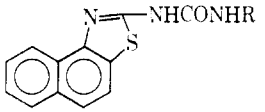
TABLE II

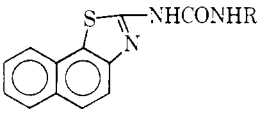
1-(2-NAPHTHO[1,2-*d*]THIAZOLYL)-3-SUBSTITUTED UREAS



No.	R	Mp, °C	Formula	Cpe virus ED_{50}, mg/kg	Drug level (mg/kg × 3) for fourfold or greater immunosuppression
61	C_6H_5	320–322	$C_{18}H_{13}N_3OS$	>100	12.5
62	1-$C_{10}H_7$	269–270	$C_{22}H_{15}N_3OS$	42	3.1
63	4-ClC_6H_4	310–311	$C_{18}H_{12}ClN_3OS$	28	100.0
64	3-ClC_6H_4	314–315	$C_{18}H_{12}ClN_3OS$	21	1.6
65	2-ClC_6H_4	321–322	$C_{18}H_{12}ClN_3OS$	49	6.2
66	2,5-$Cl_2C_6H_3$	315–316	$C_{18}H_{11}Cl_2N_3OS$	55	100.0
67	4-FC_6H_4	308–309	$C_{18}H_{12}FN_3OS$	16	12.5
68	2-FC_6H_4	318–319	$C_{18}H_{12}FN_3OS$	<16	3.1
69	4-$NO_2C_6H_4$	293–294	$C_{18}H_{12}N_4O_3S$	17	50.0
70	3-$NO_2C_6H_4$	264–265	$C_{18}H_{12}N_4O_3S$	<16	3.1
71	2-$NO_2C_6H_4$	303–304	$C_{18}H_{12}N_4O_3S$	<16	3.1
72	3,4-$Cl_2C_6H_3$	305–306	$C_{18}H_{11}Cl_2N_3OS$	18	3.1
73	4-$CH_3C_6H_4$	307–308	$C_{19}H_{15}N_3OS$	17	6.2
74	3-$CH_3C_6H_4$	317–318	$C_{19}H_{15}N_3OS$	23	3.1
75	2-$CH_3C_6H_4$	319–320	$C_{19}H_{15}N_3OS$	22	1.6
76	C_6H_{11}	245–246	$C_{18}H_{19}N_3OS$	34	3.1
77	Adamantyl	242–243	$C_{22}H_{23}N_3OS$	18	3.2

TABLE III

1-(2-NAPHTHO[2,1-*d*]THIAZOLYL)-3-SUBSTITUTED UREAS



No.	R	Mp, °C	Formula	Cpe virus ED_{50}, mg/kg	Drug level (mg/kg × 3) for fourfold or greater immunosuppression
78	C_6H_5	353–354	$C_{18}H_{13}N_3OS$	23	0.8
79	1-$C_{10}H_7$	346–347	$C_{22}H_{15}N_3OS$	<16	0.4
80	4-$CH_3C_6H_4$	369–370	$C_{19}H_{15}N_3OS$	<16	>25.0
81	C_6H_{11}	354–355	$C_{18}H_{19}N_3OS$	27	>12.5

Potential Coenzyme Inhibitors. III.[1] Some Reactions of Substituted Nicotinamide and Dihydronicotinamide Derivatives

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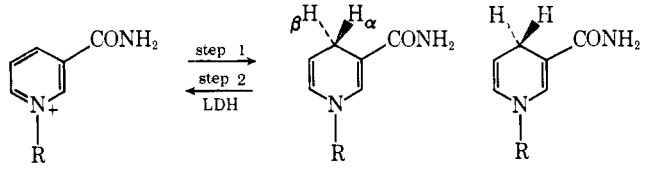
The reaction between KCN and substituted nicotinamide derivatives was examined, and a number of cyanide derivatives have been isolated. The spectroscopic evidence shows that CN^- addition occurs at the 4 position of the pyridine ring. Equilibrium constants for these reactions have been calculated from the absorption spectra, and the influence exerted by the 4-Me substituent upon the rate of addition is discussed. The H-transfer reactions between 2,6-dichlorophenolindophenol and some substituted dihydronicotinamide derivatives were examined by visible absorption spectroscopy. Rate constants for the oxidation reactions at different H^+ concentrations were calculated. The reaction rates have been related to the effects of the substituents attached to the nicotinamide ring.

The glycolytic pathway of carbohydrate metabolism involves an oxidative step in which glyceraldehyde phosphate is converted into diphosphoglyceric acid. In this reaction the pyridine ring of the cofactor (NAD, I) accepts an H atom in the β configuration giving NADH (II).[2] Cancer cells are relatively deficient in NAD and this coenzyme must be regenerated from NADH if continuous energy production is to be maintained. This is achieved by the reduction of pyruvate

(1) Previous paper in this series: A. C. Lovesey and W. C. J. Ross, *J. Chem. Soc., B*, 692 (1969).

