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N-NITROAMINES OF *AGARICUS SILVATICUS*

WILLIAM S. CHILTON and CHIEN PING HSU

Department of Chemistry, University of Washington, Seattle, Washington 98195, U.S.A.

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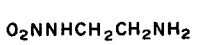
Abstract—β-Nitraminoalanine and its decarboxylation product *N*-nitroethylenediamine were identified in *Agaricus silvaticus*.

A survey of secondary amino acids in mushrooms [1] revealed the presence of two new amino acids in *Agaricus silvaticus* Vitt. ex Fr. from North Carolina and in a closely related Puget Sound species [2] sometimes referred to as *A. silvaticus*. Electrophoresis at pH 2 and 6 suggests that one of the new compounds is as acidic as aspartic acid and the other is basic. Both give the same transiently-abnormal ninhydrin reaction: initial bright yellow, turning brown and, finally, brownish purple in a few hr. The electrophoretic behavior, chromatographic mobility and identity of ninhydrin reactions suggests that the basic compound is the decarboxylation product of the acidic amino acid.

The acidic amino acid was isolated by cationic and anionic exchange enrichment, followed by chromatography and crystallization. The elemental analysis showed an unusually high nitrogen content: $C_3H_7N_3O_4$. The PMR in D_2O consisted of a two-proton doublet and a one-proton triplet typical of a β-substituted alanine. The remaining four hydrogens are exchangeable. Chemical ionization MS confirmed the molecular formula $C_3H_7N_3O_4$ (M + 1 = 150). The most distinctive feature of the MS was the dominant β-cleavage (*m/e* = 88, base peak) confirmed as a direct decomposition product of the quasimolecular ion at *m/e* 150 by observation of the metastable at *m/e* 51·7. The complementary β-cleavage ion (with H transfer) was also observed at *m/e* 60 as well as minor fragments due to loss of CO_2 (*m/e* 106) and H_2O (*m/e* 132). The elemental analysis, PMR and MS require an alanine having a β-substituent of composition HN_2O_2. Although quite a few isomeric HN_2O_2 groups are possible, only two, *N*-nitroamine and *N*-nitrosohydroxylamine, appear to have any stability. Therefore, the new amino acid would be either **1** or **2**. The two can be conveniently distinguished by UV [3]. The A of simple aliphatic *N*-nitroamines, *ca* 230 nm (ε 7000), is virtually unchanged by shifting pH from 1 to 13, while the maximum for *N*-nitrosohydroxylamines shifts from 230 nm (ε 6000) in acid or water to about 250 nm (ε 8000) in dilute NaOH. The UV spectrum of the new amino acid, 230 nm (ε 7700) remains the same at pH 1, 6 and 13. The new amino acid is therefore **1**. Its isomer **2**, alanosine, has been discovered previously in a Streptomycetes [4]. The isomeric naturally-occurring amino acids **1** and **2** are readily distinguished by PMR as well as by UV. In aqueous NaOD the α-proton triplet of alanosine occurs upfield of the β-methylene doublet, while in the PMR of β-nitraminoalanine in aq Na_2CO_3 the relative positions of these signals is just reversed.



(3)

The logical structure **3** for the other new compound was proved by synthesis of *N*-nitroethylenediamine from ethylenediamine. The mp, IR, UV and PMR of the natural and synthetic compound were identical.

One other related compound, *N*-nitroglycine, has been found as a natural product in *Streptomyces noursei* [5].

EXPERIMENTAL

Chromatographic solvents. BAW = *n*-BuOH–HOAc–H_2O, 12:3:5. MEK = sec-BuOH–*tert*-BuOH–MeCOEt–H_2O, 4:4:8:5, with 0·5% Et_2NH.

Ion exchange enrichment. A 70% EtOH extract of 9 fresh *Agaricus sp.* [2] (384 g) was absorbed on 48 ml Dowex 50W × $8H^+$. Retained amino acids were eluted in 135 ml 15% NH_3, recovered by evapn, and absorbed on 20 ml Dowex 3 (OAc^-). The effluent and one column vol of H_2O wash were set aside for isolation of *N*-nitroethylenediamine.

β-Nitraminoalanine. Glutamic acid and β-nitraminoalanine were eluted from the column with 12% HOAc. Removal of solvent gave 410 mg crystals. The crystals were extracted with 4 ml 50% EtOH and 2 ml H_2O. The combined extracts containing some glutamic acid were chromatographed on paper (BAW) to give nitraminoalamine free of glutamic acid after one crystallization. The 106 mg residue from aq EtOH extraction contained nitraminoalamine and no glutamic acid. It was recrystallized 2× to give 90 mg β-nitraminoalanine, R_f 0·15, R_{leu} 0·24 (BAW); R_f 0·24, R_{leu} 0·28 (PhOH–H_2O 4:1); R_f 0·56 (MEK). On electrophoresis at pH 6·1, 50 V/cm, 20 min, the compound was located at a position 89% of the distance from arginine to aspartic acid standards; at pH 2, other conditions identical, it co-migrated with aspartic acid.

Natural N-Nitroethylenediamine. The basic nitramine was isolated from the amino acid fraction not retained on Dowex 3 by preparative PC in BAW. The 137 mg of crystalline *N*-nitroethylenediamine was chromatographically and electrophoretically pure: R_f 0·35, R_{leu} 0·52 (BAW); R_f 0·53, R_{leu} 0·63 (PhOH–H_2O 4:1); R_f 0·85 (MEK). On electrophoresis at pH 6·1, 50 V/cm, 20 min, the compound lies 43% of the way from lysine toward aspartic acid; at pH 2, 45 V/cm, 30 min, it lies closer to the cathode than arginine or lysine. The 60 MHz PMR is a strongly coupled A_2B_2 pattern at 4·04 and 3·71 ppm (D_2O, pD 7). A KBr pellet IR had absorption at 3125 (broad), 3000–2470 (series of overtones), 1690–1530 (broad with broad maxima at 1626 and 1600), 1511, 1470 (shoulder), 1419, 1380, 1333, 1285, 1242, 1146, (shoulder), 1129, 1090, 1039, 995, 978, 888, 839, 762 and 716 cm^{-1}. UV spectrum: $\lambda_{max}^{pH_0}$ 228 nm (ε 7940); no change on addition of KOH or HCl.

Synthetic N-Nitroethylenediamine. The nitramine was synthesized by nitrating *N*-carbethoxyethylenediamine according to the procedure of ref. [6]. Its physical properties are identical to those listed for the naturally occurring nitramine.

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