Phytochemistry, Vol. 27, No. 10, pp. 3305–3306, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

SHORT REPORTS

L-2-AMINO-7-HYDROXYOCTANOIC ACID: AN AMINO ACID FROM *RUSSULA CYANOXANTHA*

YASUO AOYAGI, NAOKO NAKAMURA and TATSUYUKI SUGAHARA

Food Chemistry, Kagawa Nutrition College, Komagome 3-24-3, Toshima-ku, Tokyo, 170, Japan

(*Received* 8 *March* 1988)

Key Word Index—*Russula cyanoxantha*; Basidiomycetes; mushroom; non-protein amino acid; 2-amino-7-hydroxyoctanoic acid.

Abstract—A new amino acid, L-2-amino-7-hydroxyoctanoic acid was isolated from fruiting bodies of *Russula cyanoxantha*.

Automated amino acid analysis [1] revealed the occurrence of an unusual amino acid in fruiting bodies of the mushroom *Russula cyanoxantha* (Schw.) Fr. Compound **1** was isolated from 70% EtOH extracts of the edible mushroom by ion-exchange chromatography and Avicel cellulose chromatography.

From elemental analysis and SIMS (*m/z* 176 $[M+1]^+$), the molecular formula of **1** was estimated to be $C_8H_{17}NO_3$. The copper complex test [2] indicated that **1** is an α-amino acid. ^1H NMR in D_2O showed a three proton doublet at δ 1.17 which was attributed to the terminal methyl group. The multiplet between δ 1.2 and 1.7 (6H) accounted for three methylene groups (H-4, H-5 and H-6) and a multiplet between δ 1.7 and 2.1 (2H) was attributed to a β methylene group. The NMR also showed a triplet at δ 3.85 due to a α methyne proton and a multiplet between δ 3.6 and 4.1 which was attributed to a methyne proton strongly deshielded by an attached hydroxyl group. In addition, the ^{13}C NMR showed eight carbon signals attributable to one methyl, four methylene, two methyne and one carboxyl groups. These results suggested that the most likely structure of **1** was 2-amino-7-hydroxyoctanoic acid.

Oxidation of **1** with acidic potassium permanganate formed an amino acid **2** with M_r 173 (SIMS *m/z* 174 $[M+1]^+$) which corresponded to the elimination of two protons from **1**. ^1H NMR of **2** showed a multiplet between δ 1.2 and 1.7 (4H) due to two methylene groups (H-4 and H-5) and a two proton multiplet between δ 1.7 and 2.1 attributed to a β methylene group. A three proton singlet at δ 2.23 and triplet at δ 2.63 (2H) both deshielded by an attached carbonyl group were assigned to the terminal methyl and an H-6 methylene, respectively. A triplet at δ 3.87 (1H) was also attributed to α methyne proton. The strong absorption band at 1710 cm^{-1} in IR spectrum of **2** showed the presence of a carbonyl group in the molecule. Thus, it was possible to identify **2** as 2-amino-7-oxooctanoic acid. This fact strongly supports the above structure of **1**.

The specific rotation of **1** was more positive in acid solution than in water, indicating that the amino acid belonged to the L-series at the α asymmetric centre. The configuration of the hydroxyl group in **1** is still unknown.

EXPERIMENTAL

Mushroom. Fruiting bodies of *R. cyanoxantha* were collected from a forest in Saitama prefecture during summer 1985. The fresh fruiting bodies were freeze-dried and kept at 4° until use.

