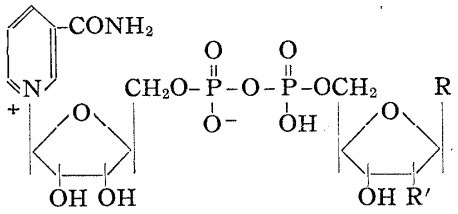
UDC 612.398.145

Synthesis of Diphosphopyridine Nucleotide Analogs and their Reaction with Dehydrogenases

Several diphosphopyridine nucleotide (DPN) analogs[1~5] have so far been prepared enzymatically, but the present writers recenty succeeded in the chemical synthesis of their new analogs in which the adenosine portion of DPN is replaced by naturally occurring nucleosides derived from RNA*1 and DNA, and their reaction with dehydrogenases was investigated.



d-DPN R=adenine, R′=H
G-DPN R=guanine, R′=OH
C-DPN R=cytosine, R′=OH
U-DPN R=uracil, R′=OH
T-DPN R=thymine, R′=H

DPN extracted from baker's yeast was decomposed enzymatically with potato pyrophosphatase[6] and the resulting NMN, after being confirmed as β-form by measurement of its optical rotation, was first reacted with d-AMP in hydrous pyridine in the presence of DCC. The product was chromatographed on Dowex-1 (formate-form), the fraction eluted with 0.01*N* formic acid (Fig. 1) was adsorbed on charcoal, and the eluate of the charcoal with 50% ethanol containing 0.5% ammonia was found to contain only d-DPN by the following tests :

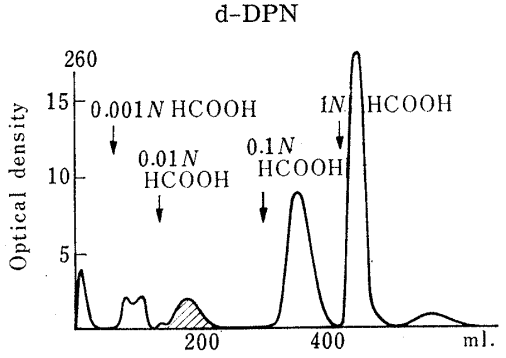


Fig. 1.

a) Paper electrophoresis (0.05*M* phosphate buffer, pH 8.0) gave only one ultraviolet absorbing spot showing the same migration value as that of DPN, b) paper electrophoresis (pH 8.0) after reaction with potato pyrophosphatase yielded two ultraviolet-absorbing spots,

*1 Abbreviations used : RNA, ribonucleic acid; DNA, deoxyribonucleic acid; NMN, nicotinamide mononucleotide; d-AMP, deoxyadenosine 5′-phosphate; DCC, dicyclohexylcarbodiimide; d-DPN, deoxy-DPN; GDH, glucose dehydrogenase; GMP, guanosine 5′-phosphate; CMP, cytidine 5′-phosphate; UMP, uridine 5′-phosphate; TMP, thymidine 5′-phosphate; G-DPN, nicotinamide guanine dinucleotide; C-DPN, nicotinamide cytosine dinucleotide; U-DPN, nicotinamide uracil dinucleotide; T-DPN, nicotinamide thymine dinucleotide; dG-DPN, deoxy-G-DPN; dC-DPN, deoxy-C-DPN; ADH, alcohol dehydrogenase; LDH, lactic dehydrogenase; DPNH, reduced DPN; G-DPNH, reduced G-DPN; T-DPNH, reduced T-DPN.

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the positions of which were in agreement with those of NMN and d-AMP. The eluate of the spot corresponding to d-AMP showed the same ultraviolet spectrum as that of this compound, c) deoxyribose : total phosphorus=1:2.28 (theoretical value, 1:2).

The concentration of d-DPN was calculated from the organic phosphorus content in the above eluate and the following constants were obtained : ε_{260} 18.0×10^3, $[\alpha]_D$ $-20.5°$. The Km of d-DPN for liver GDH was 6.8×10^{-5} (pH 7.6). The ammonium salt of d-DPN was isolated as a hygroscopic white crystalline powder by addition of acetone to the concentrated eluate.

Similarly, G-DPN, C-DPN, U-DPN, and T-DPN were also synthesized by reacting β-NMN with GMP, CMP, UMP, and TMP, respectively (Fig. 2~5). Attempts were also made to synthesize dG-DPN and dC-DPN, but their poor yield prevented their separation.

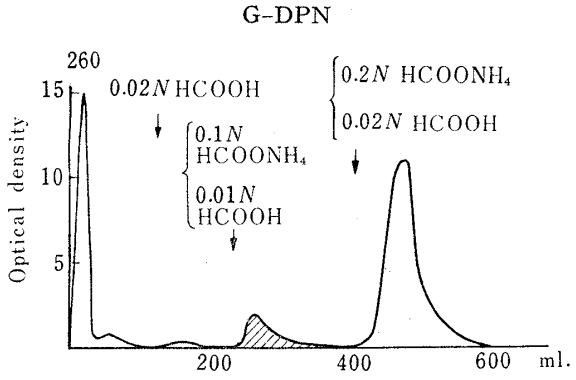

Fig. 2.

