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SHORT REPORTS

TWO γ-GLUTAMYLPEPTIDES OF ACETYLENIC AMINO ACIDS IN *TRICHOLOMOPSIS RUTILANS**

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Key Word Index—*Tricholomopsis rutilans*; Tricholomataceae; Basidiomycetes; γ-L-glutamyl-L-2-aminohex-4-ynoic acids; γ-L-glutamyl-L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid.

Abstract—Two γ-glutamylpeptides, γ-L-glutamyl-L-2-aminohex-4-ynoic acid and γ-L-glutamyl-L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid, were isolated from fruit bodies of *Tricholomopsis rutilans*. Identifications were based on elementary analysis, hydrolysis, IR spectra, optical rotation and analysis of dansyl derivatives.

Previously we reported the characterization of L-2-aminohex-4-ynoic acid [1], L-2-amino-3-hydroxyhex-4-ynoic acid (*threo* and *erythro*) [2, 3] and L-3(4-carboxy-3-furyl)alanine [4, 5] from fruit bodies of *Tricholomopsis rutilans* (Fr.) Sing. Column chromatography on Dowex 1 revealed that the fungus also contains two unknown ninhydrin-positive substances (A and B).

Both compounds A and B gave the normal violet coloration with ninhydrin. Mild hydrolysis of A with N H_2SO_4 produced L-2-aminohex-4-ynoic acid and L-glutamic acid (molar ratio, 1:1.16), which were separated on a Dowex 1-column and identified by TLC, IR, elementary analysis and optical rotation. Hydrolysis of dansyl A yielded dansylglutamic acid. Compound B was hydrolysed with N H_2SO_4, to give L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid and L-glutamic acid (molar ratio, 1:0.85). Hydrolysis products and its dansyl derivative were separated and identified as before. The results of elementary analysis supported the structures, γ-L-glutamyl-L-2-aminohex-4-ynoic acid and γ-L-glutamyl-L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid.

It is worth noting that the *threo*-isomer could not be detected in the hydrolysates of the relevant fractions of compound B, even before the crystals of B separated. The *threo*-isomer, if present, would be expected to be eluted from the Dowex 1-column in the same fractions as the *erythro*-form. The free amino acids of *threo*- and *erythro*-form occur in this fungus in the ratio 2:3 [3].

EXPERIMENTAL

Mps, determined in capillary tubes, were uncorr. and IR was measured in KBr discs. Evaporation of solvents was carried out with a rotary evaporator *in vacuo* below 40°. H_2O of crystallization was determined by drying the crystals at 105°, 2.5 mm Hg over P_2O_5.

Chromatography. Solvents used were (a) *n*-BuOH–HOAc–H_2O (63:10:27), (b) PhOH–H_2O (25:9) and (c) *n*-BuOH–MeCOEt–NH_4OH (28%)–H_2O (15:9:4:2). Avicel SF cellulose plates were used for TLC.

Isolation of peptides. The fruit bodies of *Tricholomopsis rutilans* (Fr.) Sing. (1.28 kg), collected and stored deep-frozen, were extracted with 80% EtOH (13 l.) and filtered. The filtrate was passed through a column of Amberlite IR-120 (H^+) (150 ml). After the resin was washed throughly with 80% EtOH and H_2O, the amino acids were eluted with 2 M NH_4OH (1.5 l.). The ammonia was evaporated *in vacuo* and the concentrate applied to a column of Dowex 1 × 4 ($MeCOO^-$) (4 × 80 cm). The column was eluted with 0.7 M HOAc (5.1) and then with 2 M HOAc. Peptides A and B were successively displaced with 2 M HOAc. Relevant fractions were combined, concd, decolorized with activated charcoal and treated with EtOH and Me_2CO, to give crystals, 139 mg A and 361 mg B, respectively. A: γ-L-Glutamyl-L-2-aminohex-4-ynoic acid, mp 141–2° (decomp.). Found: C, 47.79; H, 6.20; N, 10.13. $C_{11}H_{16}N_2O_5 . H_2O$ requires: C, 48.17; H, 6.62; N, 10.21%. H_2O of crystallization: 0.92 mol/mol. $[\alpha]_D^{22}$ +31.4° (H_2O; *c* 2.27). B: γ-L-Glutamyl-L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid, mp 137–8° (decomp.). Found: C, 44.53; H, 6.07; N, 9.34. $C_{11}H_{16}N_2O_6 . H_2O$ requires; C, 45.52; H, 6.25; N, 9.65%. H_2O of crystallization; 0.89 mol/mol. $[\alpha]_D^{22}$ +51.0° (H_2O; *c* 1.92).

