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L-2-AMINOHEX-4-YNOIC ACID: A NEW AMINO ACID FROM *TRICHOLOMOPSIS RUTILANS**

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Key Word Index—*Tricholomopsis rutilans*; Tricholomataceae; Basidiomycetes; L-2-aminohex-4-ynoic acid; new amino acid; synthesis.

Abstract—A new amino acid was isolated from fruit bodies of *Tricholomopsis rutilans* (Fr.) Sing. Its structure was shown to be L-2-aminohex-4-ynoic acid by elementary analysis, catalytic hydrogenation, IR and NMR spectra, and finally confirmed by chemical synthesis.

INTRODUCTION

A PAPER chromatographic survey of non-protein amino acids in fungi[1] revealed that *Tricholomopsis rutilans* (Fr.) Sing. contains three to four unusual ninhydrin-positive substances in addition to common amino acids. This paper reports the isolation and characterization of a new amino acid (TX1).

RESULTS AND DISCUSSION

Since TX1 gives a yellow to brown colour with ninhydrin and occurs near valine on a two-dimensional paper chromatogram, it appears to be neutral and unsaturated in structure. Therefore, deep frozen fruit bodies (3 kg) were extracted with ethanol and the extract was treated with Amberlite IR-120 and the amino acids eluted with 2 N ammonia. The eluate was concentrated and applied to a column of Dowex 1 to remove acidic amino acids. Further fractionations were carried out by paper and cellulose column chromatography, to give 19 mg pure crystals.

The crystals of TX1 decomposed very slowly above 199°. The elementary analysis was in good agreement with $C_6H_9NO_2$. The R_fs were 0·44 in *n*-butanol–acetic acid–water (A) (Ala 0·25, Val 0·46) and 0·72 in phenol–water (NH_3) (B) (Ala 0·58, Val 0·77). Its IR spectrum showed absorption bands characteristic of the amino acid zwitterion. Since the ninhydrin reaction of TX1 on paper was completely inhibited by the specific masking of the α-amino group with Cu^{2+},[2] TX1 was considered to be an α-amino acid. Further evidence was obtained from the determination of CO_2 and HN_3,[3] which had been evolved from TX1 after the ninhydrin reaction. The values were 1·04 and 0·65 mol/mol TX1, respectively, on the assumption that TX1 is an α-monoaminomonocarboxylic acid $C_6H_9NO_2$. The presence of conjugated double bonds was ruled out by the UV spectrum ($\lambda_{max}^{H_2O}$ 192 nm, ϵ_{max} 870). Hydrogenation

* Part III in the series "Biochemical Studies on Nitrogen Compounds of Fungi". For Part II see S. HATANAKA, *Phytochem.* **8**, 1305 (1969).

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with Adams platinum catalyst showed that TX1 absorbed 1·96 mol of hydrogen and norleucine (identified by IR analysis) was the sole reduction product.

The above results strongly suggest that TX1 is an unsaturated norleucine, with two isolated double bonds, an allenic, or an acetylenic linkage. In order to elucidate the position of the unsaturation, TX1 was oxidized with acidic $KMnO_4$. Aspartic acid was identified by TLC as the only ninhydrin-positive product. Two structures (I) and (II) were, therefore, possible.

$$CH_2{=}C{=}CH{-}CH_2{-}CH(NH_3^+){-}COO^- \quad (I)$$

$$CH_3{-}C{\equiv}C{-}CH_2{-}CH(NH_3^+){-}COO^- \quad (II)$$

Chilton *et al.*[4] reported recently the isolation of 2(*S*)-aminohex-4,5-dienoic acid (I) from the fungus *Amanita solitaria*. The identification was based on the direct comparison with a synthetic sample prepared independently by Black and Landor.[5] The IR spectrum of this amino acid shows very strong bands at 1954 and 845 cm^{-1} assigned to the allene. As our amino acid did not show any absorption at these frequencies, the structure, 2-aminohex-4-ynoic acid (II) remained as the only possible one. To confirm the existence of a triple bond, TX1 was then hydrogenated over Lindlar catalyst.[6] The result was satisfactory and the hydrogen uptake was 0·93 mol/mol TX1.

The NMR spectrum was determined in D_2O containing a small amount of 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. It showed a triplet at 8·26 τ (3H, *J* 2 Hz) attributed to three protons of the terminal methyl group. A multiplet at 7·26 τ (2H) and a triplet at 6·22 τ (1H, J 5 Hz) are assigned to two protons of the methylene and one proton of the methyne, respectively. The NMR spectrum is, thus, consistent with structure (II).

For final identification, 2-aminohex-4-ynoic acid was synthesized. Diethylformylaminomalonate[7] and 1-bromobut-2-yne[8] were condensed in the presence of NaOEt[5] and the product (diethyl 1-formylaminopent-3-yne-1,1-dicarboxylate) was obtained as needles (m.p. 95·5–96°) and analyzed for $C_{12}H_{17}NO_5$. Successive hydrolysis with alkali and acid gave (II), identical in every way (m.p., TLC, IR, NMR) with the natural material. Optical rotation measurements on the natural amino acid, in water and 3 N HCl solution, suggest that it belongs to the L-series.

Two of the four other species of *Tricholomopsis* known in Japan, namely *T. decora* (Fr.) Sing. and *T. platyphylla* (Fr.) Sing. were examined for their amino acids by paper chromatography, but this new amino acid could not be detected in them. Although norleucine is a possible precursor in the biosynthesis of 2-aminohex-4-ynoic acid, it could not be detected in the fruit bodies of *T. rutilans*.

