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CLITOCINE, A NEW INSECTICIDAL NUCLEOSIDE FROM THE MUSHROOM CLITOCYBE INVERSA

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Abstract: Chemical investigation of pest insect control agents produced by mushrooms has led to the isolation of clitocine, a new nucleoside. This compound showed strong insecticidal activity against the pink bollworm Pectinophora gossypiella. The elucidation of structure of clitocine was greatly simplified by comparisons its spectral properties to adenosine which was isolated from the same source.

Clitocybe inversa (Scop.:Fr) Quēl, is a medium sized buff-colored mushroom that is found in association with conifers in western North America. Some mycologists report it to be edible, but it is usually not eaten due to its poor quality of taste and texture. The mushroom was collected from sitka spruce forests near Arcata, California, and were immediately covered with methanol. The methanol extract was evaporated to dryness. Water was added and the aqueous solution was partitioned with hexane, chloroform and ethylacetate. Fractions were monitored in an artificial diet feeding bioassay[1] using the agricultural pest insect Pectinophora gossypiella. Insecticidal activity was found in the ethylacetate fraction. This fraction was subjected to further separation by employing DCCC (lower phase of $CHCl_3$:MeOH:H_2O, 13:7:4 v/v as mobile phase), and column chromatography using C_{18} reversed phase (MeOH:H_2O, 95:5 v/v) and Sephadex LH-20 (MeOH). This process allowed us to isolate adenosine (0.03%) and clitocine (0.001%). Clitocine exhibited biological activity while adenosine was inactive. However, close chemical similarities were apparent between the two compounds.

Adenosine was identified through its comparison to known spectral data[2]. Clitocine was found to exhibit the following physical properties: mp 228-230°C; UV (EtOH) 255 (ε 7950) and 330 nm (ε 2190); IR 3456, 1600, 1510, 1220 cm^{-1}. The ^{13}C NMR spectra of clitocine showed strong similarities to adenosine[2] (Table 1). In particular, the carbon resonances of the sugar moiety of clitocine agreed closely with the corresponding signals of adenosine. Mass spectral fragmentation patterns of these two compounds also showed strong resemblances to each other: 156 (B+2H), 184 (BH-COH) on clitocine and 136 (B+2H), 164 (BH-COH) on

Table 1. Carbon-13 chemical shift of clitocine in DMSO

Carbon no.	PPm	multiplicity
2	159.3	d
4	155.8	s
5	111.8	s
6	158.6	s
1'	83.9	d
2'	74.9	d
3'	70.0	d
4'	86.1	d
5'	60.4	t