(2) F. A. Loewus, H. R. Levy, and B. Vennesland, *J. Biol. Chem.*, **223**, 589 (1965).

to lactate and in this process an α-oriented H atom is transferred.[3]

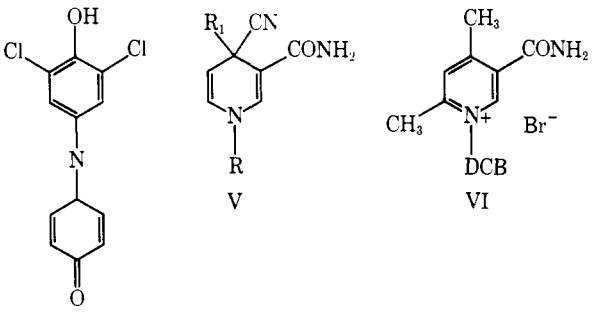


I II III

Normally the NADH produced in step 1 can be utilized in step 2. If, however, the H atom attached to C-4 of the pyridine ring of NAD is replaced by some other group R, step 1 would produce a dihydropyridine derivative (III) which cannot be used in step 2 since it contains no α-hydrogen atom. The presence of a 4-substituted NAD could therefore affect the glycolytic process which involves recycling of NAD. Numerous substituted nicotinamides are known to be incorporated into NAD *in vivo* and it should be possible to achieve the desired effect by administering a substituted nicotinamide to the tumor-bearing host.

This approach will be effective if (1) the substituted nicotinamide (N*) is incorporated into the NAD molecule to give the analog (N*AD); (2) the N*AD is a coenzyme for the glyceraldehyde to glyceric acid oxidation, otherwise respiration in normal cells may be hindered; (3) H addition to the N*AD is 1,4 and also if the stereospecificity of N*AD-mediated H-transfer reactions is the same as for those of NAD; (4) the rate of H transfer is comparable with that of the NAD–NADH system; and (5) N*AD competes favorably with NAD for association with the apoenzyme.

These aspects are being systematically examined[1,4] and it has been shown that reaction of dithionite with 4-methyl-substituted nicotinamide derivatives leads to 1,4 addition[1] just as in the reduction of NAD to NADH by the same reagent,[5] which mimics the enzymic process. Most of these points can only be tested when 4-substituted NAD derivatives are available; the synthesis of 4-Me-NAD has been achieved on a small scale.[6] In the meantime it is possible to study some of the reactions involved using model substances. The quaternary compounds used in this study have been the N-benzyl, -propoxymethyl, and -tetraacetyl-β-D-glucopyranosyl salts derived from nicotinamide and 4-methylnicotinamide, there being indications in the literature[7] that the rates of reaction of the propoxymethyl and tetraacetyl-β-D-glucopyranosyl salts more closely approach those of the natural coenzyme (NAD). Point 4 (above) is also important because the glycerophosphate "shuttle" mechanism which is available in normal cells and involves a β-specific H transfer from NADH[8] is lacking in cancer cells,[9] which have a lower NAD content. To establish whether H transfer from 4-Me-substituted dihydronicotinamide derivatives takes place at a rate comparable with that of dihydronicotinamides, the rates of H transfer from some substituted dihydronicotinamide derivatives to 2,6-dichlorophenolindophenol (IV) have now been studied. The results are discussed in the latter section of this work.



IV V VI

Cyanide Addition Reactions.—The affinity of nicotinamide salts for anions (CN^- in particular) has been used to obtain an indication of the chemical reactivity of the pyridine ring.[7] Many 3-substituted pyridinium salts react with CN^- to form adducts which are in equilibrium with the reactants. 1,4-Dihydropyridine structures (V, R_1 = H) have been proposed by San Pietro[10] on the basis of D-exchange reactions. The 4-H only undergoes exchange with the medium, and it was reasoned that this H is acidic because the cyanide group is in the α position to the H atom. Furthermore, the absorption spectra of the cyanide adducts from various pyridinium salts were similar to those of 1,4-dihydropyridines.[11,12] However, as Kosower has pointed out,[13] the D exchange may not uniquely indicate a 4 location for the cyanide group in the adduct since there is no proof that it is the adduct itself which undergoes the exchange, and no evidence bearing directly on the point of attachment of the cyanide group to the pyridine nucleus has so far been available.

