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EUSCAPHOLIDE AND ITS GLUCOSIDE FROM LEAVES OF *EUSCAPHIS JAPONICA*

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Key Word Index—*Euscaphis japonica*; Staphyleaceae; tetraketide; euscapholide; euscapholide glucoside.

Abstract—A new tetraketide, euscapholide and its glucoside were isolated from the leaves of *Euscaphis japonica*. The structures were elucidated by spectroscopic and chemical analyses. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Flavonol glycosides, anthocyanins and compounds positive to Ehrlich reagent have been isolated from the capsule of *Euscaphis japonica* (Thunb.) Kanitz [1, 2]. In the course of our studies on the constituents of subtropical plants, we examined the constituents of the leaves of the title plant harvested in Okinawa Prefecture, Japan and isolated a tetraketide, euscapholide (**1**), and its glucoside (**3**). This paper describes the isolation and structural elucidation of the new compounds.

RESULTS AND DISCUSSION

Euscapholide (**1**) and its glucoside (**3**) were isolated from the *n*-BuOH-soluble fraction of the methanolic extract obtained from the leaves of *E. japonica*.

Euscapholide (**1**) was found to have the molecular formula $C_8H_{12}O_3$ by HR-EI-mass spectrometry. The IR spectrum showed the presence of a hydroxyl group (3420 cm^{-1}) and a δ-lactone (1700 cm^{-1}). The ^1H NMR spectrum showed the presence of a secondary methyl group [δ 1.26 (H_h)] on one of the carbon atoms (C-8) having an oxygen atom, two methylene groups [δ 1.77 (H_g), 2.03 (H_f) and 2.42 (H_{e2})], a methine on C-8 [δ 4.08 (H_d)], a methine on the carbon having an acyloxy group [δ 4.65 (H_c)] and a conjugated disubstituted double bond with *Z*-geometry [δ 6.02 (H_b) and 6.92 (H_a). The ^{13}C NMR spectrum (Table 1) contained the signals for above mentioned functional groups. Acetylation of **1** with acetic anhydride and pyridine gave the monoacetate **2** [δ 2.05 (3H, *s*) and 5.13 (1H, *m*), H_d]. The planar structure was deduced as **1** by analysis of the ^1H-^1H COSY spectrum. Namely, the cross peaks were followed as $H_b \rightarrow H_a \rightarrow H_e \rightarrow H_c \rightarrow H_f, H_g \rightarrow H_d \rightarrow H_h$. The absolute stereochemistry at C-6 was elucidated as *R* from the fact that **1** showed the opposite Cotton effect [$\Delta\epsilon_{255} + 3.0$] to that of (*S*)-5,6-dihydro-6-hydroxymethyl-2H-pyran-2-one(**7**) [3]. Another asymmetric centre at C-8 was determined to have *S*-configuration by a modified Mosher's method (Fig. 1) [4]. Thus, the structure of euscapholide was elucidated as **1**.

Euscapholide glucoside (**3**) was assigned the molecular formula $C_{14}H_{22}O_8$ by negative ion HR-FAB-mass spectrometry. The ^1H and ^{13}C NMR spectra were very similar to those of euscapholide (**1**), except for the appearance of the signals due to β-glucopyranosyl moiety (Table 1 and Experimental). Thus, compound **3** was presumed to be β-glucopyranoside of **1**. Compound **3** gave the tetraacetate **4** [δ_H 2.00, 2.02, 2.03 and 2.09 (each 3H, *s*)] on acetylation with acetic anhydride and pyridine and euscapholide (**1**), $[\alpha]_D + 116.4°$ (MeOH), upon enzy-

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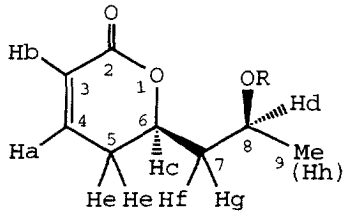
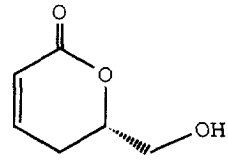


1 R=H
2 R=COMe
3 R=Glc
4 R=Glc(COMe)$_4$
5 R=(*R*)-MTPA
6 R=(*S*)-MTPA



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matic hydrolysis. Thus, the structure of euscapholide glucoside was elucidated as **3**.