In 1969 Sung *et al.*[9] reported the first examples of natural amino acids containing triple bonds, 2-amino-4-methylhex-5-ynoic acid, 2-amino-4-hydroxymethylhex-5-ynoic acid, and 2-amino-4-hydroxyhept-6-ynoic acid from the seeds of *Euphoria longan*. Several unsaturated amino acids are known in fungi; acetylenic ones, however, have not been reported before.

EXPERIMENTAL

General. M.ps, determined in capillary tubes, were uncorrected. UV spectra were recorded in H_2O and IR spectra were measured in KBr discs.

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Fungal materials. Fresh fruit bodies of *Tricholomopsis rutilans* (Fr.) Sing. were collected in September 1970, in Nagano Pref., and stored at −20° before use. Small amounts of *Tricholomopsis decora* (Fr.) Sing. and *T. platyphylla* (Fr.) Sing., were collected in July 1970 in Nara Pref.

Chromatography. Solvents were: *n*-BuOH–HOAc–H_2O (63:10:27) (A), phenol–H_2O (in NH_3 vapour) (25:39) (B), and *n*-amylalcohol–pyridine–H_2O (7:7:6) (C). 'Avicel SF' plates were used for TLC and 'Avicel SF' was adopted for column chromatography.

Isolation of the new amino acid. 3 kg fruit bodies of *T. rutilans*, stored deep-frozen, were extracted with 85% EtOH (× 5) and filtered. The filtrate (40 l.) was passed through a column of 450 ml Amberlite IR-120 (H^+). After the resin was washed thoroughly with EtOH and H_2O, the amino acids were eluted with 4·5 l. of 2 N NH_4OH. The eluate was concentrated to *ca.* 40 ml and diluted to 100 ml with 0·2 N HOAc. The concentrate was applied to a column of Dowex 1 × 4 (CH_3COO^-) and eluted with 0·2 N acetic acid. The new amino acid (TX1) appeared in neutral and basic fractions with another unknown ninhydrin-positive substance (TX2). The concentrated fractions containing TX1 were separated on paper in solvent A and 65 mg crystals were obtained. Since it was still contaminated, the isolate was further purified on a cellulose powder column with solvent C. TX1 containing fractions were extracted with H_2O, concentrated to a small volume, and crystallized thrice from 80% EtOH to give 19 mg pure sample, m.p. > 199° (decomp.). (Found: C, 56·67; H, 7·40; N, 11·37. $C_6H_9NO_2$ requires: C, 56·68; H, 7·14; N, 11·02%.) $[\alpha]_D^{28°} = -54·4°$ (*c* 1, H_2O), −29·9° (*c* 0·5, 3 N HCl).

Hydrogenation. Hydrogenations were carried out at room temp. and pressure. 4·30 mg TX1 were hydrogenated over a few mg Adams platinum oxide, suspended in 2·5 ml H_2O. To determine the absorption of hydrogen over Lindlar catalyst,[6] 4·35 mg sample and 9 mg catalyst were used together with 6 μl freshly distilled quinoline.

Degradation. 1·2 mg TX1 were dissolved in 1 ml of 10% H_2SO_4 and 1 ml of 2% $KMnO_4$ was added. After being left at 8° for 18 hr, the mixture was diluted with an equal vol. of H_2O, centrifuged and the supernatant passed through a column of 1·5 ml Amberlite IR-120 (H^+). After the resin was washed, 15 ml of 2 N NH_4OH were passed through and the eluate evaporated to dryness. The residue was dissolved in 0·03 ml H_2O and the degradation products analyzed by cellulose TLC, using solvents A and B.

Synthesis of DL-*2-amonihex-4-ynoic acid.* Sodium (0·6 g), in absolute EtOH (10 ml), was refluxed for 30 min with a solution of diethyl formylaminomalonate[7] (5 g) in absolute EtOH (12·5 ml). 1-Bromobut-2-yne[8] (3·3 g) was added with stirring and the mixture was refluxed for 5 hr. Et_2O (25 ml) and H_2O (10 ml) were added and the aqueous layer was extracted with Et_2O (2 × 10 ml). The organic phase was dried; removal of solvent *in vacuo* gave needles (6·3 g); diethyl 1-formylaminopent-3-yne-1,1-dicarboxylate (ex EtOH), m.p. 95·5–96°. (Found: C, 56·47; H, 6·42; N, 5·37. $C_{12}H_{17}NO_5$ requires: C, 56·46; H, 6·71; N, 5·49%.) Diethyl 1-formylaminopent-3-yne-1,1-dicarboxylate (2·0 g) in EtOH (10 ml) was added to a solution of NaOH (2·85 g) in H_2O (7·5 ml). After 5 hr at 20°, the solution was refluxed for 1 hr. EtOH was evaporated *in vacuo* and the remaining solution cooled in an ice-bath and acidified with conc. HCl to pH 2. The mixture was then refluxed for 1 hr, passed through Amberlite IR-120 (H^+) (50 ml) and the ammonia eluate (0·5 N, 500 ml) was evaporated. The crude crystals of (II) (0·5 g) were recrystallized from H_2O (× 3) to remove contaminating glycine. M.p. > 209° (decomp.). (Found: C, 56·95; H, 6·97; N, 10·83. $C_6H_9NO_2$ requires: C, 56·68; H, 7·14; N, 11·02%.)

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