Tetrahedron Letters, Vol.22, No.17, pp 1617 - 1618, 1981
Printed in Great Britain

0040-4039/81/171617-02$02.00/0

ISOLATION AND STRUCTURE OF CLITHIONEINE, A NEW AMINO ACID BETAINE FROM CLITOCYBE ACROMELALGA.

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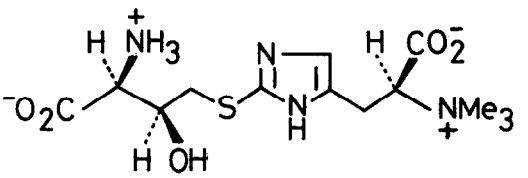
Abstract: A new amino acid betaine clithioneine was isolated from Clitocybe acromelalga. The structure was determined as 1 by spectral analyses and chemical degradations.

Our current interest in the chemical constituents of poisonous mushroom[1] led to the isolation of a new basic amino acid betaine from Clitocybe acromelalga. We wish to report in this communication the isolation and characterization of this compound for which we suggest the name clithioneine 1.

Crude clithioneine was obtained from fresh fruit bodies (16.2 kg) through sequential extraction (H_2O), precipitation (acetone), dialysis and chromatography on activated charcoal (10% EtOH). The crude clithioneine fraction (4g) was further purified by ion exchange resin Amberlite IRC-50 (2.5 % NH_4OH), paper electrophoresis at pH 4.6 (Py-AcOH buffer), cellulose TLC (MeOH-Py-H_2O=15:1:5, Rf 0.38) and finally by reversed-phase HPLC[2] (EtOH-H_2O=80:20) to yield amorphous powder[3] (38 mg), which showed dark yellow coloration with ninhydrin and yellow coloration with Pauly test[4]. Clithioneine showed the basic amino acid behavior on ion exchange resin and paper electrophoresis.



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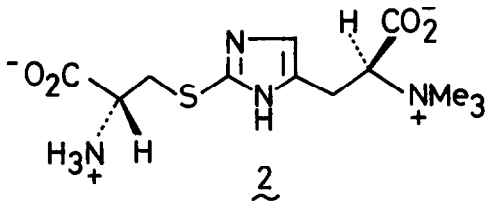
Clithioneine, $[\alpha]_D^{24}$+44.2° (C 0.5, H_2O), had a molecular formula $C_{13}H_{22}N_4O_5S$ [FD-MS; m/e 347.1334 $(M+1)^+$ calcd. for $C_{13}H_{23}N_4O_5S$ 347.1387], and showed IR absorption bands at 3600-2200, 1620 cm^{-1} (nujol). Since characteristic pH dependence in the UV spectrum [λ_{max}^{pH7} 246 nm (ε 16000) λ_{max}^{pH2} 254 nm (ε 17300), λ_{max}^{pH12} 248 nm (ε 15000)], and the ^1H NMR signal of aromatic proton [δ_{D_2O} 7.0 (s)] closely resembled those of S-(β-amino-β-carboxyethyl)-ergothioneine 2[5], it was thought probable that clithioneine might have an imidazole nucleus in a similar substitution pattern as in 2. It was supported by the ^{13}C NMR signals attributable to aromatic carbons [δ_{D_2O}

121.9 (d), 136.6 (s), 141.7 (s)]. The EI-MS spectrum did not show molecular ion peak, but the fragmentation pattern[6] was completely identical with that of ergothioneine 3. Therefore, the substituent at 5-position of the imidazole nucleus could be formulated as $-CH_2CH(\overset{+}{N}Me_3)CO_2^-$. The ^1H NMR and ^{13}C NMR spectra revealed the presence of this moiety [^1H NMR: δ_{D_2O} 3.24 (s, 9H), ^{13}C NMR: δ_{D_2O} 30.0 (t, $-CH_2-$), 54.8 (q, $-\overset{+}{N}Me_3$), 80.6 (d, $-\underline{C}H(\overset{+}{N}Me_3)CO_2^-$), 174.7 (s, $-CO_2^-$)]. The substituent at 2-position was deduced from the ^{13}C NMR spectrum [δ_{D_2O} 40.6 (t, $-SCH_2-$), 60.2 (d, $-\underline{C}H(\overset{+}{N}H_3)CO_2^-$), 71.6 (d, >CH-OH), 173.4 (s, $-CO_2^-$)].

The structure 1 deduced from spectral data was further confirmed by chemical degradation studies. Treatment of 1 with Raney-Ni yielded two ninhydrin-positive substances and one Pauly-positive substance. They were identified as L-threonine, L-α-amino-n-butyric acid and L-hercynine[7] respectively by comparison of their chromatographic behavior (cellulose TLC, reversed-phase TLC, paper electrophoresis), ^1H NMR, CI-MS and CD[8] spectra to those of the authentic samples.

These results clearly show that clithioneine is 2(S)-S-[2(S)-hydroxy-3(S)-amino-3-carboxypropyl]-ergothioneine 1.



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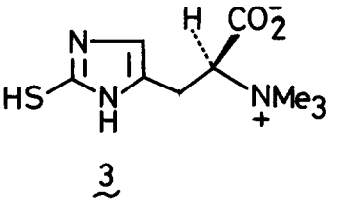

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Acknowledgement: We are indebted to Dr. T. Kanaiwa and Prof. T. Amiya (Hokkaido Institute of Pharmaceutical Science) for measurement of ^{13}C NMR spectra, and to Prof. T. Ueda (Faculty of Pharmaceutical Science of this university) for measurement of CD spectra.

References and Notes

1) K. Konno, K. Hayano, H. Shirahama, H. Saito and T. Matsumoto, Tetrahedron Letters, 481 (1977).
2) A CIG column ODS-30K (Kusano Kagakukikai Works Co. Ltd., Japan) was used under medium pressure (10-20 kg/cm^2).
3) Clithioneine has low toxicity with no death after intraperitoneal injection of 100 mg/kg to mice.
4) V.N. Reinhold, Y. Ishikawa and D.B. Melville, J. Med. Chem., 11, 258 (1968).
5) Y. Ishikawa, S.E. Israel and D.B. Melville, J. Biol. Chem., 249, 4420 (1974).
6) m/e 229, 215, 184, 140, 126, 102, 58; HR-MS m/e 229.0880 ($C_9H_{15}N_3O_2S$), 184.0858 ($C_8H_{14}N_3S$), 126.0243 ($C_5H_6N_2S$), 58.0654 (C_3H_8N).
7) The authentic sample of the this compound was synthesized according to ref. 4).
8) W. Gaffield, Chem. and Ind., 1460 (1964), J.C. Craig and S.K. Roy, Tetrahedron, 21, 391 (1965).

(Received in Japan 22 January 1981)