Walter and Kaplan[14] have found that the CN^- reaction with 4-Me-NAD was very slow (250 times smaller than for NAD) and they have suggested that the 4-Me substituent may interfere sterically with the addition to the 4 position, and that the reaction may not proceed in the usual way, but that addition may occur to other positions on the ring. Little CN^- addition takes place with 3-carbamoyl-1-(2,6-dichlorobenzyl)-4,-6-dimethylpyridinium bromide (VI). This result has also been attributed to steric hindrance by the 4-Me[12,15,16] and it is therefore important to ascertain the position of the cyanide group in the products from the reactions between CN^- and quaternary nicotinamide salts, and also the cyanide position in the 4-Me-substituted adducts. The spectroscopic data for the CN^- addition products derived from the nicotinamide and 4-methylnicotinamide salts studied in the present work are given in Table I. The uv spectra presented in Table I show that 2-cyano-1,2-dihydronicotinamide structures are inconsistent with the recorded physical

(3) N. O. Kaplan, "The Enzymes," Vol. 3, Academic Press, New York, N. Y., 1960, Chapter 12.
(4) W. C. J. Ross, *J. Chem. Soc., C*, 1816 (1966).
(5) M. B. Yarmolinsky and S. P. Colowick, *Biochim. Biophys. Acta*, **20**, 117 (1956).
(6) F. Searle (Institute of Cancer Research), private communication, 1969.
(7) K. Wallenfels, "Steric Course of Microbiological Reactions," J. & A. Churchill Ltd., London, 1959, p 10.
(8) H. R. Levy and B. Vennesland, *J. Biol. Chem.*, **238**, 85 (1957).
(9) G. E. Boxer and T. M. Devlin, *Science*, **134**, 1495 (1961).
(10) A. San Pietro, *J. Biol. Chem.*, **217**, 579 (1959).
(11) M. Marti, M. Viscontini, and P. Karrer, *Helv. Chim. Acta*, **39**, 1451 (1956).
(12) K. Wallenfels and H. Schuly, *Ann.*, **621**, 215 (1959).
(13) E. M. Kosower, ref 3, Chapter 13.
(14) P. Walter and N. O. Kaplan, *J. Biol. Chem.*, **238**, 2823 (1963).
(15) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Section 2.13.
(16) J. Biellmann and H. J. Callot, *Bull. Soc. Chim. France*, 1159 (1968).

TABLE I: SPECTRAL DATA

No.	R′	R₂	X	Uv spectra[a] λ_{max}, mμ (log ε)	Nmr spectra[b] Chemical shifts, τ[c] 2-H	4-H	5-H	6-H	4-Me	NCH_2	Others	Coupling constants, cps $J_{2,6}$	$J_{4,5}$	$J_{5,6}$	$J_{4,6}$
1	Pm	H	CN	323 (3.71)	7.16 d	4.52 q	4.87 q	6.28 o		4.60 s	3.38 t,[d] 0.99 t[d]	1.3	7.9	4.3	0.9
2	Pm	Me	CN	321 (3.82)	7.10 d		4.82 d	6.20 q	0.87 s	4.58 s	3.29 t,[d] 0.92 t[d]	1.1	8.0		
3	Tg	H	CN	322 (3.75)	7.21 d	4.50 q	4.94 q	6.35 o		5.29 m	4.7 5.6 m,[e] 3.99 m,[f] 1.18 m[g]	1.2	7.1	4.3	1.3
4	Tg	Me	CN	325 (3.80)	7.05 d		4.82 d	6.22 q	0.80 s	5.27 m	4.7 5.6 m,[e] 3.99 m,[f] 1.18 m[g]	1.3	8.2		
5[h]	Bz	H	CN	324 (3.83)	7.13 d	4.50 q	4.81 q	6.17 o		4.45 s	7.31 m[i]	1.7	7.7	4.3	1.5
6	Pm	H	H		7.16 d	3.01 q	4.79 sx	6.10 m		4.50 s	3.38 t,[d] 1.00 t[d]	1.3	8.0	4.3	1.1
7	Pm	Me	H		7.17 d	3.15 m	4.86 q	6.14 o	1.14 d	4.54 s	3.36 t,[d] 1.00 t[d]	1.3	8.3	4.6	1.0
8	Tg	H	H		7.14 d	3.06 q	4.48 sx	6.35 m		5.25 m	4.7 5.6 m,[e] 3.89 m,[f] 1.17 m[g]	1.3	7.1	4.3	1.3
9	Tg	Me	H		7.15 d	3.19 m	4.52 q	6.25 o	1.08 d	5.25 m	4.7 5.6 m,[e] 3.91 m,[f] 1.17 m[g]	1.3	8.3	4.3	1.2
10	Bz	H	H		7.17 d	3.08 q	4.76 sx	5.80 m		4.31 s	7.33 m[i]	1.5	8.0	4.3	1.5
11	Bz	Me	H		7.24 d	3.28 m	4.82 q	6.00 o	1.15 d	4.40 s	7.31 m[i]	1.7	7.7	4.6	1.3

[a] Recorded in 95% EtOH. Uv spectra for **6**–**11** given in previous paper.[4] [b] Recorded in MeCN. TMS = 0. Compounds **6**–**11** have been previously recorded in $CDCl_3$. [c] Me, 2.7–3.2, and 4.1–4.4. [d] Pm, propoxymethyl; Tg, tetraacetyl-β-D-glucopyranosyl; Bz, benzyl. [e] s, singlet; d, doublet; t, triplet; q, quartet; sx, sextet; o, octet; m, multiplet. [f] 4-Me analog not isolated (see Table II for very low equilibrium constant value).