Isolation. The dried mushrooms, 190 g (*ca* 2 kg as fresh) were extracted with 4 l of 70% EtOH × 3. The combined extract was evapd to *ca* 300 ml and defatted by extraction with Et_2O. The soln was run through a column of 1.5 l. Amberlite IR-120 (H^+ form) and, after washing with 8 l of H_2O, the amino acid were eluted with 2 M NH_4OH (10 l). The eluate was evaporated and the residue taken up in 300 ml of H_2O. The soln obtained was applied to a column of Dowex 1 × 4 (OAc^-, 500 ml) and eluted with 0.3 M HOAc. The eluate was concd and applied again to a Dowex 50w × 4 (pyridinium form, 3 × 100 cm). By elution with a pyridine–HOAc buffer gradient (0.2 M pyridine, pH 3.1 to 2.0 M pyridine, pH 5.0), the amino acids were fractionated. The relevant fractions were combined and rechromatographed on the same column. The fraction containing the compound **1** thus obtained was further fractionated by chromatography on Avicel cellulose column with *n*-BuOH–HOAc–H_2O (8:1:1 by vol.). **1** was crystallized from the concd eluate by adding EtOH and recrystallized from EtOH–H_2O (×2) to yield 220 mg, mp 206–211°; $[\alpha]_D^{25}$ +10.0 (H_2O, *c* 0.7), +29.7 (3 M HCl, *c* 0.35); (Found: C, 53.53; H, 9.94; N, 7.99. $C_8H_{17}NO_3$ requires: C, 54.54; H, 9.78; N, 7.99); SIMS *m/z*: 176 $[M+1]^+$; ^1H NMR (90 MHz, D_2O, TSP): δ 1.17 (3H, *d*, *J* = 6 Hz, H-8), 1.2–1.7 (6H, *m*, H-4, 5, 6), 1.7–2.1 (2H, *m*, H-3), 3.85 (1H, *t*, *J* = 6 Hz, H-2), 3.6–4.1 (1H, *m*, H-7); ^{13}C NMR (25 MHz, D_2O): δ 24.5 (C-8), 27.0 (C-5 or C-4), 27.2 (C-4 or C-5), 33.0 (C-3), 40.1 (C-6), 57.5 (C-2), 70.4 (C-7), 177.6 (C-1); IR ν_{max}^{KBr} cm^{-1}: 3380, 2940, 1580, 1515, 1445, 1408, 1355, 1327, 1130, 980, 860, 843, 663, 542.

$KMnO_4$ *oxidation.* A reaction mixture containing 52 mg of **1** in 10 ml of 10% H_2SO_4 and 10 ml of 2% $KMnO_4$ was left at 4° for 20 hr. This was then added to 10 ml H_2O and the filtrate was applied to a column of Amberlite IR-120 (H^+ form, 2 × 20 cm). After the resin was washed with H_2O, elution was performed with 2 M NH_4OH. The eluate was concd to dryness, redissolved

in a small vol. of H_2O and subsequently applied to a Dowex 1 × 4 resin column (OAc^- form, 2 × 8 cm). A ninhydrin positive product **2** detected in the 0.3 M HOAc eluate of the column was further purified by fractionation using a Dowex 50w × 2 resin column (pyridine form, 2 × 80 cm). **2** was pptd by adding EtOH to the concd relevant fractions, yielding 29 mg; $[\alpha]_D^{25}$ + 2.7 (H_2O, *c* 0.5), +8.8 (3 M HCl, *c* 0.25); SIMS *m/z*: 174 $[M+1]^+$; ^1H NMR (90 MHz, D_2O, TSP): δ 1.2–1.8 (4H, *m*, H-4, 5), 1.7–2.1 (2H, *m*, H-3), 2.23 (3H, *s*, H-8), 2.63 (2H, *t*, *J* = 7 Hz, H-6), 3.87 (1H, *t*, *J* = 6 Hz, H-2); IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1710, 1580, 1512, 1440, 1410, 1320, 1160, 1095, 850, 805, 655, 549.

Acknowledgement—We would like to thank Dr T. Ozawa (University of Tsukuba) for measuring ^{13}C NMR.

REFERENCES

1. Sato, E., Aoyagi, Y. and Sugahara, T. (1985) *Nippon Shokuhin Kogyo Gakkaishi* **32**, 509.
2. Larsen, P. O. and Kjaer, A. (1960) *Biochim. Biophys. Acta* **38**, 148.

