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COENZYME ANALOGS

I. PREPARATION AND PROPERTIES OF THIONICOTINAMIDE METHIODIDE

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SUMMARY

1. Thionicotinamide methiodide, a "model" for thionicotinamide adenine dinucleotide, was prepared from nicotinamide methiodide and P_2S_5.

2. The absorption spectra of thionicotinamide, thionicotinamide methiodide and 1-methyl-1,4-dihydrothionicotinamide show maxima expected for the substitution of sulfur for oxygen when these are part of the chromophore.

3. 1-Methyl-1,4-dihydrothionicotinamide undergoes a photochemical reaction in visible light in the presence or absence of oxygen.

4. 1-Methyl-1,4-dihydrothionicotinamide combines with silver nitrate; however, a 10% excess of the metal appears to be consumed when the reaction is followed spectrophotometrically and a 2-fold excess when followed amperometrically.

INTRODUCTION

KARRER and coworkers[1] first demonstrated that *N*-methylnicotinamide serves as a suitable model for the reactions of NAD^+. Replacement of the nicotinamide moiety of NAD^+ with various 3-substituted pyridines has led to the availability of a large number of dinucleotide analogs. One of the analogs which was recently made available commercially is thionicotinamide adenine dinucleotide in which sulfur is substituted for the oxygen of nicotinamide. This substitution results in the appearance of some interesting spectrophotometric properties as well as a new chemically active site. The chemical and physical properties of thionicotinamide methiodide and the photochemistry of the reduced "model" are reported in this paper.

MATERIALS AND METHODS

Thionicotinamide was synthesized from 3-cyanopyridine and hydrogen sulfide according to the method of KARRER AND SCHUKRI[2].

Thionicotinamide methiodide was prepared by a modification of the procedure of HOFMANN AND GABRIEL[3]. Nicotinamide methiodide (5.0 g), from nicotinamide and

Abbreviation: EPA, ethyl ether–isopentane–ethyl alcohol (5:3:2).

methyl iodide, was placed in a porcelain mortar and heated to 70°. Phosphorus pentasulfide (10.0 g) was added to the pyridinium salt in small portions, and the powders were thoroughly mixed under the pressure of a pestle until a tarry mass was formed. The dark material was exhaustively extracted with methanol until the extract was no longer yellow. The product was precipitated with ether and recrystallized from hot methanol–ether. Long yellow needles (35% yield) were obtained and dried at 65° under vacuum. Melting point, 198.5–199.5°. Calculated for $C_7H_9N_2SI$ (mol. wt., 280.14): C, 30.00; H, 3.24; N, 10.00. Found: C, 29.74; H, 3.49; N, 9.93.

1-Methyl-1,4-dihydrothionicotinamide was prepared by dissolving 3.0 g of thionicotinamide methiodide in 50 ml of 9% $NaHCO_3$. Sodium dithionite (5.0 g) was added in small aliquots to the solution which was maintained at 4° under a stream of helium. Orange crystals precipitated from the reaction mixture after standing in the cold for several hours, and the product was washed with cold water on a sintered-glass funnel and dried for 5 h at 65° under vacuum. Melting point, 109–110.5°. The yield was 50% of theoretical.

A methanolic solution of the product had λ_{max} 412.5 mμ, ε 10 900.

Calculated for $C_7H_{10}N_2S$ (mol. wt., 154.24): C, 54.51; H, 6.54; N, 18.17. Found: C, 54.24; H, 6.33; N, 17.94.

The photoproduct of 1-methyl-1,4-dihydrothionicotinamide was prepared by dissolving 500 mg in 1 l of water and adjusting the pH to 9.5 with NaOH. The solution was irradiated by sunlight for 3 h while being flushed with helium. The mixture was concentrated by freeze-drying, and a methanolic solution of the amorphous material was placed on Whatman 3mm paper for chromatographic separation. The solvent system used was butanol–water (3:7). The most prominent yellow band (R_F 0.76) was eluted from the paper with methanol.

Argentimetric titration of 1-methyl-1,4-dihydrothionicotinamide.

(*a*) *Amperometrically*: 1 μmole was dissolved in 30 ml of 0.1 M Tris–nitrate buffer (pH 7.4) and equilibrated in a thermostated titration vessel. The titration was carried out according to BENESCH, LARDY AND BENESCH[4]. The apparatus, described by MURAYAMA[5], consisted of a thermostated titration vessel equipped with a rotating platinum electrode, a gas inlet tube for deaerating with helium, a salt bridge to a calomel electrode, and a microburette containing 10^{-2} M silver nitrate solution. An automated time-controlled device delivered 0.1 μmole of titrant every 60 sec. A Leeds and Northrup chart-recorder having a range of 12 sensitivities, designed by MURAYAMA (manuscript in preparation), was used to follow the current during the titrations.