data, since the maximum wavelength absorption for 1,2-dihydronicotinamide derivatives is known to occur[7] at considerably longer wavelengths than those given in the table. The absence of absorption in the region 220–300 mμ indicates that 1,6-dihydronicotinamide structures are unlikely.[7]

The nmr spectra clearly show that the cyanide compounds (**1**–**5**) have 1,4-dihydronicotinamide structures. The magnitude of the coupling constants (8.0–8.2 cps) obtained from the spectra of the 4-Me-substituted cyanide derivatives (**2**, **4**) is typical of coupling across a double bond. This confirms that the cyanide compounds are not 6-cyano-1,6-dihydronicotinamide derivatives because such coupling is not possible in a 6-cyano-1,6-dihydro-4-methylnicotinamide structure and the 5,6 coupling (typical coupling constant value 3.4–4.2 cps)[1] associated with 1,6-dihydro-4-methylnicotinamide derivatives is not seen in the nmr spectra of the cyanide compounds.

The close similarities between the coupling constants and between the chemical shifts of the cyanide compounds and those of the corresponding 1,4-dihydronicotinamide derivatives[4] (**6**–**11**) confirms the 1,4-dihydronicotinamide structures of the cyanide compounds. Only the chemical shifts of the 4-protons differ between the two series and this appreciable difference is clearly attributable to the deshielding influence of the adjacent 4-cyano group. Because of the position of this group, no 4-proton resonance is observed in the spectra of the 4-Me-substituted compounds (**2**, **4**) and 4,5 and 4,6 proton coupling is not detected in these compounds.

The cyanide addition to 4-methylnicotinamide quaternary compounds to give 3-carbamoyl-4-cyano-1,4-dihydro-4-methylpyridine derivatives shows that there is little steric hindrance from the 4-Me. The lack of cyanide addition to the 4,6-dimethyl quaternary bromide (VI) therefore cannot be attributed to steric hindrance by the 4-Me as reports in the literature have previously suggested[12,15,16] but is probably due to the electronic effect from the 6-Me substituent. The inductive effect from the 6-Me should tend to reduce the positive charge on N-1 which affects the reactivity of the 4 position in pyridinium salts.[17] In agreement with this suggestion, NAD analogs such as 3-methylpyridine-AD, 3-aminopyridine-AD, and 3-acetamidopyridine-AD do not react with CN^- and this has been ascribed to the loss of the electrophilic properties of the 4 positions of the pyridine rings in these compounds.[18] Presumably for the same reason the N*AD derived from 6-aminonicotinamide (a powerful NAD antagonist and inhibitor of tumor growth[19]) undergoes none of the addition reactions typical of natural pyridine nucleotides.

In Table II the equilibrium constants for the cyanide addition reactions are given. These were calculated by the method described by Wallenfels.[7,20]

The equilibrium constants show that the 4-Me considerably weakens the reactivity of the 4 position toward addition. The k values of the 4-Me compounds are reduced to between 0.05 and 0.09% of the values for

(17) R. A. Barnes, F. Brody, and P. R. Ruby, "Chemistry of Heterocyclic Compounds," Interscience Publishers, Inc., New York, N. Y., 1960 Part 1, Chapter 1.

(18) G. M. Anderson and N. O. Kaplan, *J. Biol. Chem.*, **234**, 1226 (1959).

(19) L. S. Dietrich, L. A. Kaplan, I. M. Friedland, and D. S. Martin, *Cancer Res.*, **18**, 1272 (1958).

(20) K. Wallenfels and H. Dieckmann, *Ann.*, **621**, 166 (1959).

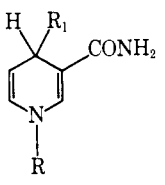
TABLE II

EQUILIBRIUM CONSTANTS AT 25.0°

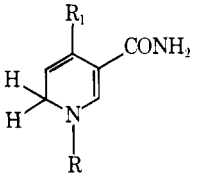
R_1[a]	R_2	X	λ_{max}, mμ	k_5, mole
Pm	H	CN	328	513 ± 51
Pm	Me	CN	331	0.36 ± 0.04
Tg	H	CN	319	4030 ± 500
Tg	Me	CN	325	1.98 ± 0.25
Bz	H	CN	344	6.46 ± 0.29
Bz	Me	CN	344	0.0056 ± 0.0005

[a] Pm, propoxymethyl; Tg, tetraacetyl-β-D-glucopyranosyl; Bz, benzyl.

the corresponding compounds lacking 4-Me. These results indicate that 4-Me-NAD derivatives which might be formed *in vivo* from 4-methylnicotinamide compounds may provide only weak competition with the natural coenzyme in the oxidative stage of glycolysis, and the resulting concentration of 1,4-dihydro-4-methylnicotinamide derivatives produced could be much lower than the NADH concentration in the tumor cells. However, by incorporating substituents, which could form covalent bonds with groups adjacent to the active site of the enzyme, into 4-Me-substituted nicotinamides, the derived N*ADs should be preferentially bound to the enzyme (compare the work of Baker[21] on the design of irreversible antagonists). This approach to increasing the involvement of substituted N*ADs and the use of ring substituents which confer greater reactivity toward 1,4 addition is now being investigated.



