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Unexpected Enzymatic $\underline{N}^1$-Glycosidation of Benzotriazoles as Evidenced by ^{15}N-NMR Spectroscopy

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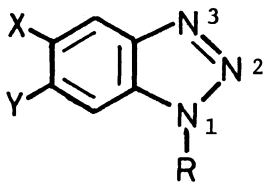
In contrast to $\underline{N}^2$-glycosidation for indazoles, benzotriazoles were found to undergo a regiospecific $\underline{N}^1$-glycosidation in the NADase-catalyzed transglycosidation reaction. The $\underline{N}^1$-ribosylated structure of benzotriazole dinucleotides obtained was established on the basis of ^{15}N-NMR spectral data.

In previous papers,[1] we have described the direct synthesis of various indazole adenine dinucleotides from NAD and indazole bases using porcine brain NADase-catalyzed transglycosidation. In that case, essentially only one dinucleotide was produced and the site of glycosidation was exclusively shown to be N^2-atom on the basis of the UV spectral properties of the indazole moieties. Our attention was then focused on a benzotriazole (BT) system having three ring-nitrogen atoms which can undergo a possible transglycosidation. It is thus of deep interest to examine whether the NADase-catalyzed reaction occurs or not and if occurs, which nitrogen is ribosylated. This communication describes unexpected regiospecific $\underline{N}^1$-glycosidation of some BT compounds that proceeds in the presence of NAD and porcine brain NADase.



1 X = Y = H
2 X = CH_3, Y = H
3 X = NO_2, Y = H
4 X = Cl, Y = H
5 X = Y = CH_3

a: R = H
b: R = adenosine diphosphoribosyl
c: R = 5'-phosphoribosyl

The enzymatic reaction was performed for BT bases (1a — 5a) in a similar manner as reported previously[1] and corresponding BT dinucleotides (1b - 5b) were isolated in good yields.[2] Judging from the $\underline{N}^2$-glycosidation for indazoles, the site of glycosidation in BT dinucleotides was initially expected also to be N^2-atom around which no essential steric hindrance occurs. On the contrary, the UV spectral pattern of BT moieties of respective dinucleotides showed remarkable similarities to that of corresponding original BT bases which exist mainly in $\underline{N}^1$-H form.[3] In order to examine the unexpected trends more clearly, we used ^{15}N-NMR spectroscopy[4] that can offer definitive informations on a distinction between a ribosylated and a non-ribosylated nitrogens. The spectrum of BT mononucleotide (1c)[5] showed three sharp and intense peaks I, II, and III at 377.3, 343.0, and 235.2 ppm, respectively. Such signal patterns

were also observed for other substituted BT nucleotides (2c – 5c).[6] Most downfield peak I (δ 377.3), the chemical shift of which is characteristic of a dicoordinated central nitrogen in the triazole system,[7] can readily be assigned to the "pyridine"-type N^2-atom. Thus, peak III (δ 235.2) is assignable to the ribosylated N^1-atom and peak II (δ 343.0) to another "pyridine"-type N^3-atom on the basis of the established criterion[8] that signals for substituted "pyrrole"-type nitrogens occur at higher field than for "pyridine"-type ones. If compound 1c took a N^2-ribosylated symmetrical quinonoid structure, peak I should be expected to occur at much higher field in the vicinity of peak II and peak III should be shifted by ca. 40 ppm to lower field.[8,9] In addition, four phenyl protons of 1c exhibited, in the ^{1}H-NMR spectrum, separate four signals (two doublets and two double doublets) instead of a symmetrical splitting pattern.[10] These observations lead to the conclusion that

$$-N^1H-N^2=N^3- \rightleftharpoons -N^1=N^2-N^3H- \quad (1)$$

there occurred a regiospecific N^1-ribosylation for the BT system in contrast to a N^2-ribosylation for indazoles.

Considering the facts that the transglycosidation did not take place for indole, but certainly took place for benzimidazole, the difference in regio-specificity of BT and indazole systems may be closely related to an autotropic rearrangement between two forms in the triazole (Eq. 1). In any case, it is noteworthy that the BT system was found to undergo a regiospecific N^1-glycosidation in the NADase-catalyzed transglycosidation reaction of NAD. Further studies are now under way to clearly account for the reaction mechanism.

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References

1) S. Tono-oka, I. Sekikawa, and I. Azuma, Chem. Lett., 1983, 805; S. Tono-oka, Y. Tone, V.E. Marquez, D.A. Cooney, I. Sekikawa, and I. Azuma, Bull. Chem. Soc. Jpn., 58, 309 (1985).
2) 1b: 80%, 2b: 76%, 3b: 66%, 4b: 43%, 5b: 33% (based on NAD used). Satisfactory spectral and analytical data were obtained for all these new compounds.
3) J. Eluguero, C. Marzin, A.R. Katritzky, and P. Linda, "The Tautomerism of Heterocycles," Academic Press, Inc., New York (1976), p. 295.
4) Determined in DMSO-d_6 solution (0.3 – 0.4 M) containing chromium (III) tris (acetyl acetonate) (10^{-2} M) on a Bruker MSL-400 spectrometer at 40.561 MHz with nitromethane as external standard, with later conversion (conversion factor, 380.23 ppm) to liquid ammonia standard.
5) Obtained by phosphodiesterase-catalyzed hydrolysis of compounds 1b – 5b.
6) E. g. 371.9, 334.4, and 232.9 ppm for 2c; 380.5, 339.0, and 234.0 ppm for 3c.
7) G.C. Levy and R.L. Lichter, "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy," John Wiley & Sons, Inc., New York (1979).
8) W. Philisborn and R. Mueller, Angew. Chem., Int. Ed. Engl., 25, 383 (1986).
9) S. Nagarajan, S.R. Wilson, and K.L. Rinehart, Jr., J. Org. Chem., 50, 2174 (1985).
10) In case of N^2-substitution, an AA'BB'-type signals are enough expected to occur.

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