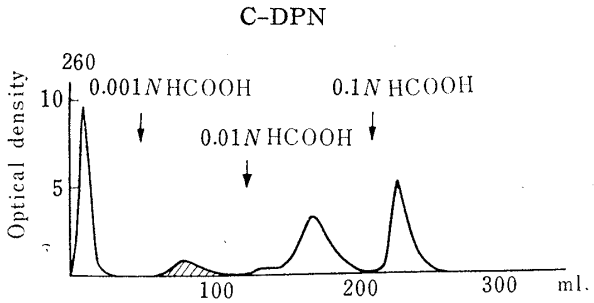

Fig. 3.

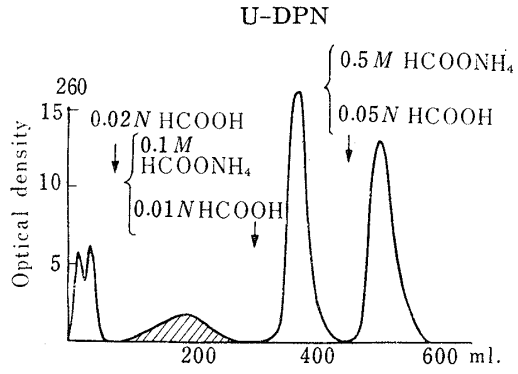

Fig. 4.

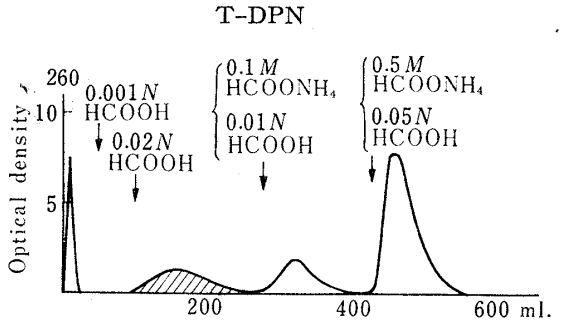

Fig. 5.

Next, reaction of the five DPN-analogs thus obtained with dehydrogenases was examined. The comparative rate of reaction of DPN-analogs with that of DPN is summarized in Table I. U-DPN and T-DPN were not reduced in the yeast ADH system and they did not inhibit the reduction of DPN in the same system. In contrast, U-DPN reacted with liver GDH more rapidly than DPN. The reaction of DPN with muscle LDH was investigated after reduction of DPN with GDH, because the equilibrium of DPN + lactate $\rightleftarrows$ DPNH + pyruvate was markedly shifted to DPN side. DPNH and G-DPNH were equally reactive in this case. T-DPNH was only ca. 30% as reactive as DPNH and it did not affect the oxidation of DPNH in the same system.

Table I. Rate of Reaction of the DPN Analogs to that of DPN in the Dehydrogenase Systems

Dehydrogenase \ Analogs	DPN	d-DPN	G-DPN	C-DPN	U-DPN	T-DPN
Yeast ADH	100	10	4	4	0	0
Liver GDH	100	90	95	90	125	35
Muscle LDH	100	60	90	80	80	30

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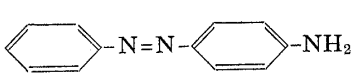
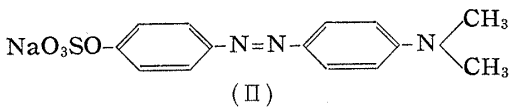
Metabolites of *p*-Dimethylaminoazobenzene in Rat Bile

Since the work of Stevenson and his co-workers[1] on the metabolism of *p*-dimethylaminoazobenzene, a carcinogenic aminoazo dye, many reports have been published about it and the work was reviewed by Miller and Miller.[2] Some new metabolites of this dye were also found by Ishidate and Hashimoto[3] in this laboratory. Majority of the past work concerned with urinary metabolites and, since it has been observed that many conjugated and non-conjugated aminoazo dyes were excreted in a rat bile during liver perfusion technique originated by Ishidate and Nakajima,[4] examination using a whole animal to detect these metabolites in rats bile was carried out. A solution of 15 mg. of *p*-dimethylaminoazobenzene dissolved in 1 cc. of olive oil was injected into the stomach of a rat through a catheter. After 1 hour, the rat was anesthetized with Nembutal, and a polyethylene tube with external diameter of ca. 1 mm. was inserted into the bile duct by surgical operation. The bile was collected for the following 5 hours, spotted directly on Toyo Roshi filter paper No. 51, and subjected to chromatography with a solvent system of PrOH-BuOH-H_2O (2:3:5).

The paper chromatogram revealed more than eight kinds of aminoazo dyes and these were subjected to qualitative analyses; color reaction with 2*N* hydrochloric acid, Ehrlich reagent, or Gibbs reagent, hydrolysis of conjugated form with *β*-glucuronidase or diastase, separation by partition chromatography over silica gel, adsorption chromatography on alumina, or paper electrophoresis, and measurement of absorption spectra. By comparison with synthesized aminoazo dyes, the main metabolites in the bile were identified with the following seven kinds of dyes :


(I)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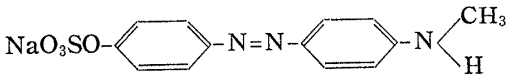
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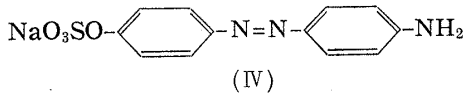
(IV)

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3) M. Ishidate, Y. Hashimoto : This Bulletin, **10**, No. 2 (1962).
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