(*b*) *Spectrophotometrically*: 3 μmoles of 1-methyl-1,4-dihydrothionicotinamide was diluted to 50 ml with 0.1 M Tris–nitrate buffer (pH 7.4). The absorption spectrum was scanned from 500 to 300 mμ prior to titration and within 3 min after each addition of silver nitrate. The concentrated (0.1 M) silver nitrate was added by means of a micropipette manufactured by Roger Gilmont Instruments, Inc. Each addition of silver nitrate to the thione was accompanied by the addition of an equivalent amount of titrant to the blank.

Absorption spectra were measured on a Cary Recording Spectrophotometer, Model 14, having a thermostated cell compartment.

Emission properties were examined on a modified Aminco-Bowman Spectrophotofluorometer equipped with a cell compartment designed to accommodate a

Dewar flask with quartz windows to allow examination of rigid glass solutions at low temperatures.

Solvents were Spectroquality grade purchased from Matheson, Coleman and Bell.

RESULTS

Spectral properties

Thionicotinamide: The absorption spectrum of thionicotinamide in methylene chloride is shown in Fig. 1. The inset illustrates the long-wavelength absorption band with an expanded extinction-coefficient scale. The oscillator strength of the band was calculated to be $f \cong 0.008$. When the spectrum was examined in methanol, the long-wavelength maximum had shifted to the 380-mμ band. The weak band disappeared completely when the pyridine nitrogen was quaternized in thionicotinamide methiodide (see below).

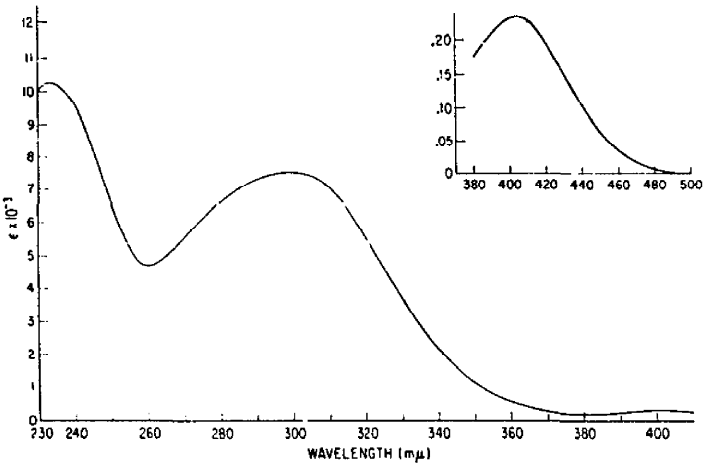

Fig. 1. Absorption spectrum of $9.43 \cdot 10^{-5}$ M solution of thionicotinamide in methylene chloride. The inset represents the long-wavelength absorption of a more concentrated solution ($1.886 \cdot 10^{-3}$ M).

The magnitude of the oscillator strength[6], the "blue shift" on increasing solvent polarity[7], and the disappearance of the band in the methahalide[8], are in accord with the assignment of $n \rightarrow \pi^*$ transition to the longest-wavelength band. The more intense bands at 300 and 232.5 mμ are absent in nicotinamide and are in the vicinity of absorption maxima reported for other thionamides[9].

Thionicotinamide methiodide: Attempted alkylation of thionicotinamide with methyl iodide generally resulted in the production of a strong mercaptan odor and isolation of nicotinamide methiodide from the reaction mixture. Similar results were reported in the reaction of thionicotinamide with dichlorobenzyl bromide[10]. Successful synthesis of thionicotinamide methiodide was effected by treating nicotinamide methiodide with P_2S_5 (see METHODS).

The absorption spectrum of thionicotinamide methiodide is illustrated by the dashed curve in Fig. 2. The long-wavelength band which was present as a shoulder in thionicotinamide is no longer detectable. The 261-mμ maximum is found in the absorption spectra of a large number of 3-substituted pyridinium salts[11].

1-Methyl-1,4-dihydrothionicotinamide: RAFTER AND COLOWICK[12] demonstrated

that reduction of nicotinamide methiodide with sodium dithionite takes place at the *para* position of the pyridine ring. Dithionite reduction of thionicotinamide methiodide yielded an orange crystalline product whose absorption spectrum is shown by the solid curve in Fig. 2. The position of the long-wavelength absorption maximum near 415 mμ is consistent with the hypsochromic shift of 40–50 mμ usually observed when oxygen is replaced by sulfur as part of the chromophore[13]. (The absorption maximum of 1-methyl-1,4-dihydronicotinamide is at 365 mμ.)

