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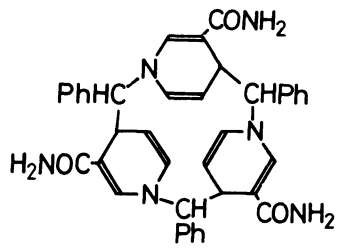
REACTIONS OF NAD^+-MODEL COMPOUNDS WITH BASE. THE REVISED STRUCTURE OF DITTMER'S TRIMER

Yutaka OHNISHI*
Sagami Chemical Research Center, Nishi-Ohnuma, Sagamihara, Kanagawa 229
Hiroshi MINATO, Kentaro OKUMA, and Michio KOBAYASHI
Department of Chemistry, Tokyo Metropolitan University, Setagaya, Tokyo 158

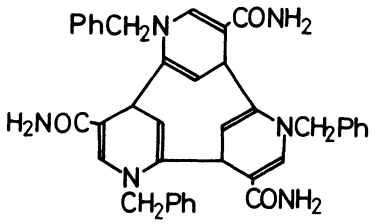
The structure of the cyclic trimer obtained by the reaction of N-benzylnicotinamide cation with base was established. A plausible mechanism involves the removal of the amide hydrogen with base, the attack of the amide anion at C-4 of another pyridinium ion, and the formation of a 12-membered ring.

Reactions between nucleophiles and quarternary salts of nicotinamide are of interest in connection with the biological behavior of the oxidized forms of pyridine nucleotides.[1] Dittmer and Kolyer treated 1-benzyl-3-carbamoylpyridinium chloride with NaOH in EtOH-H_2O (72/28 v/v/) at room temperature and obtained a trimer to which they assigned Structure I.[2] Two of the authors reported that their experimantal findings are accounted for not by Structure I but by Structure II.[3] In this communication, we wish to present the evidence showing that Dittmer's trimer has Structure III.

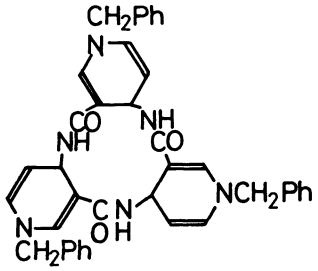
Dittmer's trimer was prepared by the method described in the literature[2] and showed the same melting point, molecular weight, and elemental analysis data as those reported. When this trimer was dissolved in CD_3OD, disappearance of the PMR signal of the amide proton was not observable because this compound is unstable in solution and the proton-deuterium exchange of the amide hydrogen is not fast. Fig. 1 compares the PMR spectrum (Varian XL-100) of Dittmer's trimer and that of its deuterated analog obtained from the reaction of 1-benzyl-3-carbamoylpyridinium-4-d. These highly-resolved spectra appear to be not consistent with Structures I and II, and Structure III is suggested as the alternative.



I



II



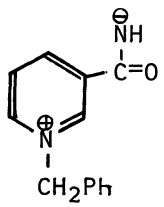
III

The signal at δ 5.89 which is absent in the spectrum of the deuterio compound must be due to the proton on C-4 of the pyridine ring which splits signals of the amide proton and the proton on C-5 into doublet of doublet. The signals of the proton on C-5 (δ 4.86) are further split into a quartet by coupling with the proton on C-6 (δ 6.04). The irradiation at δ 6.04 simplified the doublet signal at δ 7.16, supporting the idea that the signal at δ 7.16 is due to the proton on C-2. Chemical shifts and coupling constants are reasonable compared to those of related known compounds.[4] Furthermore, the ^{13}CMR spectra (Varian-100-15A-TT-100) with the off-resonance proton decoupling technique show unambiguously that Structure III is correct. The experiment with the deuterio compound established that the amide nitrogen attacked not C-2 nor C-6 but C-4 of the N-benzylnicotinamide cation.