VII



VIII

H-Transfer Reactions of Dihydronicotinamide Derivatives.—The dihydronicotinamide derivatives investigated were the 1,4- and 1,6-dihydro compounds (VII, R_1 = Me or H) and (VIII, R_1 = Me or H) where the N substituents were benzyl, propoxymethyl, and tetraacetyl-β-D-glucopyranosyl.[1] In view of the potential biological involvement of the enzymatically reactive 1,6-NADH,[22] the 1,6-dihydro compounds were studied. In order to investigate the effect of an electron-withdrawing group at the 4 position, upon the rate constant, the 4-cyano-1,4-dihydronicotinamide derivatives (V, R_1 = H or Me, R = propoxymethyl) were also studied.

The spectroscopic method used was that described by Wallenfels and Gellrich,[23] observing the change of oxidizing agent concentration with time. 2,6-Dichlorophenolindophenol (redox potential +0.217 V) was chosen as the oxidizing agent because the rate of oxidation for dihydronicotinamides is generally slow enough to provide accurate absorption measurements, and the relatively fast side reactions which are present in other reagents are absent in 2,6-dichlorophenolindophenol.[7,23] Rate constants for the H-transfer reactions of the dihydronicotinamide derivatives studied in the present work are presented in Table III.

Rate constants for the benzyl compound (VII, R = benzyl, R_1 = H) and the glucopyranosyl compound (VII, R = tetraacetyl-β-D-glucopyranosyl, R_1 = H) at pH 7 at 25° have also been determined by Wallenfels and Gellrich,[23] and are approximately of the same order of magnitude as the corresponding values in Table III. The reaction mechanism of the oxidation process has been discussed in detail by these authors.[23] As the ease of H^- transfer is related to the electron density in the heterocyclic ring, the rate constants should be markedly affected by the groups attached to N-1. In agreement with this, the values in Table III increase in the same order as for the electron-donating properties of the 1 substituents, *i.e.*, benzyl > propoxymethyl- > tetraacetyl-β-D-glucopyranosyl.

In compounds with the same N substituent, the highest rate constants are displayed by the 1-alkyl-1,6-dihydro-4-methylnicotinamide derivatives (VIII, R_1 = Me). The high rates of H transfer are predictable because these structures resemble allylic systems where the methyl group facilitiates double-bond rearrangement (*e.g.*, as in the acid-catalyzed interconversion of *cis*- and *trans*-crotyl alcohol *via* but-3-en-2-ol).[24] The significant effect of the 4-Me substituent in accelerating the 6-H^+ release can be seen from a comparison with the rate constants for the 1,6-dihydronicotinamide derivatives (VIII, R_1 = H) without the 4-Me, where the oxidation rate is much lower.

There is little difference between the rate constant values for the 1,4-dihydronicotinamide derivatives when R_1 is Me or H. In these cases, H^- elimination may involve electron displacement from either of the two double bonds conjugated to the N-1 lone pair. Such electromeric effects should be more important than the weaker electron-repelling effect of the 4-Me[25] in determining the rate constant value. The results show that the 4-Me does not exert an effective inhibiting influence upon oxidation as the H-transfer rates are little affected by the 4-Me substituent. It is therefore possible that the rates of H transfer from NADH and its 4-Me analog could be of the same order. Such a result is desirable for the chemotherapeutic approach discussed earlier, since otherwise respiration in normal cells may be hindered after administration of a 4-methylnicotinamide derivative.

Some authors[23,26] have investigated 2,6-dichlorophenolindophenol oxidations over various ranges of H^+ concentrations and the influence of the pH of the medium upon the value of the rate constant has been discussed by Wallenfels and Gellrich.[23] The reactions are pH dependent and considerable variations in rate may be achieved by slight alterations in the H^+ concentration of the reaction medium. The rate constants for the H-transfer reactions at various pH values were therefore recorded in the present work. When R_1 was Me or H, the reaction rate increased as the pH decreased, indicating that the oxidation process was facilitated in more acidic media. With an electron-with-

(21) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., Chapter 8.

(22) K. Chakraverty and S. Chaykin, *Biochim. Biophys. Res. Commun.*, **15**, 262 (1964).

(23) K. Wallenfels and M. Gellrich, *Ann.*, **621**, 149 (1959).