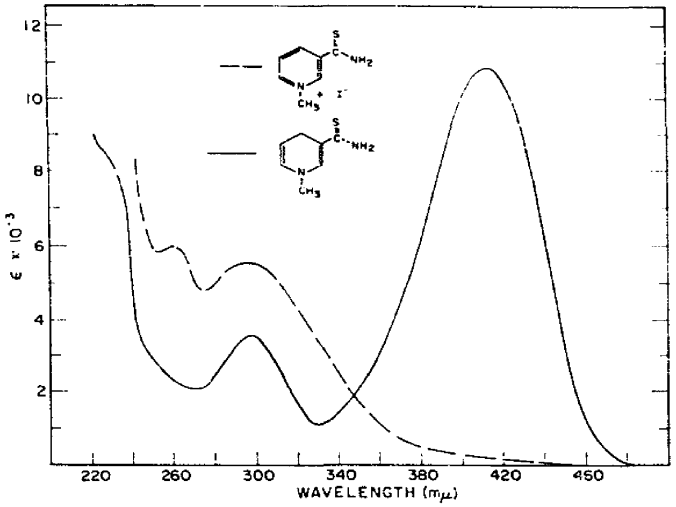

Fig. 2. Absorption spectrum of thionicotinamide methiodide (dashed curve) and 1-methyl-1,4-dihydrothionicotinamide (solid curve).

A summary of the absorption maxima and their corresponding extinction coefficients is given in Table I.

Fluorescence properties. The emission of 1-methyl-1,4-dihydrothionicotinamide in methylene chloride and methanol was examined using an exciting wavelength of 415 mμ. There was no detectable fluorescence at room temperature; however, in EPA rigid glass solution at 77° K, emitted light was easily seen with a maximum near 495 mμ. On warming to room temperature the intensity of the emitted light was undetectable.

TABLE I

ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS OF THIONICOTINAMIDE, THIONICOTINAMIDE METHIODIDE AND 1-METHYL-1,4-DIHYDROTHIONICOTINAMIDE

Compound	*Solvent*	λ_{max} (mμ)	ε
Thionicotinamide	Methylene chloride	403.2	230
		300	7600
		232.5	10 300
Thionicotinamide methiodide	Methanol	297.5	5500
		261	6000
1-Methyl-1,4-dihydro-thionicotinamide	Methanol	412.5	10 900
		297.5	3500

thionamide as if it were present in the thiolimide form. Recent studies on the absorption of tautomeric thiones have demonstrated that C=S is preferred to the thiolenol form[17,18] in the absence of an approaching alkyl halide.

The absorption maxima of thionicotinamide, thionicotinamide methiodide and 1-methyl-1,4-dihydrothionicotinamide are consistent with the hypsochromic shift of 40–50 mμ when oxygen is replaced by sulfur as part of the chromophore[13]. The persistence of the 295–300-mμ band in these three compounds prompts the assignment of the absorption band to the C=S group.

The thionamide function was also reactive with potassium cyanide after the thionicotinamide methiodide–cyanide adduct was formed. Absorption studies of the product with excess cyanide suggested the hydrolysis of the thione to nicotinamide methiodide. The ease of hydrolysis and the reactivity of the thionamide is sufficient warning that reagents generally used in studying the reactions of NAD^+ or its model compounds cannot be used indiscriminately.

Photochemical transformation of thiocarbonyl compounds has been examined in visible and ultraviolet light[19]. In the presence of visible light and oxygen, oxidation of the thiones to the corresponding ketones takes place (*e.g.*, thiobenzophenone → benzophenone). By analogy, 1-methyl-1,4-dihydronicotinamide should have been the photoproduct obtained by irradiation of the thionamide. The oxygen compound has been well characterized by its absorption and fluorescence properties and by its instability in acid. The photoproduct does not show any of these properties.

On the basis of the optical properties of thiones BUROWAY[20,21] proposed that they may exist in a free-radical form to a small extent. This suggestion has also been made on the basis of the phosphorescent behavior of thiobenzophenone and the tendency for many thiones to polymerize[20]. The photochemical reaction may also be initiated by free radicals.

Complex formation of 1-methyl-1,4-dihydrothionicotinamide with silver nitrate resulted in a product whose absorption spectrum was very similar to that of the photoproduct. SCHONBERG AND STOLPP[23] proposed the structure of the silver perchlorate complex of diaryl thiones to be

$$[Ar_2C{-}S{-}Ag]^+ ClO_4^-$$

The structure of the silver complex of the dihydrothionicotinamide might similarly be written as

$$\left[\begin{array}{c} Py{-}C{-}S{-}Ag \\ | \\ NH_2 \end{array}\right]^+ X^-$$

Cysteine has been found to consume silver nitrate in a 10–12 % excess as measured amperometrically[21]. SLUYTERMAN[24] proposed that the consistently high value was associated with the presence of a free amino group. The reduced thionamide similarly consumed a large excess of silver nitrate when the titration was followed spectrophotometrically. However, when the titration was followed amperometrically, a ratio of 2 $AgNO_3$: 1 thione was observed. This 2-fold excess was not accounted for by complexation at sites other than the sulfur atom. A longer time interval elapsed between additions of silver nitrate in the spectrophotometric assay permitting equilibration with the sulfur atom than the 60 sec between additions in the amperometric method. This may account for the discrepancy in the two methods.

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