The reaction of 1-phenyl-3-carbamoylpyridinum salt with NaOH in EtOH-H_2O gave a similar product; m.p. 210°(dec) and elemental analysis (Calcd as $C_{12}H_{10}N_2O$, C, 72.71, H, 5.05, N, 14.14; Found, C, 72.62, H, 5.02, N,14.13). Upon treatment with HCl it regenerated the starting material as its benzyl analog did, and gave the spectrum shown in Fig. 2. In this case, the signals of the protons on the amide and on C-2 are much separated from those of aromatic protons, and the spectrum can easily be analyzed. When this N-phenyl trimer was treated with CD_3OD, the doublet signal at the lowest field was quenched, and consequently it was assigned to the amide proton. The assignments of PMR and CMR spectra of the trimeric N-benzyl and N-phenyl derivatives are shown in Fig. 3.

Dittmer and Kolyer reduced the N-benzyl trimer with H_2/Raney-Ni and obtained a compound, having m.p. 260-261°, but its structure has not been established yet.[2] The determination of the structure of this partially-reduced compound should help ascertain the structure of the trimer. By using the same procedure[2] we obtained crystals melting at 262-263°(dec) with the molecular weight 578 (in $CHCl_3$)(Calcd 647), and found that it is the 1,4,5,6-tetrahydropyridine derivative shown in Fig. 3. The chemical shifts and the integral ratios of the protons in its PMR spectrum are consistent with the structure shown in Fig. 3, in which the C_5-C_6 bond of the N-benzyl trimer III has been reduced. Other products conceivable by partial reduction of I, II, or III are not consistent with the data obtained. Although the proton-deuterium exchange technique could not be used for identification of the amide proton, the decoupling of the signal at δ 6.83 or δ 5.08 revealed that the doublet signal at δ 6.83 split with a large coupling constant(J=10 Hz) can be assigned to the amide proton. Moreover, the irradiation at δ 1.84 simplified the collapsed broad signal at δ 5.08 into a clear doublet and converted the complex signals at δ 2.9-3.5 to a broad but clear AB quartet (J_{AB}= 13 Hz, $\nu_0\delta_{AB}$= 26.7). The CMR data are consistent with the structure shown in Fig. 3.

The most reasonable precursor of the trimer is not the pyridinium ylide[2] nor the 6-hydroxy compound[3] but the pyridinium betaine shown here. A similar amide anion of NAD^+ may be a precursor of NAD^+-carbonyl adduct, which is formed more readily in frozen system than in liquid system and is assumed to be an intermediate in NAD^+-dependent biological hydrogen transport.[5]



