. Preparative high performance liquid chromatography (HPLC) was carried out on an LC-09 (Nihon Bunseki Kogyo). For column chromatography, Diaion HP-20 (Mitsubishi Kasei), silica gel (Merck), Toyopearl HW-40s (Toyo Soda) and Sephadex G-10 (Pharmacia) were used.

Extraction and Purification Fresh capsules (1.5 kg) of *Euscaphis japonica*, collected from Shiga prefecture, were immersed three times in methanol at room temperature for 3 d. Combined extracts were evaporated *in vacuo* to produce a gummy extract (63.3 g). The extract was suspended in water and filtered. The filtrate was subjected to Diaion HP-20 column chromatography with water to give three fractions (1—3). Fraction 2 (26.93 g) was repeatedly chromatographed on silica gel columns and eluted with isopropanol–water (15 : 1) and $CHCl_3$–MeOH–H_2O (6 : 4 : 0.5) to give five fractions (4—8). The fraction 5 (3.84 g) was rechromatographed on a Sephadex G-10 using water to give euscamine A (**1**) (100 mg). Rechromatography of the fraction 8 (669 mg) on cellulose with $CHCl_3$–MeOH–H_2O (7 : 3 : 1, upper layer) and Toyopearl HW-40s with water gave the crude fraction of euscamine B (168 mg). After methylation of this fraction with diazomethane, the reaction mixture was purified by column chromatography on a silica gel ($CHCl_3$–MeOH–H_2O, 8 : 2 : 0.2) to give **2** (21 mg).

Euscamine A (1) A white powder (mp 195—200 °C (dec.)), $[\alpha]_D^{23}$ −5.3° (*c* = 0.8, H_2O). IR (KBr, cm^{-1}): 3450 (OH), 1686 (COOH), 1680, 1625 (secondary amide). Negative FAB-MS *m/z*: 215 $(M)^-$. HR-MS *m/z*: 215.3612 $(M)^+$. Calcd for $C_9H_{13}NO_5$: 215.3626. ^{1}H-NMR (D_2O) δ: 2.05 (1H, ddd, *J* = 7.0, 10.0, 13.0 Hz, 3-H), 2.45 (1H, ddd, *J* = 6.5, 9.0, 13.0 Hz, 3-H), 2.59 (1H, ddd, *J* = 6.5, 10.0, 13.0 Hz, 2-H), 2.61 (1H, ddd, *J* = 7.0, 9.0, 13.0 Hz, 2-H), 4.15 (1H, dd, *J* = 2.0, 15.0 Hz, 6′-H), 4.22 (1H, dd, *J* = 2.0, 15.0 Hz, 6′-H), 4.59 (1H, dddd, *J* = 2.0, 4.0, 4.0, 14.0 Hz, 5′-H), 4.72 (1H, dddd, *J* = 2.0, 4.0, 4.0, 14.0 Hz, 5′-H), 6.07 (1H, ddd, *J* = 2.0, 4.0, 4.0 Hz, 2′-H), 6.12 (1H, ddd, *J* = 2.0, 4.0, 4.0 Hz, 4′-H). ^{13}C-NMR: Table 1.

Euscamine B (2) A white powder (mp 120—123 °C (dec.)) $[\alpha]_D^{23}$ −9.8° (*c* = 0.5, MeOH). IR (KBr, cm^{-1}): 3400 (OH), 1736 (ester), 1655 (tertiary amide). EI-MS *m/z*: 272 $(M)^+$, 256 $(M-NH_2)^+$, 213 $(M-COOCH_3)^+$, 129 $(C_6H_{11}NO_2)^+$, 98 $(C_5H_6O_2)^+$. HR-MS *m/z*: 272.3365. Calcd for $C_{12}H_{20}N_2O_5$: 272.3398. ^{1}H-NMR (CD_3OD) δ: 1.95 (2H, dt, *J* = 7.0, 7.0 Hz, 3-H), 2.26 (2H, t, *J* = 7.0 Hz, 4-H), 3.23 (1H, t, *J* = 7.0 Hz, 2-H), 3.30 (3H, s, NCH_3), 3.73 (3H, s, OCH_3), 4.14 (2H, m, 6′-H), 4.51 (1H, dddd, *J* = 2.0, 4.0, 4.0, 13.0 Hz, 5′-H), 4.65 (1H, dddd, *J* = 2.0, 4.0, 4.0, 13.0 Hz, 5′-H), 6.05 (1H, ddd, *J* = 1.5, 2.0, 3.0 Hz, 4′-H), 6.35 (1H, m, 2′-H). ^{13}C-NMR: Table 1.

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References

1) Ishikura N., *Bot. Mag. Tokyo*, **84**, 1 (1971).
2) Takahashi K., Kawaguchi S., Nishimura K., Kubota K., Tanabe Y., Takani M., *Chem. Pharm. Bull.*, **22**, 650 (1974).
3) Grove M. D., Weisleder D., Daxenbichler M. E., *Tetrahedron*, **29**, 2715 (1973).
4) Inoue T., Konishi T., Kiyosawa S., Fujiwara Y., *Chem. Pharm. Bull.*, **42**, 154 (1994); Inoue T., Iwagoe K., Konishi T., Kiyosawa S., Fujiwara Y., *ibid.*, **38**, 3187 (1990).
5) Cheng D. L., Cao X. P., *Phytochemistry*, **31**, 1317 (1992); Inada A., Kobayashi M., Murata H., Nakanishi T., *Chem. Pharm. Bull.*, **35**, 841 (1987); Konishi T., Shoji J., *ibid.*, **29**, 2807 (1981); Tori K., *Kagaku No Ryoiki Zokan*, **125**, 221—245, (1980); Stoll A., Renz J., Bark A., *Helv. Chim. Acta*, **33**, 1877 (1950).
6) Horsley W., Sternlicht H., Cohen J. S., *J. Am. Chem. Soc.*, **92**, 680 (1970).
7) Kruger G. J., Plessis L. M., Grobbelaar N., *J. South Afr. Chem. Inst.*, **29**, 24 (1976).