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D_S-*ERYTHRO*-2-AMINO-4-ETHOXY-3-HYDROXYBUTANOIC ACID FROM THE FRUITING BODIES OF THE EDIBLE MUSHROOM, *LYOPHYLLUM ULMARIUM*

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Key Word Index—*Lyophyllum ulmarium*; Tricholomataceae, mushroom; amino acid; D_S-*erythro*-2-amino-4-ethoxy-3-hydroxybutanoic acid.

Abstract—A new β-hydroxy amino acid isolated from the fruiting bodies of *Lyophyllum ulmarium* was identified as D_S-*erythro*-2-amino-4-ethoxy-3-hydroxybutanoic acid by chemical degradation and spectroscopic analyses.

In the previous paper [1], we reported the occurrence of a unique amino acid, 2-amino-3,4-dihydroxybutanoic acid (**2**) having the D_S-*erythro* configuration, in the edible mushroom, *Lyophyllum ulmarium*. The mushroom accumulated **2** at relatively high concentrations (*ca* 1 mg/g fr. wt of the fruiting bodies of the edible stage) together with small amounts of an unknown ninhydrin-positive compound (**1**), which was detected on the chromatogram of the amino acid analyser (system I [2]) as a single peak emerging between the elution positions of aspartic acid and threonine. The compound **1** isolated by ion-exchange chromatography and prep. PC was found to be a neutral α-amino acid [1] having M_r 163 (CI/MS *m/z*, 164 $[M+1]^+$). The IR spectrum (1120 cm^{-1}) showed the presence of the ether linkage in the molecule. The ^1H and ^{13}C NMR spectra were closely similar to those of **2** [1] except for the extraneous signal corresponding to the isolated ethyl group, suggesting that **1** was the 3- or 4-ethoxy derivative of **2**. Formation of **2**, 2-amino-4-hydroxybutanoic acid (**3**) and 2-aminobutanoic acid (**4**) by the reduction with HI–red P [3] also supported the above conclusion. The 4-ethoxy structure of **1**, that is, 2-amino-4-ethoxy-3-hydroxybutanoic acid, was confirmed by GC/MS of the TMSi derivative of the isolated **1**, which gave the $[M]^+$ ion at *m/z* 379 (tri-TMSi-**1**) and the decisive fragmentation pattern.

The configuration of the isolated **1** was determined using the HI–red P reduction products **2** and **3**. The product **2** showed the same chromatographic behaviour as those of D_S- and L_S-*erythro*-**2** reported previously [1] on the amino acid analyser (system I) and TLC (solvent 3), indicating that the isolated compound was the *erythro* form. When the optical property of product **3** was examined by the chromatographic method of Manning and Moore [4], 98% of the L_S-leucyl peptide of the product **3** was recovered as L_S-leucyl-D_S-**3**. Small amounts of L_S-**3** observed may be due to the racemization at C-2 during the reduction process. On the basis of above evidence, the compound isolated from the fruiting bodies of *Lyophyllum ulmarium* was identified as D_S-*erythro*-2-amino-4-ethoxy-3-hydroxybutanoic acid, which is a hitherto unknown natural product.

EXPERIMENTAL

General methods. Amino acid analyses were performed by the automatic amino acid analyser equipped with the resin-buffer system I [2, 5] and GC/MS of the TMSi derivatives was done by the same method described previously [1]. PC and TLC (cellulose) were developed with the following solvent systems: PhOH–H_2O (4:1, w/w, solvent 1), *n*-BuOH–HOAc–H_2O (4:1:1, solvent 2), *n*-BuOH–Me_2CO–conc NH_4OH–H_2O (50:6.25:6.25:37.5, solvent 3 [6]).

Mushroom. The freshly harvested fruiting bodies of *Lyophyllum ulmarium* (edible stage, cap diameter *ca* 1.5–2.0 cm; one fruiting body, *ca* 2.5 g fr. wt) were supplied from a commercial grower.

Isolation of **1**. The fruiting bodies of the mushroom (1 kg fr. wt) were homogenized and extracted with EtOH (3 l.). The extract was applied to a column of Amberlite IR-120B (H^+, 800 ml) to obtain the amino acid fraction. The amino acids were fractionated using columns of Dowex 1 × 4 (AcO^-, 150 ml) and Dowex 50WX8 (H^+, 150 ml) according to the same manner as described previously [2]. Compound **1** was recovered in the fraction containing D_S-*erythro*-2-amino-3,4-dihydroxybutanoic acid, threonine and serine [1]. It was further purified by prep PC with solvent 2 and was isolated as a colourless residue (*ca* 40 mg) after the evaporation of the aqueous solution (hardly yielding a