(24) W. G. Young and J. S. Franklin, *J. Amer. Chem. Soc.*, **88**, 785 (1966).

(25) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, New York, N. Y., 1953, p 70.

(26) S. J. Leach, J. H. Baxendale, and M. G. Evans, *Aust. J. Chem.*, **6**, 409 (1953).

TABLE III
RATE CONSTANTS (K) FOR HYDROGEN-TRANSFER REACTIONS AT 25.0°

No.	R_1	R[a]	pH	K,[b] L./mol. min.	K,[c] L./mol. min.	$t_{1/2}$, min.	$[PyH]_0$[d] × 10^{-4}, mol.
VII	H	Bz	6.24	1027 ± 13	1066 ± 20	1.3 ± 0.1	5.0
VII	H	Bz	6.47	752 ± 9	730 ± 15	1.9 ± 0.1	5.0
VII	H	Bz	6.81	455 ± 5	447 ± 10	3.1 ± 0.1	5.0
VII	H	Bz	6.98	354 ± 3	347 ± 10	4.0 ± 0.1	5.0
VII	Me	Bz	6.24	894 ± 18	924 ± 20	1.5 ± 0.1	5.0
VII	Me	Bz	6.47	636 ± 9	630 ± 10	2.2 ± 0.1	5.0
VII	Me	Bz	6.81	451 ± 9	462 ± 10	3.0 ± 0.1	5.0
VII	Me	Bz	6.98	368 ± 9	364 ± 10	3.8 ± 0.1	5.0
VIII	H	Bz	6.24	205 ± 2	210 ± 10	13 ± 1	2.5
VIII	H	Bz	6.47	131 ± 1	139 ± 10	20 ± 1	2.5
VIII	H	Bz	6.81	106 ± 1	110 ± 10	25 ± 1	2.5
VIII	H	Bz	6.98	99 ± 1	92 ± 7	30 ± 1	2.5
VIII	Me	Bz	6.24	4357 ± 46	4472 ± 100	0.62 ± 0.05	2.5
VIII	Me	Bz	6.47	3307 ± 46	3262 ± 50	0.85 ± 0.05	2.5
VIII	Me	Bz	6.81	1953 ± 46	1980 ± 50	1.40 ± 0.05	2.5
VIII	Me	Bz	6.98	1373 ± 46	1320 ± 50	2.11 ± 0.05	2.5
VII	H	Pm	6.24	68.8 ± 0.9	69.3 ± 2.0	40 ± 1	2.5
VII	H	Pm	6.47	53.1 ± 0.9	55.4 ± 2.0	50 ± 1	2.5
VII	H	Pm	6.81	31.3 ± 0.8	32.6 ± 2.0	85 ± 1	2.5
VII	H	Pm	6.98	25.2 ± 0.7	24.9 ± 2.0	111 ± 1	2.5
VII	Me	Pm	6.24	48 ± 1	49 ± 2	14 ± 1	10.0
VII	Me	Pm	6.47	41 ± 4	42 ± 2	16 ± 1	10.0
VII	Me	Pm	6.81	34 ± 1	36 ± 2	20 ± 1	10.0
VII	Me	Pm	6.98	28 ± 1	30 ± 2	23 ± 1	10.0
VIII	H	Pm	6.24	54.6 ± 1.1	57.7 ± 2.5	12 ± 1	10.0
VIII	H	Pm	6.47	35.0 ± 1.2	34.6 ± 2.0	20 ± 1	10.0
VIII	H	Pm	6.81	18.4 ± 0.5	18.7 ± 0.5	37 ± 1	10.0
VIII	H	Pm	6.98	14.4 ± 0.5	14.7 ± 0.5	47 ± 1	10.0
VIII	Me	Pm	6.24	778 ± 9	693 ± 10	4.0 ± 0.5	2.5
VIII	Me	Pm	6.47	567 ± 9	554 ± 10	5.0 ± 0.5	2.5
VIII	Me	Pm	6.81	280 ± 9	276 ± 10	10.0 ± 0.5	2.5
VIII	Me	Pm	6.98	158 ± 4	154 ± 5	18.0 ± 0.5	2.5
VII	H	Tg	6.24	4.21 ± 0.15	4.34 ± 0.25	245 ± 2.5	6.5
VII	H	Tg	6.47	3.69 ± 0.10	3.67 ± 0.20	290 ± 2.5	6.5
VII	H	Tg	6.81	3.12 ± 0.10	3.13 ± 0.20	340 ± 2.5	6.5
VII	H	Tg	6.98	2.76 ± 0.10	2.77 ± 0.20	385 ± 2.5	6.5
VII	Me	Tg	6.24	2.48 ± 0.01	2.48 ± 0.02	430 ± 5	6.5
VII	Me	Tg	6.47	1.57 ± 0.01	1.58 ± 0.02	680 ± 4	6.5
VII	Me	Tg	6.81	1.17 ± 0.01	1.17 ± 0.02	910 ± 5	6.5
VII	Me	Tg	6.98	1.06 ± 0.01	1.06 ± 0.02	1000 ± 5	6.5
VIII	H	Tg	6.24	9.9 ± 0.5	9.9 ± 0.5	70 ± 2	10.0
VIII	H	Tg	6.47	8.2 ± 0.5	8.1 ± 0.5	85 ± 2	10.0
VIII	H	Tg	6.81	4.8 ± 0.2	4.9 ± 0.2	140 ± 2	10.0
VIII	H	Tg	6.98	3.6 ± 0.2	3.6 ± 0.2	195 ± 3	10.0
VIII	Me	Tg	6.24	110 ± 4	106 ± 9	4.5 ± 0.2	13.0
VIII	Me	Tg	6.47	71 ± 4	82 ± 8	6.5 ± 0.2	13.0
VIII	Me	Tg	6.81	49 ± 4	48 ± 6	11.1 ± 0.2	13.0
VIII	Me	Tg	6.98	40 ± 4	40 ± 5	14.5 ± 0.2	13.0
V	H	Pm	6.24	0.48 ± 0.02	0.48 ± 0.02	360 ± 3	40.0
V	H	Pm	6.47	0.63 ± 0.05	0.63 ± 0.05	275 ± 2	40.0
V	H	Pm	6.81	1.35 ± 0.05	1.30 ± 0.05	143 ± 2	40.0
V	H	Pm	6.98	1.86 ± 0.02	1.84 ± 0.02	37 ± 1	40.0

