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STRUCTURE AND SYNTHESIS OF CLITIDINE, A NEW PYRIDINE NUCLEOSIDE FROM CLITOCYBE ACROMELALGA

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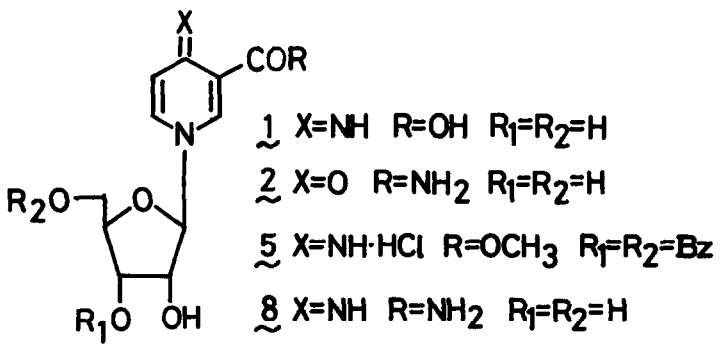
As a part of studies on physiologically active substances produced by mushrooms, we examined constituents of Clitocybe acromelalga (Japanese name Dokusasako). It has long been known in Japan that accidental ingestion of this toadstool produces markedly increased hyperemia and hyperesthesia in fingers and toes. We describe here isolation and characterization of a new pyridine nucleoside 1 for which we suggest the name clitidine.

Crude clitidine was obtained in 0.036% yield from frozen fruit bodies through sequential extraction (H_2O), precipitation (acetone), dialysis and chromatography on Sephadex G-10. The crude crystals were further purified by preparative paper chromatography (n-BuOH-H_2O saturated), paper electrophoresis at pH 4.6 (Py-AcOH buffer) and finally by recrystallization from water. The pure sample had elemental composition $C_{11}H_{14}O_6N_2.H_2O$, mp 189-191°, $[\alpha]_D^{24°}$ -50.6° (H_2O, c= 10.6mg/ml), exhibited uv maxima at 271nm (logϵ 4.09, H_2O), 267nm (4.16, pH 2), 271nm (4.18, pH 12), and showed ir absorption bands at 3300-2200, 1660, 1585, 1065, 1030 cm^{-1} (nujol). The nmr spectrum (in DMSO) indicated presence of a pentose moiety [δ 3.70 (2H, bs), 4.08 (3H, bs), 5.59 (1H, d, J=5Hz)] and a 3,4-disubstituted pyridine ring [δ 6.90 (1H, d, J=7Hz), 8.24 (1H, dd, J=7, 1.5), 8.7 (1H, d, J=1.5)]. The δ values of aromatic protons suggested that the substituents at C-3 and C-4 are electron attractive and electron donating respectively. Therefore, structure 1 or 2 was tentatively assigned to clitidine. Of these alternatives, formula 1 seemed more probable since it explained more reasonably the mass spectrum [m/e 138.0423 (100%, A), 121.0347 (13%, B), 93.0476 (13%, C)].

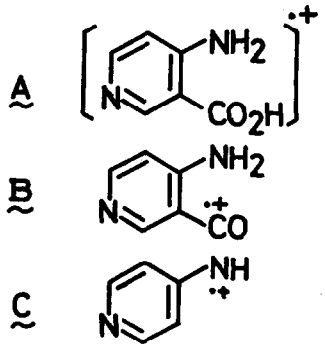


1 X=NH R=OH R_1=R_2=H

2 X=O R=NH_2 R_1=R_2=H

5 X=NH·HCl R=OCH_3 R_1=R_2=Bz

8 X=NH R=NH_2 R_1=R_2=H



The structure 1 was verified by an unambiguous synthesis. Condensation of methyl 4-aminonicotinate[1] 3 (47mg) with 3,5-di-O-benzoyl-D-ribofuranosyl chloride[2] 4 (97mg) was carried out in CH_2Cl_2 at ambient temperature for 1 day to give 5 [mp 160-161°; nmr δ (CD_3OD) 3.73 (3H, s), 3.5-4.0 (5H, m), 5.64 (1H, dd, J=6, 3Hz), 6.0 (1H, d, J=6), 7.11 (1H, d, J=8), 7.2-8.2 (10H, m), 8.31 (1H, dd, J=8, 2), 8.93 (1H, d, J=2); ir (nujol) 3440, 1730, 1665, 1270, 1100 cm^{-1}] (75mg, 82%), which was deblocked by treatment with Et_3N-H_2O-MeOH (1:4:11) solution at rt overnight. Clitidine thus obtained (60mg, 89%) was completely identical with the natural product (mp, ir, nmr, ms, paper chromatography and paper electrophoresis).

The question that in the above reaction the 4-amino group of 3 was ribosylated instead of the N-atom of pyridine nucleus was eliminated by the following series of the reactions. Methyl 4-chloronicotinate[1] 6 (75mg) was ribosylated with 4 in a similar manner to the reaction (3+4→) and the resulting product 7 was without purification treated with NH_3-MeOH (rt, 1 day) to give 8 [nmr δ (D_2O) 3.93 (2H, bs), 4.31 (3H, bs), 5.74 (1H, d, J=4.5Hz), 7.04 (1H, d, J=7), 8.23 (1H, dd, J=7, 1.5), 8.78 (1H, d, J=1.5)] (34mg, 26%) as a glass. On the other hand 8 was obtained also by treatment of 5 with NH_3-MeOH (rt, 1 day) and these two products from 5 and 7 were not distinguishable spectroscopically, paper chromatographically and paper electrophoretically.

Scince ribosylation employing 4 is known to give predominantly β-anomers[3], clitidine is formulated as 1,4-dihydro-4-imino-3-carboxy-1-(β-D-ribofuranosyl)pyridine 1[4,5].

Studies on the physiological activity of clitidine are now in progress.

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3) M. Jorman and W.C.J. Ross, J. Chem. Soc. (c), 199 (1969).
4) The β-configuration at anomeric position of clitidine was further confirmed enzymatically. The nicotinamide group of NAD was replaced by 3 in the presence of NADase from pig brain and the 4-amino NAD analogue thus produced yielded clitidine by further treatment with enzymes. S. Tonooka, A. Sasaki and S. Kakimoto, The 35th annual meeting of The Chem. Soc. Japan, Abstracts II, p. 684 (1976).
5) Although no naturally occuring pyridine nucleoside and nucleotide are known except for NAD and clitidine, a number of pyridine nucleosides have been synthesized and their physiological activities have been examined.
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