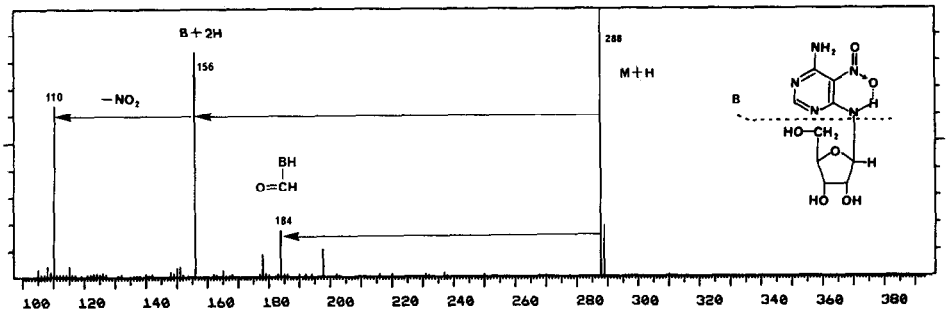

Figure 1. The CI-MS (NH_3) spectrum of clitocine

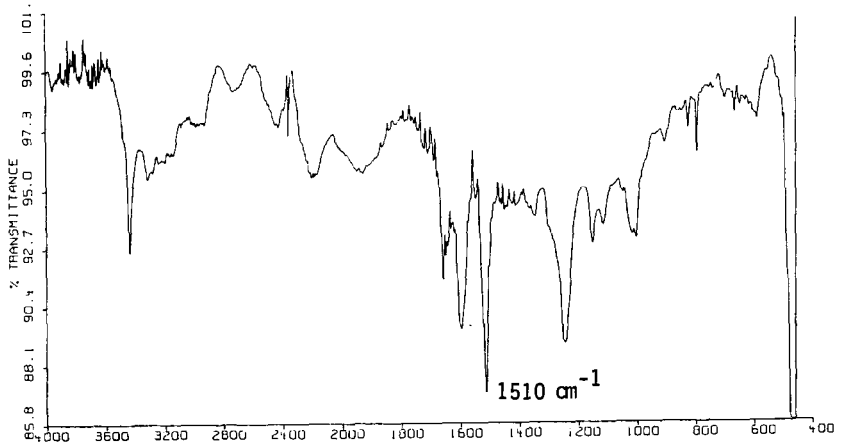

Figure 2. The FT-IR spectrum of clitocine

adenosine[3]. However, they differed by 20 mass units, as is shown in Figure 1. Clitocine lacked the signal corresponding to the C-8 carbon of adenosine. The greater mass of clitocine (20 amu) and its lack of adenosine's C-8 suggest the lacking carbon was replaced by nuclei totaling 32 amu in mass. Analysis of the IR spectrum of clitocine showed the characteristic absorption band at 1510 cm^{-1} indicative of a nitro group[4] (Fig. 2). These observations are consistent with the compound resulting from biosynthetic oxidation of adenosine to remove the C-8 carbon followed by oxidation of the N-7 nitrogen to a nitro group.

