STRUCTURE AND PROPERTIES OF THE ACETONE ADDUCTS OF 1-n-PROPYL NICOTINAMIDE IODIDE*

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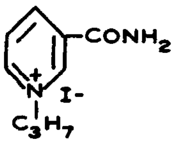
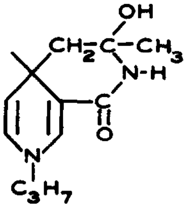
Carbonyl derivatives of 1-methyl nicotinamide iodide and NAD have been studied by spectrophotometry and fluorescence (Huff, 1947). The structure of these derivatives was proposed on the basis of their ultraviolet spectra resemblance to the 1,4-dihydronicotinamide derivatives (Burton *et al.*, 1957). Recently, NAD^+ carbonyl addition compounds were shown to be formed by keeping commercial samples of NAD^+ in frozen weakly alkaline solutions (Dolin *et al.*, 1963). To our knowledge, no proof of structure for the acetone adducts of the 1-substituted nicotinamide cations has as yet been described.



I



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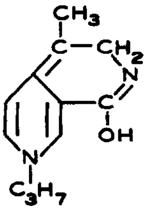
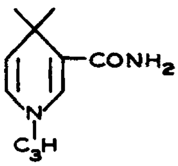


III



IV

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Addition of acetone to 1-n-propyl nicotinamide iodide--One liter of acetone was added to 30 g. of (I) in 200 ml. of 0.5 M Tris, pH 11. To the stirred solution, 12 ml. of 7 M KOH were added in a 1 hr. period. To obtain (II), 1/2 the volume of the reaction mixture was shaken with 1,500 ml. ether. The ether-acetone layer was dried with 450 g. of anhydrous Na_2SO_4 for 30 min. and evaporated in vacuum at room temperature to a volume (about 40 ml.) in which a crystalline compound comes out. Recrystallization in a minimum volume of boiling methanol yields (II) (1-2 g.), M.P. 127°-127.5°. To obtain (III), the remaining volume of the reaction mixture was left standing at room temperature for 24 hours. Compound (III) was isolated as above. Yield 2-3 g. M.P. 186°-187°; MW = 206 (theory; MW = 204), as determined by osmometry in isopropanol at 40°. Both compounds gave good elemental analysis.

The structures of (II) and (III) were determined by comparison with the structure of (IV) on the Varian A-60 spectrometer using $CDCl_3$ solutions containing tetramethylsilane, as internal reference (Fig. 1). In all three spectra, the signals around $\delta = 0.90$, 1.60 and 3.05 are due to $-CH_2-\underline{CH_3}$, $-\underline{CH_2}-CH_3$ and $-N-\underline{CH_2}-CH_2-$protons, respectively.

In the spectrum of (IV), the resonance at $\delta = 3.16$ corresponds to the methylene protons at C_4. The pair of triplets at $\delta = 4.70$ is due to H_5. This pattern is caused by spin-coupling of H_5 to H_6 and the methylene protons at C_4 with coupling constants of 8 and 3.4, respectively, while the pair of quartets at $\delta = 5.74$ represents H_6. This proton, besides being spin-coupled to H_5, is also coupled to protons at C_4 and C_2 with the same coupling constants, namely 1.8 cps. The split peak at $\delta = 7.02$, a typical value for a proton β

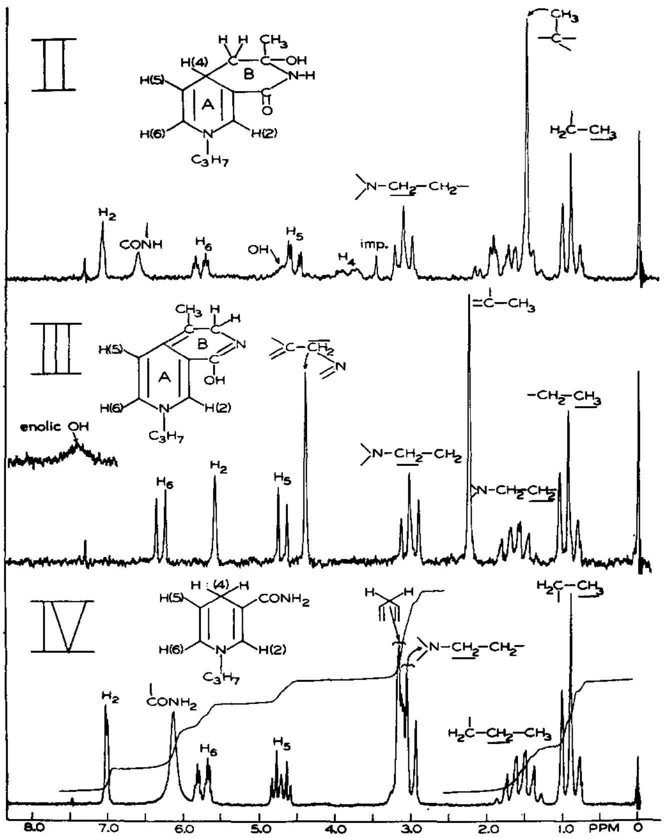

Fig. 1. NMR spectra

to a carbonyl group, is due to H_2. The broad signal at $\delta = 6.13$ represents the $CONH_2$ group protons.