[a] Bz, benzyl; Pm, propoxymethyl; Tg, tetraacetyl-β-D-glucopyranosyl. [b] Graphical slope method. [c] Half-time method. [d] $[PyH]_0$ is the initial concentration of dihydronicotinamide derivative.

drawing group in the 4 position of the nicotinamide ring, the H transfer from a dihydronicotinamide derivative should be impeded, as the electronic activation at the 4 position caused by the 1-alkyl group should be offset by the deactivating properties of the 4 substituent. In agreement with this, the rate constant values of the 4-cyano-1,4-dihydro derivative (V, R_1 = H, R = propoxymethyl) were considerably reduced compared to the corresponding 4-H and 4-Me compounds (VII, R_1 = H, R = propoxymethyl and VII, R_1 = Me, R = propoxymethyl), and also as the reaction medium became more acidic, the oxidation process was retarded as shown by the rate constants given in Table III. As expected, no reaction was detected between the oxidizing agent and the 4-cyano-1,4-dihydro-4-methyl derivative (V, R_1 = Me, R = propoxymethyl) because of the absence of a reactive H at the 4 position, consistent with the mechanism proposed by Wallenfels and Gellrich.[23]

Wallenfels[7] has compared the rate constants for the H-transfer reactions of a number of dihydronicotinamide compounds with the CN^- affinity constants of

the corresponding quaternary salts. It was found that the oxidation rate constants decreased in the same order of substituents in which the affinity constants increased. If the oxidation rate constants (K) for the 1,4-dihydronicotinamide derivatives at pH 7 (Table III) are compared with the CN^- addition constants (k) for the corresponding quaternary salts given in Table II, it is seen that the 4-Me derivatives, and the three compounds lacking 4-Me, form two separate series, where the rate constant K decreases in the substituent order R = benzyl, propoxymethyl, tetraacetyl-β-D-glucopyranosyl, while the CN^- affinity constant k increases. These results are therefore similar to those of Wallenfels.[7]

Experimental Section

The H-transfer reaction is represented by the equation, PyH + Ind + H^+ → Py^+ + $IndH_2$. PyH represents the dihydronicotinamide derivative and Ind represents the 2,6-dichlorophenolindophenol. When the dihydronicotinamide compound is in excess, the reaction becomes kinetically of the first order, and the rate equation is then[27]

$$K = \frac{1}{t[\mathrm{PyH}]_0} \ln \frac{[\mathrm{Ind}]_0}{[\mathrm{Ind}]_t} \quad (1)$$

where $[Ind]_0$ is the initial concentration of 2,6-dichlorophenolindophenol, $[Ind]_t$ is the concentration after time t, and $[PyH]_0$ is the initial concentration of the dihydronicotinamide derivative. By rearrangement

$$\log [\mathrm{Ind}]_t = -\frac{[\mathrm{PyH}]_0 K t}{2.303} + \log [\mathrm{Ind}]_0 \quad (2)$$