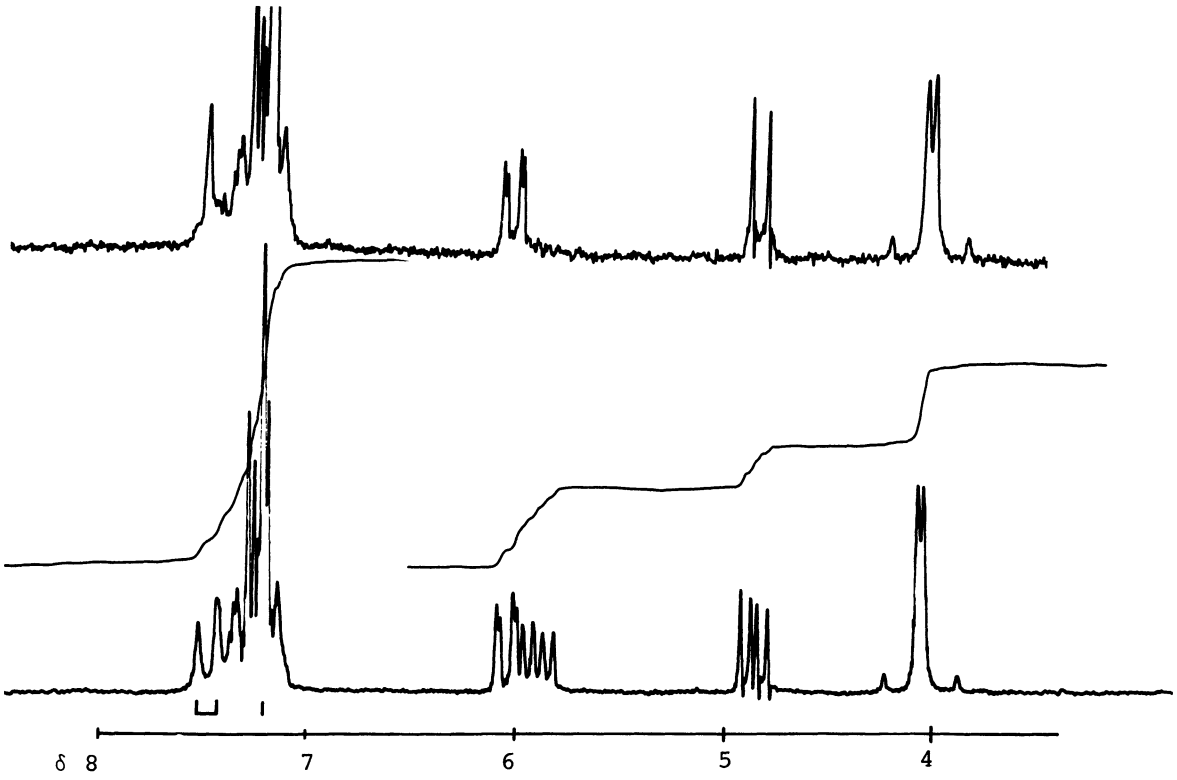


Fig.1. PMR spectra of the trimer of 1-benzyl-3-carbamoylpyridinium ion (Dittmer's trimer)(lower) and its deuterio derivative(upper).

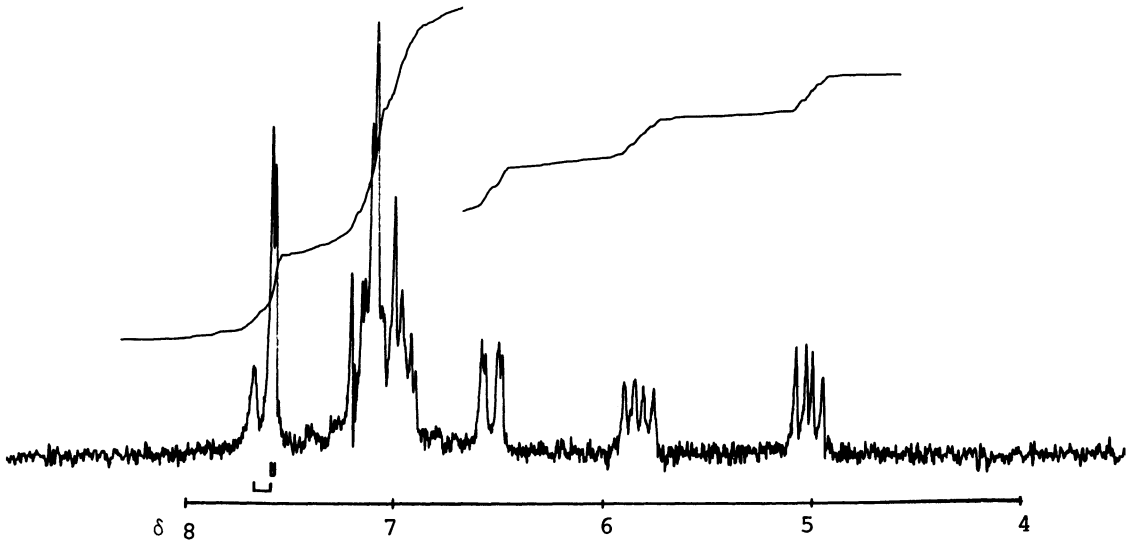


Fig. 2. PMR spectrum of the trimer of 1-phenyl-3-carbamoylpyridinium ion.