EXPERIMENTAL

General

NMR: ^{1}H (400 MHz) and ^{13}C (100 MHz) with TMS as int. standard; FABMS: PEG-400 as matrix; CC: Dianion HP-20 (Mitsubishi Kasei) and silica gel 60 (230–400 mesh, Merck); TLC: silica gel 60 F_{254} (0.25 mm and 0.5 mm in thickness); prep. HPLC: Cosmosil 10 C_{18}, 20 × 250 mm; solvent: MeOH–H_2O 1:4, 6 ml min^{-1}, detection 210 nm.

Plant material

The plant material was collected at Kunigamison, Okinawa Prefecture, Japan in July 1994 and identified as *Euscaphis japonica* (Thunb.) Kanitz by one of the authors (A. T.). A voucher specimen (9407-4) was deposited in the Herbarium of Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine.

Table 1. ^{13}C NMR data for euscapholide (**1**) and its glucoside (**3**). (CD_3OD, 100 MHz)

C	1	3
2	166.9 (164.2)*	167.1
3	121.3 (121.2)	121.3
4	148.3 (145.4)	148.3
5	30.2 (29.3)	30.4
6	77.5 (76.8)	77.1
7	44.7 (43.5)	42.6
8	64.8 (65.0)	74.6
9	23.6 (23.6)	22.2
1′		104.2
2′		75.2
3′		78.2
4′		71.6
5′		77.9
6′		62.8

* Chemical shifts in parentheses are for chloroform-d.

Isolation

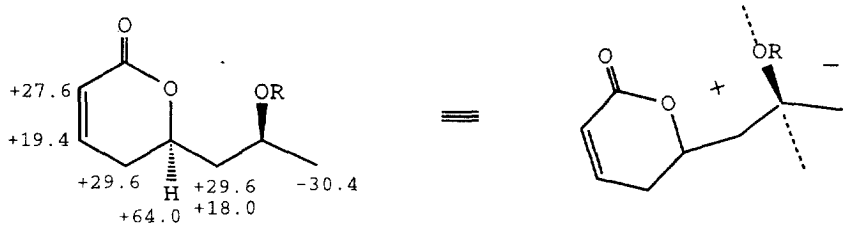
Dried leaves (2.9 kg) of *E. japonica* were extracted (×2) with MeOH (45 l) at room temp for three weeks. The combined methanolic extract was concentrated *in vacuo*. The residue was dissolved in 90% aq. MeOH (2.1 l) and the solution washed with *n*-hexane (1 l × 3). The aq. MeOH layer was concentrated *in vacuo* and the residue was suspended in H_2O (1 l). The suspension was extracted with EtOAc (1 l × 3) and *n*-BuOH (1 l × 3), successively. The *n*-BuOH extract was concentrated *in vacuo* to give a residue (77 g) which was chromatographed on Dianion HP-20 (a highly-porous synthetic resin [Φ = 78 mm, L = 536 mm] with stepwise increases 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)]. Frs. of 500 ml being collected.

Frs 14–17 were combined and concentrated *in vacuo* to give a residue (10.7 g) which was chromatographed over silica gel ($CHCl_3$–MeOH with increasing amounts of MeOH). The fractions which contained a spot with R_f 0.35 on TLC ($CHCl_3$–MeOH–H_2O 15:6:1) were collected and the residue (418.7 mg) was purified by prep. HPLC to give euscapholide glucoside (**3**) (128.4 mg).

Frs 18–23 were combined and concentrated *in vacuo* to give a residue (12.2 g), which was repeatedly chromatographed over silica gel ($CHCl_3$–MeOH, Et_2O and finally $CHCl_3$–MeOH) to give euscapholide (**1**) (1.21 g), R_f 0.27 (Et_2O) and 0.29 ($CHCl_3$–Me_2CO 4:1).

*Euscapholide (**1**)*

Colourless oil, $[\alpha]_D^{30}$ +115.5° (MeOH; *c* 1.52). IR ν_{max} ($CHCl_3$) cm^{-1}: 3420, 1700, 1230, 1200, 1050, 1025; UV λ_{max} (MeOH) nm (log ϵ): 209 (3.89); ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, *d*, *J* = 6.3 Hz, 9-H_3), 1.77 (1H, *ddd*, *J* = 14.6, 5.4 and 4.4 Hz, 7-H_1), 2.03 (1H, *dt*, *J* = 14.6 and 7.8 Hz, 7-H_1), 2.42 (2H, *m*, 5-H_2), 4.08 (1H, *m*, 8-H), 4.65 (1H, *m*, 6-H), 6.02 (1H, *dt*, *J* = 10.1 and 1.7 Hz, 3-H) and 6.92 (1H, *ddd*, *J* = 10.1, 5.4 and 3.4 Hz, 4-H); ^{13}C NMR:



(**5**) R=(*R*)-MTPA
(**6**) R=(*S*)-MTPA

△δ values in Hz
(δ*S*-δ*R*, 400MHz)

Fig. 1.