The corresponding equation given by Wallenfels and Gellrich[23] is in error, as the first term on the right-hand side has no negative sign attached. The reaction was followed by observing the decrease in 2,6-dichlorophenolindophenol visible absorption at 640 mμ with time, and the rate constant K was calculated from the slope of the graph of log $[Ind]_t$ against time. K was also obtained by recording the time $t_{0.5}$ at which $[Ind]_0$ was reduced by one-half, and substituting $[Ind]_0/2$ for $[Ind]_t$ in eq 1 to give eq 3. The spectra were recorded at constant H^+ concentrations by

$$K = \frac{\ln 2}{t_{0.5}[\mathrm{PyH}]_0} \quad (3)$$

the use of phosphate buffer solutions of known pH.[28] Absorption spectra were recorded on a Unicam SP 800A spectrophotometer linked to an SP 21 slave recorder. The temperature was maintained at 25.0° by a Shandon K2 Ultra-Thermostat. Dihydronicotinamide derivatives were prepared and used on the same day for the oxidation experiments. Reactant concentrations were 0.57×10^{-4} mol of 2,6-dichlorophenolindophenol and 2.5×10^{-4} to 4.0×10^{-3} mol of dihydronicotinamide derivative in 0.007 mol of 1:1 aqueous–methanolic phosphate buffer solution.[28] Melting points were determined in open capillary tubes and are corrected. Compounds whose elemental analyses are indicated only by symbols showed values within 0.4% of the theoretical values. The nmr spectra were recorded with a Perkin-Elmer R 10 spectrometer (60 Mcps), TMS = 0. Cyanide equilibrium constants were obtained by the method of Wallenfels,[7,20] quaternary salt concentrations being 10^{-4} M in H_2O, CN^- concentrations being 6×10^{-3} to 2×10^{-1} M in H_2O. Evaporations were carried out under reduced pressure.

1-(Tetraacetyl-β-D-glucopyranosyl)-3-carbamoyl-4-cyano-1,4-dihydropyridine.—A solution of 3 g (0.0058 mole) of 1-(tetraacetyl-β-D-glucopyranosyl)-3-carbamoylpyridinium bromide[29] in 20 ml of H_2O was added to a solution of 10 g of KCN in 20 ml of H_2O at 0°. Anhydrous Na_2SO_4 (100 g) was added and the mixture was stirred. The slurry was extracted with MeCN, the extracts were filtered, and the filtrate was evaporated. The residue was recrystallized from Et_2O–MeOH to give 1.8 g (90%) of yellow cubes, mp 39–41°. *Anal.* ($C_{21}H_{25}N_3O_{10}$) C, H, N.

In a similar way, **1-(tetraacetyl-β-D-glucopyranosyl)-3-carbamoyl-4-cyano-1,4-dihydro-4-methylpyridine** was prepared from 1 g (0.0019 mole) of 1-(tetraacetyl-β-D-glucopyranosyl)-3-carbamoyl-4-methylpyridinium bromide[7] and gave 0.65 g (94%) of creamy needles, mp 72–74°. *Anal.* ($C_{22}H_{27}N_3O_{10}$) C, H, N.

1-Benzyl-3-carbamoyl-4-cyano-1,4-dihydropyridine.—A solution of 2 g (0.0069 mole) of 1-benzyl-3-carbamoylpyridinium bromide[30] in 100 ml of H_2O was added to a solution of 12 g of KCN in 100 ml of H_2O at 0°. The mixture was shaken for 10 min and the precipitate was collected and washed with ice–H_2O followed by Et_2O. Recrystallization from EtOH yielded 1.4 g (88%) of colorless needles, mp 141–142°. *Anal.* ($C_{14}H_{13}N_3O$) C, H, N.

3-Carbamoyl-4-cyano-1,4-dihydro-1-propoxymethylpyridine.—A solution containing 0.4 g (0.0017 mole) of 3-carbamoyl-1-propoxymethylpyridinium chloride[7] in 100 ml of H_2O at 0° was added to a solution of 10 g of KCN in 100 ml of H_2O at 0°. The mixture was extracted with $CHCl_3$ and the organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated. Recrystallization of the residue from Et_2O–MeOH gave 0.2 g (51%) of colorless needles, mp 86–87°. *Anal.* ($C_{11}H_{15}N_3O_2$) C, H, N.

In a similar way, **3-carbamoyl-4-cyano-1,4-dihydro-4-methyl-1-propoxymethylpyridine** was prepared from 0.5 g (0.002 mole) of 3-carbamoyl-4-methyl-1-propoxymethylpyridinium chloride[7] and gave 0.1 g (20%) of colorless needles, mp 185–186°. *Anal.* ($C_{12}H_{17}N_3O_2$) C, H, N.

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(27) S. Glasstone, "Textbook of Physical Chemistry," Macmillan and Co., Ltd., London, 1964, p 1057.

(28) G. Kortum and J. Bockris, "Textbook of Electrochemistry," Elsevier Publishing Co., Amsterdam, 1951, p 744.

(29) L. J. Haynes and A. Todd, *J. Chem. Soc.*, 303 (1950).

(30) C. Ukita, D. Mizuno, and S. Kosaka, *J. Pharm. Soc. Japan*, **73**, 111 (1953).