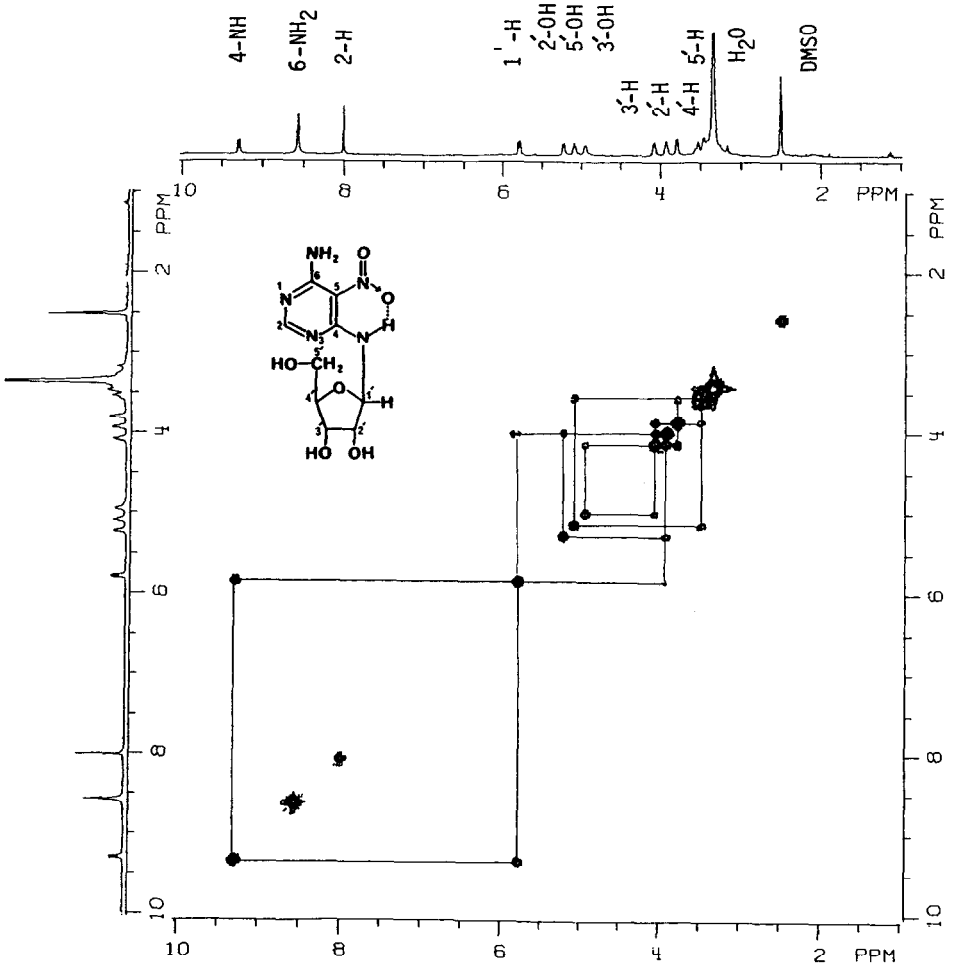

Figure 3. The 300 MHz ^{1}H-^{1}H 2D NMR spectrum of clitocine in DMSO-d_6

The 300 MHz ^{1}H-NMR spectrum of clitocine showed resonances typical of a ribose moiety [δ 5.8 (1H, d.d) for 1'-H, 4.1 (1H, d.d) for 3'-H, 3.9 (1H, d.d) for 2'-H, 3.8 (1H, m) for 4'-H, 3.5 (2H, m) for 5'-H, 5.2 (1H, d) for 2'-OH, 5.1 (1H, m) 5'-OH and 4.95 (1H, d) for 3'-OH] and a 4,5.6-trisubstituted-pyrimidine moiety [9.3 (1H,d) for 4-NH, 8.55 (2H, s) for 6-NH_2 and 8.0 (1H, s) for 2-H]. The assignment, corresponding to the proton NMR signals of ribose were readily established by ^{1}H-^{1}H 2D-NMR. The proton assignment of the 4-N<u>H</u> (δ 9.3) was also facilitated by the ^{1}H-^{1}H 2D-NMR correlation of (4)-N<u>H</u>-(1')-C<u>H</u>-(Fig. 3). The appearance at low field of the NH proton signal can be explained by strong hydrogen bonding between NO_2 and NH groups.

The proton assignment of the 6-N<u>H</u>$_2$ (δ 8.55, 2H) was confirmed by comparison with the NMR spectrum of its acetate derivative. Clitocine was acetylated in the usual manner to give a tetra-acetate having resonances at δ 2.2, 2.13, 2.1 ppm due to 2'-,3'-,5'-OC-C<u>H</u>$_3$ and δ 1.57 ppm due to 6-NHCOC<u>H</u>$_3$. In addition, a broad signal at δ 8.46 ppm integrating for only one proton indicated an amide proton (-N<u>H</u>COCH$_3$).

All the above data indicated clitocine to be 6-amino,5-nitro-4-imino-[ribofuranosyl] pyrimidine. Similarity in structure between clitocine and adenosine suggest clitocine may have similar properties and could act as an adenosine analog. Recently, Konno <u>et al</u> isolated a new amino acid betaine and a pyridine nucleoside from <u>Clitocybe acromelalga</u>[5,6]. However, nucleosides containing nitro functions are quite rare, and we believe this to be the first report of a naturally occurring nitro-containing nucleoside.

Acknowledgement. We thank Dr. D. Largent and Dr. B. Thompson for identification of the mushroom.

References and Notes

1. Various concentrations of test samples were applied to α-cellulose, and added to solid nutrients, vitamins and agar components of a meridic artificial diet. Newly molted larvae were placed on the diet in a plastic vials. Daily observations of molting and growth were made. I. Kubo, J. A. Kloche and S. Asano, J. Insect Physiol., <u>29</u>, 307 (1983).
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6. K. Konno, H. Shirahama and T. Matsumoto, Phytochemistry, <u>23</u>, 1003 (1984).
7. The insecticidal and other biological activity data of clitocine will be published elsewhere.

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