Table 1; CD (MeOH, *c* 1.41×10^{-4} M): Δε (nm) + 3.0 (255); HR-EIMS *m/z* 156.0789 $[M]^+$ ($C_8H_{12}O_3$ requires 156.0786).

Euspholide glucoside (**3**)

Amorphous powder, $[\alpha]_D$ + 36.4° (MeOH, *c* 2.47). UV ν_{max} (MeOH) nm (log ε): 209 (3.98); IR ν_{max} (dry film) cm^{-1}: 3387, 1703; ^1H NMR (CD_3OD): δ 1.33 (3H, *d*, *J* = 5.9 Hz, 9-H_3), 1.81 (1H, *dt*, *J* = 14.2 and 5.9 Hz, 7-H_1), 2.12 (1H, *dt*, *J* = 14.2 and 7.1 Hz, 7-H_1), 2.39 (1H, *ddt*, *J* = 18.6, 11.2 and 2.7 Hz, 5-H_1), 2.57 (1H, *br.dt*, *J* = 18.6 and 4.9 Hz, 5-H_1), 3.14 (1H, *dd*, *J* = 8.3 and 7.8 Hz, H-2'), 3.66 (1H, *dd*, *J* = 11.5 and 4.9 Hz, 6'-H_1), 3.85 (1H, *br.d*, *J* = 11.5 Hz, 6'-H_1), 4.03 (1H, *m*, 8-H), 4.32 (1H, *d*, *J* = 7.8 Hz, 1'-H), 4.74 (1H, *m*, 6-H), 5.97 (1H, *dd*, *J* = 9.8 and 1.5 Hz, 3-H), 7.04 (1H, *ddd*, *J* = 9.8, 5.9 and 2.4 Hz, 4-H); ^{13}C NMR: Table 1; HR-FABMS (negative) *m/z*: 317.1229 $[M-H]^-$ ($C_{14}H_{21}O_8$ requires 317.1237).

Euscapholide monoacetate (**2**)

Euscapholide (**1**) (51.5 mg) was dissolved in a mixture of Ac_2O (0.5 ml) and pyridine (0.5 ml) and the mixture was left for 24 hr at room temp. Excess MeOH was added and the solution was concentrated *in vacuo*. The residue was purified by silica gel CC ($CHCl_3$–Me_2CO) to give the monoacetate **3** (55.9 mg) as a colourless oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 1710, 1630, 1370, 1220, 1040; ^1H NMR ($CDCl_3$): δ 1.31 (3H, *d*, *J* = 6.4 Hz, 9-H_3), 1.85 (1H, *ddd*, *J* = 14.6, 6.3 and 4.9 Hz, 7-H_1), 2.05 (3H, *s*, OAc), 2.19 (1H, *ddd*, *J* = 14.6, 8.1 and 6.6 Hz, 7-H_1), 2.33 (1H, *ddt*, *J* = 18.8, 11.7 and 2.4 Hz, 5-H_1), 2.47 (1H, *dt*, *J* = 18.8 and 4.7 Hz, 5-H_1), 4.53 (1H, *m*, 6-H), 5.13 (1H, *m*, 8-H), 6.02 (1H, *dd*, *J* = 9.8 and 1.5 Hz, 3-H), 6.90 (1H, *ddd*, *J* = 9.8, 5.9 and 2.4 Hz, 4-H); HR-EIMS *m/z* 198.0866 $[M]^-$ (Calculated for $C_{10}H_{14}O_4$:198.0892).

(R)-MTPA ester (**5**) *from euscapholide* (**1**)

