

## L-2-AMINO-4-METHYLPIMELIC ACID: A NEW AMINO ACID FROM *LACTARIUS* SPECIES\*

SHIN-ICHI HATANAKA,† HITOSHI IIZUMI‡ and ATSUKO TSUJI

Department of Biology, College of General Education, The University of Tokyo,  
Komaba, Meguro-ku, Tokyo 153, Japan

and

ROLF GMELIN

Institut für Pharmakognosie, Freie Universität Berlin, 1 Berlin-Dahlem 33,  
Königin-Luise-Strasse 2-4, West Berlin

(Received 8 January 1975)

**Key Word Index**—*Lactarius quietus*; Russulaceae; Basidiomycetes; L-2-amino-4-methylpimelic acid; synthesis.

**Abstract**—A PC survey revealed the occurrence of an unusual amino acid in fruit bodies of the mushroom *Lactarius quietus* Fr. and a related species. This was identified as L-2-amino-4-methylpimelic acid, which has not been previously reported as a naturally occurring compound.

Results of elementary analysis of the purified isolate were in good agreement with the formula  $C_8H_{15}NO_4$ . On cellulose TLC the amino acid chromatographs between alanine and valine in phenol-H<sub>2</sub>O and moves a little further than valine in *n*-BuOH-HOAc-H<sub>2</sub>O. Determination of CO<sub>2</sub> and NH<sub>3</sub> after reaction with ninhydrin [1], potentiometric titration, as well as the copper complex-test [2], showed that it is an  $\alpha$ -aminodicarboxylic acid. From the above results and its NMR-spectrum, the most probable structure is 2-amino-4-methylpimelic acid.

For the final confirmation of the structure, DL-2-amino-4-methylpimelic acid was synthesized by the condensation of diethyl (3-bromoisobutyl)malonate, prepared from its chloro derivative [3], and diethyl acetamidomalonate in the presence of sodium ethoxide, followed by hydrolysis in 11 M HCl. The  $R_f$ -values on TLC with several

different solvents, the elution volumes from Dowex 1-columns and the MS of the natural and synthetic samples were the same.

The structural studies mentioned above were carried out on the isolate from the unidentified *Lactarius* sp., because only a small amount of the sample was obtained from *Lactarius quietus*. Both isolates had the same elution volumes in an amino acid analyzer, IR-spectra, mobilities on TLC and fragmentation patterns in MS.

The values of optical rotation determined under two different pH conditions showed that the natural amino acid belongs to the L-series. The configuration of the methyl group in the natural amino acid is still unknown.

### EXPERIMENTAL

**Isolation.** Fruit bodies of *Lactarius* sp. were collected and immediately dried at 40–50° (fr.wt 2.75 kg). They were then extracted repeatedly with 80% EtOH (15:51), the combined extracts treated with Amberlite IR-120(H<sup>+</sup>) (500 ml) and the amino acids eluted with 2 N NH<sub>4</sub>OH (4:81). NH<sub>3</sub> was removed by evaporation under red pres and the resultant syrup was fractionated on a column of Dowex 1 (MeCOO<sup>−</sup>, 42 × 840 mm) using 0.15 N MeCOOH. 2-Amino-4-methylpimelic acid was eluted just before  $\alpha$ -aminoadipic acid. On

\* Part XII in the series *Biochemical Studies on Nitrogen Compounds of Fungi* for Part XI see Hatanaka, S.-I. and Katayama, H. *Phytochemistry*, in press.

† To whom correspondence should be addressed.

‡ Present address: Ocean Research Institute, The University of Tokyo.

concn of the relevant fractions, crude crystals separated (412 mg), which were recrystallized from  $\text{Me}_2\text{CO}-\text{H}_2\text{O}$ . mp  $189-191^\circ$  (decomp)  $[\alpha]_D^{25} -6.3^\circ$  (c 1.1,  $\text{H}_2\text{O}$ ),  $+10.9^\circ$  (c 0.55, 3 N HCl). Elementary analysis: C, 50.43; H, 7.57; N, 7.50.  $\text{C}_8\text{H}_{15}\text{NO}_4$  requires: C, 50.78; H, 7.99; N, 7.40%. PMR: (in  $\text{D}_2\text{O}$ , DSS as an internal standard):  $\delta(\text{ppm})$  0.97 (d,  $-\text{CH}_3$ ), 1.7 (broad,  $-\text{CH}_2-\text{CH}(\text{Me})-\text{CH}_2-$ ), 2.45 (t,  $\text{DOOC}-\text{CH}_2-$ ), 3.82 [t,  $-\text{CH}(\text{ND}_2)-\text{COOD}$ ].

From a small amount of sample of *Lactarius quietus* Fr.\* (dry wt 14.5 g), 2 mg of 2-amino-4-methylpimelic acid were isolated in the same way described above.

*Synthesis of DL-2-amino-4-methylpimelic acid.* Diethyl (3-chloroisobutyl)malonate [3] (31.5 g) and KBr (28.5 g) in MeOH (30 ml) were refluxed for 6 hr.  $\text{H}_2\text{O}$  (100 ml) and  $\text{Et}_2\text{O}$  (80 ml) were added and the  $\text{H}_2\text{O}$ -layer extracted with  $\text{Et}_2\text{O}$  ( $\times 2$ , 60 ml). The  $\text{Et}_2\text{O}$  extract was dried and distilled to give diethyl (3-bromoisobutyl)malonate (13.5 g), bp  $153-156.5^\circ$  (probably impure). Na (0.4 g) was dissolved in EtOH (6 ml) and diethyl acetamidomalonate (3.8 g) added. The mixture was then refluxed for 30 min and diethyl (3-bromoisobutyl)malonate (4.8 g) was added by drops over 100 min under reflux, and refluxing was continued for a further 20 hr.  $\text{H}_2\text{O}$  (5 ml) and  $\text{Et}_2\text{O}$  (20 ml) were added and the organic layer was concentrated. 11 M HCl (40 ml) was added to the syrup and re-

fluxed for 6 hr. After the bulk of the HCl had been removed by evaporation, the product was passed through Amberlite IR-120 ( $\text{H}^+$ ) (20 ml). The amino acids were eluted with 2 N  $\text{NH}_4\text{OH}$  (200 ml) and  $\text{NH}_3$  was removed by evaporation. A large amount of glycine was removed by fractionation with Dowex 1 ( $\text{MeCOO}^-$ ,  $26 \times 700$  mm). DL-2-Amino-4-methylpimelic acid, yield: 75 mg. Recrystallized sample (ex aq.  $\text{Me}_2\text{CO} \times 3$ ) mp  $173-7^\circ$  (decomp).

*Acknowledgements*—We wish to thank Professor T. Okamoto, Faculty of Pharmaceutical Sciences, The University of Tokyo, for his kind help with the MS. Our thanks are also due to Professor T. Tsumita and Miss T. Osawa, Institute of Medical Science, The University of Tokyo, for the potentiometric titration and analysis with an amino acid analyzer and Dr. K. Koyano, Department of Chemistry of our campus for NMR.

#### REFERENCES

1. Linko, P. (1955) *Suomen Kemistikehti* **28B**, 96.
2. Larsen, P. O. and Kjær, A. (1960) *Biochim. Biophys. Acta* **38**, 148.
3. Kazanskii, B. A. and Lukina, M. Yu. (1952) *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk* 1951, **47**, (cited in *Chem. Abstr.* **46**, 4491).

\* Vouchers are deposited in the Department of Biology, College of General Education, The University of Tokyo.