Phytochemistry 1974 Vol 13 pp 2813 to 2814 Pergamon Press Printed in England

L-3-(3-CARBOXYFURAN-4-YL)ALANINE, A NEW AMINO ACID FROM THE MUSHROOM *PHYLLOTOPSIS NIDULANS**

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(*Received* 7 *April* 1974)

Key Word Index—*Phyllotopsis nidulans*, Basidiomycetes, fungi, furan, amino acid

Abstract—A new amino acid has been discovered in uncombined form in extracts of the fruiting bodies of the mushroom, *Phyllotopsis nidulans* Chemical and spectroscopic data support formulation of the structure as L-3-(3-carboxyfuran-4-yl)alanine

INTRODUCTION

BASIDIOCARP extracts of the mushroom, *Phyllotopsis nidulans* (Pers. ex Fr.) Sing. were examined during the course of a search for new amino acids in higher fungi.[1,2] 2D PC revealed the presence of an unusual ninhydrin-positive compound which absorbed in UV light. This paper describes its identification as L-3-(3-carboxyfuran-4-yl)alanine (**1**).

RESULTS AND DISCUSSION

Initial studies with partially purified solutions using small ion-exchange columns indicated that the new amino acid had an excess of acidic functional groups. Comparison of the UV spectra of these solutions with those of model compounds led to the conclusion that a furan ring was involved. Purification of the amino acid by ion-exchange and PC techniques yielded a crystalline substance, $C_8H_9NO_5$ (FW 199.2) This formula was supported by the fact that a Chemical Ionization Mass Spectrum gave a parent peak at m/e = 200. From this, it was formulated as a furanylalanine with an extra carboxyl group on the furan ring. Final resolution of the structure was based on the NMR spectrum of the dimethyl ester hydrochloride in D_2O solution Besides confirming the presence of the alanine side chain, this spectrum permitted assigning the carboxyl group and the β-alanyl side chain to the 3 and 4 positions, respectively, of the furan ring. Comparison of the pattern of furan ring proton signals with those of model furan derivatives showed that the

* Presented in part before the 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill, 18, April 1967 The investigation was supported by a grant (GM-13325) from the U S Public Health Service

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[1] LEVENBERG, B (1968) *J Biol Chem* **243,** 6009

[2] DOYLE, R R and LEVENBERG, B (1968) *Biochem* **7,** 2457

protons are present at positions 2 and 5 of the furan ring in the new amino acid. The optical rotation was not measured because of the small amount of purified material available, but the amino acid was shown to belong to the L series by chemical degradation to L-aspartic acid. The latter product was determined enzymatically by the MDH-GOT method[3] and confirmed by its elution position on the amino acid analyzer.

EXPERIMENTAL

M.ps were determined on a Mel-Temp apparatus and are corrected.

Paper chromatography. Descending techniques were employed throughout using Whatman No. 3 paper. Solvent systems were: (A) n-BuOH–HOAc–H_2O (18:2:5) and (B) n-BuOH–MeCOEt–10N NH_3 (5:3:2). Amino acids were detected by dipping in ninhydrin in acetone (0.2%, w/v) containing about 5000 ppm of redistilled 2,4,6-collidine. Colors were allowed to develop at room temp. Approximate R_f values of the new amino acid are (A) 0.30 and (B) 0.045.

Source and preparation of the mushroom extract. Basidiocarps of *Phyllotopsis nidulans* were collected from Southeastern Michigan in Sept. 1963, 1964 and 1966. Fresh, washed mushroom caps (1 kg) were broken up into a large capacity Waring Blendor. MeOH (1.2 l.) 0.043 N in H_2SO_4, was added and the caps were blended for 1.5 min. The extract, after filtration through cheesecloth, had a volume of 1.7 l. and a pH of 2.5. The extracts were pooled and evaporated under vacuum at 40–45° to attain a 5–10 fold concn. The conc. extract was then stored at −17° until needed.

Isolation. Crude mushroom extract (150 ml) was thawed and adjusted to pH 6.5 with 1 N KOH. The extract was added slowly to a column containing 1 kg of Amberlite IRA-400 (Cl^-) anion exchange resin. The resin was then washed thoroughly with H_2O. Elution of the amino acid was accomplished with 0.0025 N HCl at a flow rate of approx. 1 litre per day. The eluate was monitored by UV spectroscopy; after several days the typical shoulder at 240 nm appeared and those fractions absorbing in this region were combined and evaporated to a small vol. under vacuum at 30–35°. The conc. eluate was further purified by preparative PC in solv. A. The UV-absorbing areas were cut out, eluted with H_2O and the eluate concn. The compound was finally crystallized by the addition of acetone.

Properties. The product had m.p. 227–228°, $\lambda_{max}^{H_2O}$ 241 nm (ε 3500) at pH 1.5. The NMR spectrum (D_2O, as di-Me ester · HCl) shows α-hydrogen resonances at τ 5.50 and β-hydrogen doublets at τ 6.61 and 6.68 with a splitting pattern typical for β-substituted alanines.[4] Peaks at τ 1.76 and 2.36 agree in position and fine structure with those of model furans substituted in the 3 and 4 positions. The compound can be located on chromatograms by its UV absorbing property or by its steel-blue color with ninhydrin/collidine. Potentiometric titration with NaOH gives approximate pK values of 3.75 (Eq. wt. 211) and 10.0 (Eq. wt. 204). The amino acid is stable to hydrolysis by 3 N HCl for 15 min at 100° but is rapidly destroyed by heating with saturated $Ba(OH)_2$.

Analyses. Found: C, 48.6; H, 4.75; N, 7.09; O, 41.2. $C_8H_9NO_5$ (FW 199.2) requires: C, 48.2; H, 4.56; N, 7.03; O, 40.2. Nitrogen analysis by the Van Slyke method gave value of 7.2%, in good agreement with the above analysis by the Kjeldahl method.

Chemical degradation. Approx. 6 μmols of the purified amino acid were benzoylated, and the benzoyl derivative was oxidized at room temp. using 0.1 M $KMnO_4$. After chromatographic purification, the oxidation product was debenzoylated by acid hydrolysis and shown to be L-aspartic acid by quantitative analysis using the MDH-GOT procedure.[3]

Plant documentation. Voucher specimens of *Phyllotopsis nidulans* (RD 64) are on deposit in The University of Michigan Herbarium, Dr. Robert L. Shaffer, curator.

Acknowledgements. We thank Mrs. Doris Kaczmarek and Mrs. Linda Carl for their excellent technical assistance and Drs. A. H. Smith and R. L. Shaffer and Mrs. Florence Hoseney for collecting the fungi. Special thanks are also due to Dr. Jules Shafer for assistance with the potentiometric titration and to Dr. Henry Fales for running the mass spectrum. Fungus identification was done by Dr. Alexander H. Smith, University Herbarium, The University of Michigan.

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