Euscapholide (**1**) (22.3 mg) was dissolved in CH_2Cl_2 (1 ml). (*R*)-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) (146.7 mg), dimethylaminopyridine (42.3 mg) and dicyclohexylcarbodiimide (DCC) (126.0 mg) were added successively to the solution and the mixture was stirred for 5 min under ice cooling. The mixture was diluted with EtOAc (15 ml) and the solution was washed with 5% HCl, satd $NaHCO_3$ and satd NaCl successively. The dried EtOAc extract was concentrated *in vacuo* to give a residue which was purified by prep. TLC ($CHCl_3$–Me_2CO 9:1) to give the (*R*)-MTPA ester **5** (40.3 mg). ^1H NMR ($CDCl_3$):δ 1.44 (3H, *d*, *J* = 6.4 Hz, 9-H_3), 1.88 (1H, *ddd*, *J* = 14.2, 6.4 and 4.9 Hz, 7-H_1), 2.19 (3H, *m*, 4-H_2, 7-H_1), 3.56 (3H, *d*, *J* = 1.0 Hz, OMe), 4.30 (1H, *m*, 6-H), 5.34 (1H, *m*, 8-H), 5.97 (1H, *br.d*, *J* = 9.8 Hz, 3-H), 6.77 (1H, *m*, 4-H), 7.38 (3H, *m*), 7.52 (2H, *m*); HR-FABMS (negative): *m/z* 371.1087 (Calculated for $C_{18}H_{18}O_5F_3$: 371.1107).

(S)-MTPA ester (**6**) *from euscapholide* (**1**)

Euscapholide (**1**) (21.8 mg) was esterified with (*S*)-MTPA as described above to give the (*S*)-MTPA ester **6** (37.8 mg). ^1H NMR ($CDCl_3$): δ 1.36 (3H, *d*, *J* = 6.3 Hz, 9-H_3), 1.93 (1H, *dt*, *J* = 14.7 and 5.9 Hz, 7-H_1), 2.26 (1H, *dt*, *J* = 14.7 and 7.1 Ht, 7-H_1), 2.32 (2H, *m*, 5-H_2), 3.51 (3H, *d*, *J* = 1.0 Hz, OMe), 4.46 (1H, *m*, 6-H), 5.36 (1H, *m*, 8-H), 6.02 (1H, *br.d*, *J* = 9.8 Hz, 3-H), 6.84 (1H, *m*, 4-H), 7.41 (3H, *m*), 7.51 (2H, *m*); HR-FABMS (negative): *m/z* 371.1136 $[M-H]^-$ (Calculated for $C_{18}H_{18}O_5F_3$: 371.1107).

Euscapholide glucoside tetraacetate (**4**)

Compound **3** (5.7 mg) was acetylated as above to give the tetraacetate **4** (7.7 mg) as an amorphous powder. IR ν_{max} ($CHCl_3$) cm^{-1}: 1740, 1370, 1220, 1040; ^1H NMR ($CDCl_3$): δ 1.32 (3H, *d*, *J* = 6.4 Hz, 9-H_3), 1.73 (1H, *ddd*, *J* = 14.2, 6.8 and 5.4 Hz, 7-H_1), 2.00, 2.02, 2.03, 2.09 (each 3H, *s*, 4 × OAc), 2.37 (2H, *m*, 5-H_2), 3.71 (1H, *m*, 5'-H), 4.05 (1H, *m*, 8-H), 4.14 (1H, *dd*, *J* = 12.2 and 2.5 Hz, 6'-H_1), 4.25 (1H, *dd*, *J* = 12.2 and 5.4 Hz, 6'-H_1), 4.49 (1H, *m*, 6-H), 4.58 (1H, *d*, *J* = 7.8 Hz, 1'-H), 4.95 (1H, *dd*, *J* = 9.8 and 7.8 Hz, 2'-H), 5.05 (1H, *t*, *J* = 9.8 Hz, 4'-H), 5.19 (1H, *t*, *J* = 9.8 Hz, 3'-H), 6.04 (1H, *dd*, *J* = 9.8 and 2.0 Hz, 3-H), 6.89 (1H,

m, 4-H); HR-FABMS (negative): *m/z* 485.1657 [M–H]$^-$ (Calculated for $C_{22}H_{29}O_{12}$: 485.1659).

Enzymatic hydrolysis of euscapholide glucoside (3)

Compound **3** (20.1 mg) was dissolved in EtOH–H_2O (1:9, 10 ml) and β-glucosidase (ex almond) (purchased from Toyobo Co. Ltd., Osaka, Japan) (17.6 mg) was added. The mixture was incubated at 37° for 24 hr. The solution was extracted with EtOAc (10 ml × 3). The EtOAc extracts were washed with sat. NaCl aq. solution, dried and evaporated *in vacuo* to give the aglucone (7.2 mg) as a colourless oil, $[\alpha]_D^{21}$ +116.4° (MeOH, *c* 0.36), HR-EIMS: *m/z* 156.0736 [M]$^+$ (Calculated for $C_8H_{12}O_3$: 156.0786). The compound was identified with the authentic sample of euscapholide (**1**) by comparisons of IR and ^1H and ^{13}C NMR spectra.

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