Potential Coenzyme Inhibitors. Part II.[1] Reduction of 4-Methylnicotinamide Derivatives by Sodium Dithionite and Sodium Borohydride †

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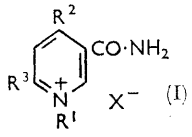
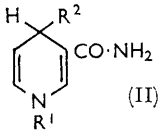
The preparation of some 4-methylnicotinamide derivatives is described, and the dithionite and borohydride reduction products are compared with those from the corresponding nicotinamide derivatives. The u.v. absorption and ^{1}H n.m.r. spectra show that in each case 1,4-dihydronicotinamides are formed from the dithionite reactions, whilst borohydride reductions yield 1,6-dihydro-derivatives.

In Part I [1] the rationale for examining 4-substituted nicotinamide derivatives as stereospecific inhibitors of glycolysis was discussed. For this approach to selective tumour growth inhibition to be valid it is essential that 1,4-addition to the substituted pyridinium structure should occur.


(I)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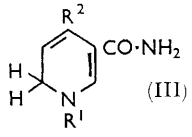
(III)

Wallenfels and Schuly [2] have reported that reduction of 3-carbamoyl-1-(2,6-dichlorobenzyl)-4,6-dimethylpyridinium chloride (I; R^1 = 2,6-dichlorobenzyl, $R^2 = R^3$ = Me, X = Cl) by sodium dithionite, which effects 1,4-addition of hydrogen to the pyridine ring of NAD, affords a 1,2-dihydro-derivative. They suggested that the presence of a 4-methyl substituent hinders 1,4-addition. Walter and Kaplan [3] were unable to reduce 4-methyl-NAD with sodium dithionite. In view of these indications that 1,4-addition of hydrogen cannot occur if the 4-position of the pyridine ring carries a methyl substituent, we have examined the reduction of the model substances (I; R^1 = propoxymethyl, tetra-acetyl-β-D-glucopyranosyl, or benzyl, R^2 = H or Me, R^3 = H, X = Cl or Br).

By u.v. and ^{1}H n.m.r. spectral measurements it has been established that 1,4-addition does occur when 4-methyl substituted pyridinium compounds are reduced with dithionite. Reduction with sodium borohydride was shown to give the expected 1,6-dihydro-compounds.

Reduction of the quaternary compounds (I) by sodium dithionite gave dihydro-compounds (II) whilst sodium borohydride gave dihydro-compounds (III) (see Table 1). The hypsochromic effect of a 4-methyl substituent in 1,4-dihydropyridines has already been noted [4] and there is a similar but smaller effect on the long wavelength band of the 1,6-dihydropyridines.

The proton assignments for the model compounds (II; R^2 = H) and (III; R^2 = H) are in good agreement with

† Throughout this paper pyridine derivatives are numbered with the carbamoyl group in the 3-position.

[1] Part I, W. C. J. Ross, *J. Chem. Soc.* (*C*), 1966, 1816.
[2] K. Wallenfels and H. Schuly, *Annalen*, 1959, **621**, 215.
[3] P. Walter and N. O. Kaplan, *J. Biol. Chem.*, 1963, **238**, 2823.
[4] D. Hofmann, E. M. Kosower, and K. Wallenfels, *J. Amer. Chem. Soc.*, 1961, **83**, 3314; J. A. Berson and E. Brown, *ibid.*, 1955, **77**, 444.

