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ENZYMATIC SYNTHESIS OF CLITIDINE[1)]

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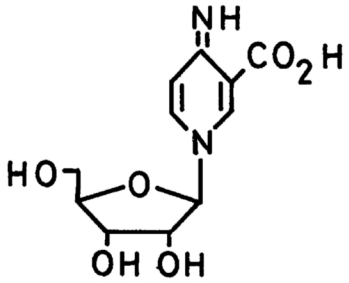
Clitidine (1) was synthesized by successive enzymatic cleavages of a new NAD-analogue, which was in turn prepared in a good yield from β-NAD and methyl 4-aminonicotinate utilizing the trans-glycosidation action of pig-brain NADase. The present synthesis establishes the β riboside structure of 1.

Recently, Konno et al[2)] have isolated a pyridine nucleoside, clitidine, from a toadstool, Clitocybe acromelalga, and its structure was found out to be 1. Since clitidine is the first naturally-occurring pyridine nucleoside containing an additional substituent to nicotinic acid, the compound is expected to have some physiological and pharmaceutical significance. This communication describes the enzymatic preparation[3)] of a new NAD-analogue (2a) in which nicotinamide group is replaced by methyl 1,4-dihydro-4-iminonicotinate[4)], and synthesis of clitidine through successive enzymatic cleavages[5)] of the demethylated analogue (3) obtained from 2a.



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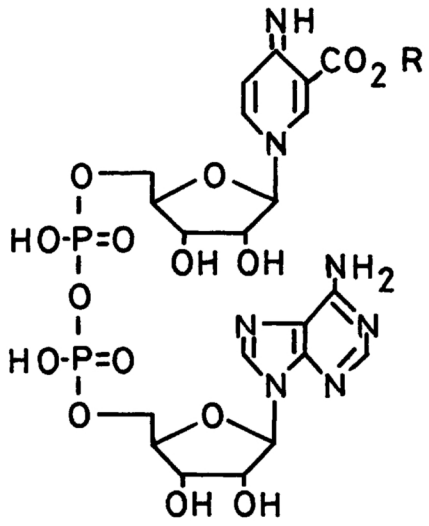
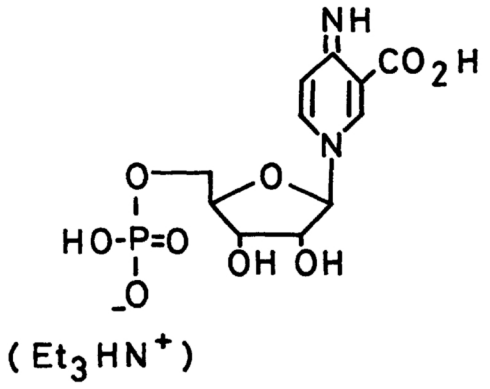
The ester-analogue was prepared as follows. A reaction mixture containing 0.05 M Tris-HCl buffer (pH 7.4, 40 ml), methyl 4-aminonicotinate[6)] (1.6 mmole, 243 mg), β-NAD (0.4 mmole, 265 mg) and pig-brain NADase[7)] (4.3 U[8)], 20 ml) was incubated for 8 hr at 37°. After removal of protein (50% TCA, 2 ml), cold acetone (300 ml) was added to the clear supernatant to afford crude hygroscopic white powder (240 mg). Column chromatography[9)] of the crude product with

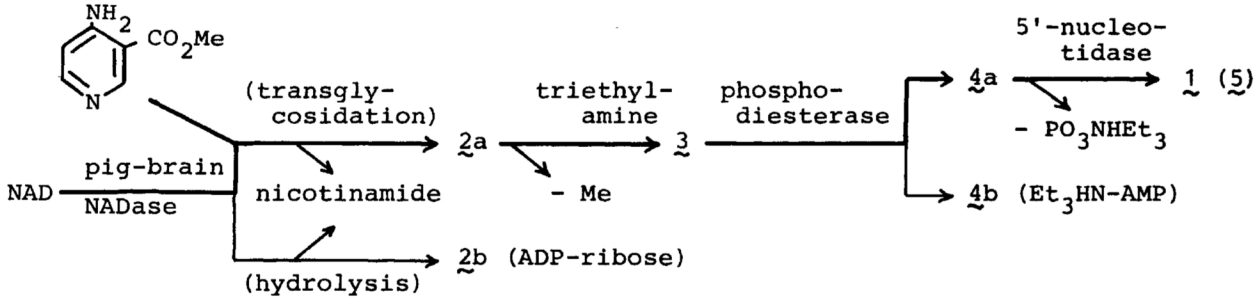




2a R = Me

3 R = H

4a



ammonium formate, followed by repeated lyophilization, gave two purified substances, 2a (142 mg)[10] and 2b (64 mg). 2b was identical with ADP-ribose by comparison with an authentic sample. The structure of 2a was characterized by the following spectral data. $[\alpha]_D^{18}$ = -23° (c = 2.1, H_2O). ν_{max}(KBr): 3300-2800, 1715, 1655, 1200, 1100, 1065 cm^{-1}. λ_{max} 264 nm (logε 4.2, H_2O). δ(60 MHz, D_2O): 3.95 (3H, s, CO_2CH_3), 4.2-4.7 (10H, bs), 5.64 (1H, d, J = 5.0 Hz, py-C_1'-H), 6.00 (1H, d, J = 5.5 Hz, ad-C_1'-H), 6.90 (1H, d, J = 7.5 Hz, py_5-H), 8.08 (1H, s, ad_2-H), 8.13 (1H, dd, J = 7.5, 1.5 Hz, py_6-H), 8.32 (1H, s, ad_8-H), 8.75 (1H, d, J = 1.5 Hz, py_2-H).