$R^1-CH_2-CH(R^2)-CH(NH_2)COOH$

1 $R^1 = OEt$ $R^2 = OH$
2 $R^1 = OH$ $R^2 = OH$
3 $R^1 = OH$ $R^2 = H$
4 $R^1 = H$ $R^2 = H$

crystalline form); PC: R_fs 0.75 (solvent 1); 0.43 (solvent 2); RR_ss on the amino acid analyser (system I); 0.86 and 1.19 (relative to serine and aspartic acid, respectively); CI/MS (isobutane, probe) 200 eV, *m/z* (rel. int.): 164 $[M+1]^+$ (16), 116 $[M-47]^+$ (100); EI/MS (GC) of TMSi-1: 20 eV, *m/z* (rel. int.): 379 $[M]^+$ (0.6), 364 $[M-15]^+$ (9), 320 $[TMSiOCHCH(NHTMSi)COOTMSi]^+$ (3), 291 $[M-OTMSi]^+$ (68), 262 $[M-COOTMSi]^+$ (29), 219 $[TMSiNH{=}CHCOOTMSi+1]^+$ (100), 218 $[TMSiNH{=}CHCOOTMSi]^+$ (93), 161 $[CH_3CH_2OCH_2CHOTMSi]^+$ (21); ^{13}C NMR (25.05 MHz, D_2O, TMS): δ14.3 (*q*, C-2'), 58.0 (*d*, C-2), 67.4 (*t*, C-1'), 67.9 (*d*, C-3), 71.1 (*t*, C-4), 171.4 (*s*, C-1); ^1H NMR (100 MHz, D_2O, TMS): δ1.20 (3H, *t*, J = 7 Hz, H-2'), 3.56 (2H, *q*, J = 7 Hz, H-1'), 3.67 (2H, partial overlap with H-1', H-4), 3.93 (1H, *d*, J = 4 Hz, H-2), 4.28 (1H, *dd*, J = 4, 4 Hz, H-3); IR ν_{max}^{KBr} cm^{-1}: 1050 (*m*), 1120 (*s*), 1380 (*s*), 1570 (*s*), 1630 (*s*), 1660 (*s*), 3400 (*s*).

Chemical degradation. Reduction with HI–red P was carried out according to ref. [3]. The reduction products were separated by prep. PC with solvent 2 and determined by GC/MS and the amino acid analyses (system I). The yields of the products were as follows: **2**:**3**:**4** = 0.5:0.3:0.1 [molar ratios to original **1** (1.0)].

Determination of the stereochemical properties of the products **2** *and* **3**. The stereoisomers of the product **2** were determined by the amino acid analyser (system I); RR_ss (relative to L_s-serine): the product **2**; 0.71, synthetic D_s- and L_s-*erythro*-**2**; 0.71, synthetic D_s- and L_s-*threo*-**2**; 0.69. The optical properties of the products **3** was examined by the chromatographic method of ref. [4] using the analytical system described previously [5]; RR_ss (relative to L_s-leucine): L_s-leucyl peptide of the product **3**, 1.13; synthetic L_s-leucyl-D_s-**3**, 1.13; synthetic L_s-leucyl-L_s-**3**, 1.61.

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ACETOGENINS FROM THE AQUATIC PLANT *ELODEA CANADENSIS*

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Key Word Index—*Elodea canadensis*; Hydrocharitaceae; acetogenins.

Abstract—13-(2-furyl)-Tridec-12*E*-en-1-yne and (7*S*)-hydroxyhexadeca-8*E*,10*Z*,13*Z*-trienoic acid have been isolated from *Elodea canadensis* in addition to the already known 13-(2-furyl)-tridec-1-yne, hexadec-11*Z*-enoic, hexadeca-7*Z*,10*Z*,13*Z*-trienoic and (10*R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoic acids.

INTRODUCTION

In connection with a systematic study [1] of the aquatic species distributed in Italy, we recently described the isolation of a novel tetraterpene alcohol (**1**) [2] from *Elodea canadensis*. In this paper we now report the chemical investigation of other metabolites from the same source.

RESULTS AND DISCUSSION

The plants, collected in the Botanical Garden of the University of Naples, were air-dried and extracted with cold ether to afford a residue that, after treatment with charcoal, was separated into acidic and neutral fractions by conventional procedures.

The neutral fraction was chromatographed on neutral alumina to give three groups of fractions. The first group, eluted with petrol, consisted of two compounds (**2** and **3**) which were separated by prep. TLC. Compound **2** showed spectroscopic feature identical with those reported for the already known avocadynofuran [3]. Compound **3** had a molecular peak at *m/z* 244 and absorptions in the IR spectrum at 3280, 2100, 1590, 1500, 1260, 1145 and 1050 cm^{-1}. The ^1H NMR spectrum showed three furanic