Hydrolysis. A soln of peptide A (85 mg) in N H_2SO_4 (4 ml) was heated for 3.5 hr at 100° and the soln was treated with Amberlite IR-120 (H^+) (5 ml). The ammonia eluate was evapd to dryness, dissolved in 0.5 M HOAc (1 ml), and the mixture applied to a column of Dowex 1 × 4 ($MeCOO^-$, 1.2 × 40 cm), followed by fractionation with 0.5 M HOAc (Fr. No. 1–30, each 7 ml) and 1 M HOAc (Fr. No. 31–). Fractions of L-2-aminohex-4-ynoic acid (Fr. No. 5–8), L-glutamic acid (Fr. No. 32–37) and unchanged peptide A (Fr. No. 61–76) were combined, separately. The molar ratio of the hydrolysis products, determined colorimetrically, was 1:1.16. Evaporation of the solvent gave L-2-aminohex-4-ynoic acid (24.5 mg), L-glutamic acid (23.4 mg) and peptide A (13.5 mg), respectively. L-2-aminohex-4-ynoic acid. Mp >202° (decomp.) (lit. [1], >199° (decomp.)). Found: C, 56.64; H, 7.22; N, 10.81. Calc. for $C_6H_9NO_2$: C, 56.68; H, 7.14; N, 11.02%. $[\alpha]_D^{22}$ −44.2° (H_2O; *c* 0.77) (lit. [1], $[\alpha]_D^{28}$ −54.4°. (H_2O; *c* = 1). L-glutamic acid. mp >200° (decomp.) (lit. [6], 211–3° (decomp.). Found: C, 40.62; H, 6.29; N, 9.55. Calc. for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52%. $[\alpha]_D^{22}$ +8.9° (H_2O; *c* = 1.17) (lit. [6], $[\alpha]_D^{25}$ +12.0° (H_2O; *c* = 2)). Peptide B (41 mg) was hydrolysed and the products were separated under the same conditions as peptide A. The crystals of L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid (8.45 mg) and L-glutamic acid (9.6 mg) were obtained

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and the molar ratio was 1:0.85. L-*erythro*-2-amino-3-hydroxy-hex-4-ynoic acid. Mp >135° (decomp.) (lit. [3], 140–54° (decomp.)). Found: C, 50.08; H, 6.38; N, 9.57. Calc. for $C_6H_9NO_3$ C, 50.35; H, 6.34; N, 9.79%, $[\alpha]_D^{22}$ −20.0 (H_2O; c = 1.61) (lit. [3], $[\alpha]_D^{28}$ −24° (H_2O; c = 1.151) L-glutamic acid. Mp >197° (decomp.) Found: C, 40.54; H, 6.21; N, 9.49%. $[\alpha]_D^{22}$ +10.3° (H_2O; c = 1.19).

Dansylation [7, 8]. Samples were dissolved in 0.5 M $NaHCO_3$ to give a final concn of 10 mM of each. To 0.1 ml of this soln, 0.1 ml dansyl chloride soln (5.5 mg in 2 ml cold Me_2CO) was added. The tubes were covered with Parafilm and the reaction mixture was allowed to stand for 1 hr at 37°. The reaction was stopped by adding 20 μl HCO_2H (85%) and the mixture diluted ×20 with H_2O. In the case of the peptides half vol. (0.1 ml) of the reaction mixture was hydrolysed in N H_2SO_4 (1 ml) for 4 hr at 100°. To an aliquot (0.1 ml), EtOAc (0.2 ml) was added and shaken. The upper layer was analysed by TLC on polyamide (5 × 5 cm) with the solvents, 1.5% HCO_2H in H_2O, C_6H_6–HOAc (9:1) and EtOAc–HOAc–MeOH (20:1:1). The chromatogram, dried by air, was examined under UV light (365 and 254 nm).