Phytochemistry, Vol. 27, No. 10, pp. 3306–3307, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

β-HYDROXY-L-VALINE FROM *PLEUROCYBELLA PORRIGENS*

YASUO AOYAGI and TATSUYUKI SUGAHARA

Food Chemistry, Kagawa Nutrition College, Komagome 3-24-3, Toshima-ku, Tokyo, 170, Japan

(*Received* 8 *March* 1988)

Key Word Index—*Pleurocybella porrigens*; basidiomycetes; mushroom; non-protein amino acid; β-hydroxy-L-valine.

Abstract—β-Hydroxy-L-valine was isolated from fruiting bodies of *Pleurocybella porrigens* (Fr.) Sing.

In a previous paper [1], we reported the free amino acid contents of fruiting bodies of 113 mushroom species, as determined by automatic amino acid analysis of their 70% ethanol extracts. In the course of the experiment, some of the mushrooms were found to contain several unusual amino acids. This paper describes the isolation and identification of an unknown ninhydrin positive compound, hereafter termed as **1**, from the fruiting bodies of the edible mushroom, *Pleurocybella porrigens* (Fr.) Sing.

Compound **1** was detected on the chromatogram of the amino acid analyser (Li-citrate buffer system) and was completely overlapped with threonine. The two were distinguishable by TLC (silica gel and Avicel cellulose). The isolation of **1** was accomplished by chromatography using several ion-exchange resins, Avicel cellulose and silica gel, followed by crystallization. From the elemental analysis and SIMS, the molecular formula of **1** was estimated to be $C_5H_{11}NO_3$. The ninhydrin reaction of **1** on paper was completely inhibited by Cu^{2+}, showing that **1** is a α-monoamino acid. On the ^1H NMR of **1** (in D_2O), one *gem*-dimethyl group (δ 1.26 and 1.47) and one α-methyne singlet (δ 3.65), which indicated the β carbon atom to be fully substituted, were observed. The absorption band at 1167 cm^{-1} in the IR showed the presence of a tertiary hydroxyl group in the molecule [2]. These facts strongly suggested that **1** is β-hydroxyvaline. This was further supported by the formation of valine from the reduction with HI-red P [3], and the detection of glycine in the degradation product by barium hydroxide [4].

Optical rotation measurements on **1** performed in H_2O and 6 M HCl solution showed the shift to more positive rotations in acid associated with an L configuration. The IR of **1** correlated well with that of a synthetic specimen of β-hydroxy-L-valine [5].

Although β-hydroxy-L-valine was first found as a constituent of the antibiotic YA-56 [6, 7], it is thought that the evidence presented here is the first to demonstrate its natural occurrence in a free form.

EXPERIMENTAL

Mushroom. Fruiting bodies of *P. porrigens* were collected from a forest in Nagano prefecture during autumn 1985. The fresh fruiting bodies were washed with H_2O and then freeze-dried. They were kept at 4° until use.

General. Chromatography solvents were *n*-BuOH–HOAc–H_2O (8:1:1, by vol.; solvent 1), *n*-BuOH–HOAc–H_2O (4:1:2, by vol.; solvent 2), PrOH–H_2O (7:3, by vol.; solvent 3).

Isolation. The freeze-dried fruiting bodies (670 g) were extracted with 70% EtOH (× 3) and filtered. The filtrate (30 l) was passed through a column (5 × 90 cm) of Amberlite IR-120 (H^+). After the resin was washed with 70% EtOH and H_2O, the amino acids were eluted with 2 M NH_4OH. The eluate was evapd to dryness and dissolved in 200 ml of H_2O. The concentrate was applied to a column (5 cm × 75 cm) of Dowex 1 × 4 (OAc^-). Compound **1** was detected in the neutral and basic amino acid fractions eluted with 0.1 M HOAc from the column. The amino acid fraction was concentrated and subsequently chromato-