In spectrum of (II), the resonances at $\delta = 4.53$, 5.76 and 7.05 correspond to H_5, H_6 and H_2, respectively, and are very similar to the ones in (IV), indicating that the ring A, as well as the carbonyl group at C_3, are the same in (II) and (IV). The resonance patterns for H_5 and H_6 in (II), however, change from the pairs of triplets and quartets in (IV) to pairs of doublets and triplets, respectively, in (II), indicating that C_4 has only one proton rather than two as in (IV). The signal due to this proton is at $\delta = 3.72$. This proton is further coupled to adjacent CH_2 protons. The latter two protons, being non-equivalent, have different spin-coupling constants to H_4, whose resonance is split by a large spin-coupling of 12 cps. by one of the CH_2 protons and a small coupling of 4 cps. by the other. The resonances due to this CH_2 proton are located around $\delta = 1.9$, a typical value. The peaks at $\delta = 6.57$ and 4.66, representing one proton each, are due to the labile protons $-\overset{O}{\overset{\|}{C}}-\overset{|}{N}-\underline{H}$ and $-CH_2-\underset{N=}{\overset{Me}{C}}-\underline{OH}$, respectively. The tall peak at $\delta = 1.47$ is caused by $Me-\underset{OH}{C}-CH_2-$. The spectrum of (III) shows signals at $\delta = 2.23$ and 4.29 due to $=C-Me$ and $=C-CH_2-N=$ protons, respectively, characteristic values for protons in such environments. The doublets having spin-coupling constants of 7 cps. at $\delta = 4.68$ and 6.30 correspond to H_5 and H_6, respectively. The chemical shift for H_6 to a lower field may be attributed to the greater conjugation in (III) as compared to (IV). The resonance at $\delta = 5.57$ is due to H_2. This upfield shift indicates the absence of a carbonyl group at C_3. The broad peak at $\delta = 12.40$ representing one proton is due to the enolic OH.

The ultraviolet spectra of (II) and (III) are not the same (Fig. 2). In diluted aqueous buffered solutions, (II) dissociates reversibly and almost to completion into (I) and

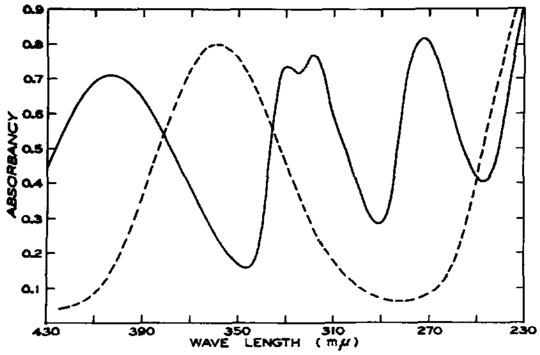

Fig. 2. Absorption spectra of II (---) and III (——) 10^{-4} M methanolic solutions.

acetone (Fig. 3). The dissociation rate of (II) increases with the increasing pH of the medium up to pH 9, on which the dissociation of (II) is completely independent of alkalic concentration.

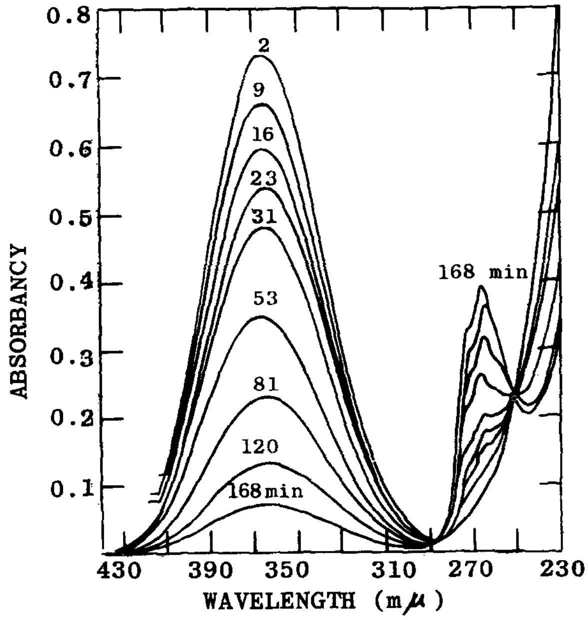

Fig. 3. Dissociation of II (10^{-4} M) in 0.1 M Tris, pH 9. At neutral pH or in methanol, (II) does not dissociate and at acid pH, it is irreversibly destroyed. Unlike (II), (III) does not dissociate. These compounds rapidly reduce a cold solution of $AgNO_3$. Compounds (II) and (III) are fluorescent. Using the Aminco-Bowman Spectrofluorometer, (II) has an emission band at 460 mμ when exited at 360 mμ; (III) has an emission band at 485 mμ with exitation peaks at 285, 340 and 410 mμ. Thus some of the fluorophors responsible for the fluorescence of (I) in alkaline acetone solutions are (II) and (III).

With methods described in a previous publication (Ludowieg and Levy, 1963), (II) or (III) reduces NAD^+ to NADH by direct hydrogen transfer. Reductions were carried out in weakly alkaline solution mixtures at the final concentration of 10^{-2} M for each component. When the reaction was performed with NAD-nicotinamide-4-t, the isotope was found equally distributed at both sides of C-4 of the nicotinamide ring of the reduced nucleotide.

References

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