Chromatographic data. R_{Ala}-values of γ-L-glutamyl-L-2-amino-hex-4-ynoic acid on cellulose using solvents (a) and (b) were 1.58 and 1.1 respectively and those of its hydroxylated form 0.9 and 0.73, respectively.

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A NEW AMIDE FROM *PIPER OFFICINARUM*

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Key Word Index—*Piper officinarum*; Piperaceae; *N-iso*butyl eicosa-*trans*-2-*trans*-4-*cis*-8-trienamide.

During investigations of the constituents of the fruits of *Piper officinarum*, we have reported previously the presence of 3 new compounds namely, Me piperate [1], *N-iso*butyl-trideca-13-(3,4, methylenedioxyphenyl)-2,14, 12-trienamide [2] and *N-iso*butyl docosa-*trans*-2-*trans*-4-*cis*-10-trienamide [3] (filfiline). We now wish to report the structure of another new *iso*butylamide from the same source.

The petrol extract of the fruits, on repeated column chromatography over neutral Al_2O_3 and repeated recrystallization, gave a white waxy crystalline compound mp 67–67.5°. The compound analysed for $C_{24}H_{43}NO$ (found C, 79.82; H, 12.34; N, 3.61; calc. for $C_{24}H_{43}NO$: C, 80.2; H, 12.08 and N, 3.59%). The UV spectrum (MeOH) λ_{max} 259 nm, indicated the presence of a conjugated system related to sorbamide [4, 5]. IR (KBr) showed characteristic bands for —NH, (3300, 3080 cm^{-1}), —C=O (1625 cm^{-1}), —C=C_1 (1660 cm^{-1}), —C=C_2 (1620 cm^{-1}), —(CH=CH)$_2$ (997 cm^{-1}) and no peak in the region 960–965 cm^{-1}; indicating the presence of a *trans*-2-*trans*-4-dienamide system [4, 5]. The 100 MHz PMR ($CDCl_3$) spectrum showed a doublet at 0.9 δ (9H, J = 6 Hz) which has been assigned to —C(CH_3)$_2$ and terminal —CH_3 protons, a broad singlet at 1.28 due to methylene groups (18H), a multiplet between 1.8 and 2.22 (6H) is attributed to allylic protons, a triplet centred at 3.13 accounts for two protons (N—CH_2—C—), typical for *iso*butylamides, which is replaced by a doublet after D_2O exchange.

The PMR also showed a triplet at 5.33 due to two *cis* protons (—CH=CH—), a doublet at 5.78 (J = 16 Hz) is attributed to one α-olefinic proton adjacent to a carbonyl group. The multiplet between 5.65 to 6.24 accounts for two (γ, δ) olefinic protons and a broad multiplet at 7.2 is attributed to one (β) olefinic proton.

Because of the conjugated nature of the carbonyl with two double bonds, as shown by the IR and UV spectra of the compound, it appears that one of the double bonds (isolated) is located elsewhere in the chain. The appearance of a triplet at 5.33 (2H) in the PMR spectrum indicated the presence of identical olefinic protons of an isolated double bond and were thus assigned as *cis*. The $KMnO_4$ oxidation of the compound to dodecanoic acid showed the exact location of this *cis* isolated double bond in the molecule.

MS of the compound with fragments at *m/e* 361, (M^+), 333, 289, 261, 254, 236, 223, 208, 192, 180, 156, 152, 121, 115, 96, 95 and 81 supported its structure 1 as the *iso*-butylamide of eicosa -2,4,8-trienoic acid.