Hydrolysis of 2a (150 mg) with 0.2 M triethylamine (H_2O-MeOH) at 40° for 12 hr, followed by chromatography with ammonium formate, afforded 3 (138 mg). The structure of 3 was characterized by the PMR spectrum which showed the same

pattern as that of 2a except for the disappearance of the signal due to methyl group.

In order to obtain mononucleotide (4a), 3 was treated with phosphodiesterase (PDE). A mixture of 0.2 M Tris-HCl buffer (pH 9.2, 4 ml), 3 (150 mg), 0.2 M $MgCl_2$ (0.1 ml) and PDE (from snake venom, 2 U[8], 0.2 ml) was incubated for 5 hr at 37°, followed by chromatography with triethylammonium hydrogen carbonate (pH 8.0), to afford 4a (78 mg) and 4b (77 mg). 4b was identical with the monotriethylammonium salt of an authentic 5'-adenylic acid. 4a had one phosphoryl group (colorimetric method[11]) and one triethylammonium group (PMR-spectrum). The structure of 4a was characterized by the following spectral data. $[\alpha]_D^{18}$ = -12° (c = 0.60, H_2O). λ_{max} 268 nm (H_2O). $\delta(D_2O)$: 1.30 (9H, t, J = 7.5 Hz), 3.20 (6H, q, J = 7.5 Hz), 4.1 (2H, bs), 4.4 (3H, bs), 5.70 (1H, d, J = 5.0 Hz), 7.05 (1H, d, J = 7.5 Hz), 8.40 (1H, dd, J = 7.5, 1.5 Hz), 8.65 (1H, d, J = 1.5 Hz).

Then, a mixture of 0.1 M glycine buffer (pH 9.0, 3.5 ml), 4a (90 mg), 0.2 M $MgCl_2$ (0.2 ml) and 5'-nucleotidase (from snake venom, 15 U[8]) was incubated for 6 hr at 37°, followed by chromatography with 0.02 M triethylammonium hydrogen carbonate, to afford 5 [55 mg, mp 192°(dec)] in a quantitative yield. 5 was formulated as $C_{11}H_{14}O_6N_2 \cdot H_2O$ from elemental analysis and its spectral data were as follows. $[\alpha]_D^{18}$ = -54° (c = 0.37, H_2O). ν_{max}(nujol): 3400-3000, 1665, 1580, 1190 cm^{-1}. λ_{max} 271 nm (logε 4.12, H_2O). $\delta(D_2O)$: 3.9 (2H, bs), 4.3 (3H, bs), 5.67 (1H, d, J = 5.0 Hz), 6.92 (1H, d, J = 7.5 Hz), 8.10 (1H, dd, J = 7.5, 1.5 Hz), 8.70 (1H, d, J = 1.5 Hz). The mixed melting point of 5 with an authentic clitidine (1) showed no depression, and the spectral properties were also in good agreement with those reported by Konno et al[2].

Thus, clitidine was obtained in a good yield from β-NAD (41 %). The β-configuration of clitidine at the anomeric position was supported firmly, since the enzymatic reaction proceeds stereospecifically. In addition, the loss of the quarternary pyridinium ion character of 2a was shown by the following observations. 2a was no longer attacked by hydrolase action of NADase, while other pyridine analogues[12] with quarternary pyridinium linkage were susceptible to the enzyme. The signals of PMR spectra due to the anomeric proton (py-$C_1'\underline{H}$) of the former and its cleavage products (3 to 5) appeared at significantly higher field (δ5.64 - δ5.70) than those of the latters (δ6.00 - δ6.10). The ribose moiety of clitidine could not be detected by an orcinol reaction[13], while both the two ribose moieties

of NAD were sensitive to the reaction.

A probable availability of the enzymatic method for the synthesis of other pyridine nucleosides would also be anticipated. Further investigations are in progress.

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References and Notes

1) A part of this study was presented at the 35th Annual Meeting of The Chemical Society of Japan, Sapporo, September 1976.
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3) Preparation of NAD-analogues by enzymatic methods: P. Walter and N. O. Kaplan, J. Biol. Chem., 238, 2823 (1963), and references cited therein.
4) No detectable amount of NAD-analogue (acid-form) was formed from NAD and 4-aminonicotinic acid.
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7) N. O. Kaplan, "Methods in Enzymology", vol. 2, Academic Press, New York, (1955), p. 660.
8) 1 U is the amount of enzyme cleaving 1 μmole of corresponding substrate per minute.
9) All column chromatographies were carried out on DEAE-Sephadex A-25 in this study.
10) Satisfactory analytical data were obtained for this compound.
11) G. R. Bartlett, J. Biol. Chem., 234, 466 (1959).
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