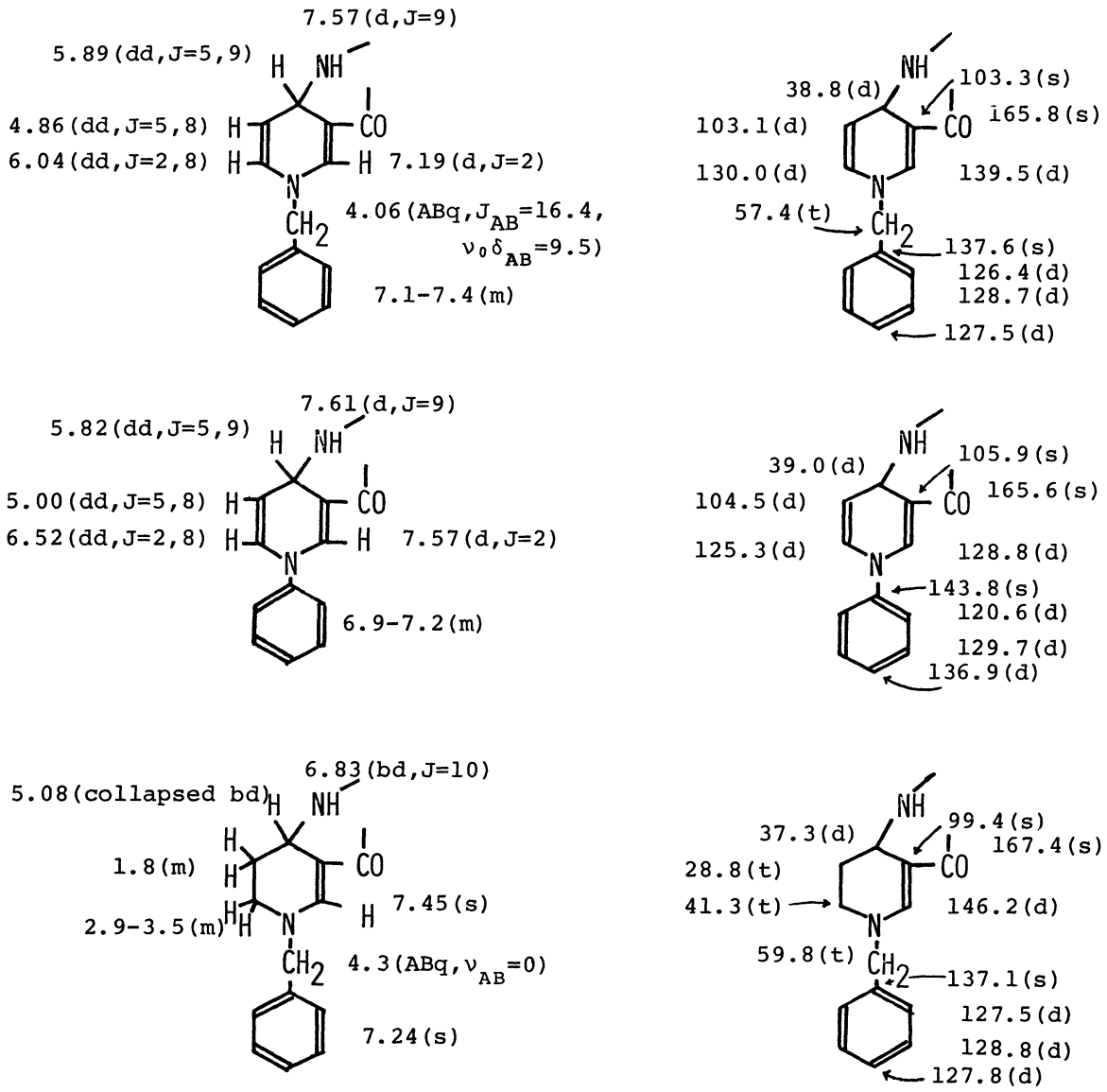


Fig. 3. Assignments of PMR and CMR signals. (The numbers represent chemical shifts, δ, from TMS in $CDCl_3$).

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