TABLE 1

U.v. absorption spectra

	$\lambda_{max.}$ (mμ)	ε	$\lambda_{max.}$ (mμ)	ε
1 (I; R^1 = Pm, R^2 = R^3 = H) ...	266	3548		
2 (I; R^1 = Pm, R^2 = Me, R^3 = H)	263	3357		
3 (I; R^1 = Tg, R^2 = R^3 = H) ...	266	4957		
4 (I; R^1 = Tg, R^2 = Me, R^3 = H)	261	4870		
5 (I; R^1 = Bz, R^2 = R^3 = H)	264	4266		
6 (I; R^1 = Bz, R^2 = Me, R^3 = H)	263	4017		
7 (II; R^1 = Pm, R^2 = H)			338	5134
8 (II; R^1 = Pm, R^2 = Me)			326	6058
9 (II; R^1 = Tg, R^2 = H)			331	6205
10 (II; R^1 = Tg, R^2 = Me)			317	4593
11 (II; R^1 = Bz, R^2 = H)			352	6353
12 (II; R^1 = Bz, R^2 = Me)			339	6460
13 (III; R^1 = Pm, R^2 = H)	262	4800	343	5100
14 (III; R^1 = Pm, R^2 = Me)	261	7280	339	4500
15 (III; R^1 = Tg, R^2 = H)	262	2444	338	7076
16 (III; R^1 = Tg, R^2 = Me)	253	5330	332	4914
17 (III; R^1 = Bz, R^2 = H)	267	6178	358	5617
18 (III; R^1 = Bz, R^2 = Me)	266	8228	350	5687

Abbreviations: Pm propoxymethyl, Tg tetra-acetyl-β-D-glucopyranosyl. For 1 and 2 X = Cl; for 3—6 X = Br.

the data recorded for 1,4-dihydronicotinamides [5,6] and 1,6-dihydronicotinamides [6] respectively. The dithionite reduction products derived from 4-methylnicotinamide salts are readily identified as 1,4-dihydronicotinamide derivatives, as in each n.m.r. spectrum, the 4-methyl resonance is split into a doublet by coupling with a single proton; the magnitude of the coupling constant (J 6·0—6·2 c./sec.) confirms that the proton concerned is at the 4-position. The 4-proton resonance appears as an octet because of the coupling with both the 5-proton ($J_{4,5}$ 4·3—4·9 c./sec.) and the 4-methyl protons (J 6·0—6·2 c./sec.).

The allocations for the 1,4-dihydro-4-methylnicotinamide derivatives are presented in Tables 2 and 3. The similarities in coupling constant and chemical shift values between these compounds and the corresponding unsubstituted nicotinamide derivatives, further confirms the assignments.

The small effect on the chemical shifts caused by the introduction of a 4-methyl group into the ring has also been noticed in the spectrum of 4-methylpyridine.[7]

The n.m.r. spectra of the borohydride reduction products show that 1,6-dihydronicotinamide derivatives are formed from the reactions. The presence of the 4-methyl group (a singlet resonance) simplifies the analysis of the spectra, since the proton coupling from the 4-position, which is present in the unsubstituted systems, is not seen in the 4-substituted compounds. 1,2-Dihydro-4-methylnicotinamide structures would be inconsistent with the observed proton coupling (J 3·4—4·2 c./sec.), as coupling of a 5- or 6-proton with the methylene protons at the 2-position in a 1,2-dihydro-4-methylnicotinamide derivative should give much lower

TABLE 2

N.m.r. spectra (τ values for solutions in deuteriochloroform)

	2-H	4-H	5-H	6-H	4-Me	N·CH_x	CH_2	Other shifts
(II; R^1 = Pm, R^2 = H)	2·82	6·86	5·21	4·04		5·51	8·40	6·61,[a] 9·09[b]
(II; R^1 = Pm, R^2 = Me)	2·81	6·75	5·16	4·00	8·86	5·47	8·41	6·63,[a] 9·09[b]
(II; R^1 = Tg, R^2 = H)	2·84	6·84	5·52			4·76	5·84	7·87,[c] 7·92,[d] 4·4—5·3,[e] 6·12[f]
(II; R^1 = Tg, R^2 = Me)	2·82	6·71	5·48	3·89	8·92	4·76	5·81	7·90,[c] 7·97,[d] 4·6—5·3,[e] 6·10[f]
(II; R^1 = Bz, R^2 = H)	2·81	6·82	5·24	4·25		5·70		2·68[g]
(II; R^1 = Bz, R^2 = Me)	2·74	6·62	5·18	4·12	8·85	5·61		2·69[g]
(III; R^1 = Pm, R^2 = H)	2·90	4·09	5·23	5·88		5·57	8·41	6·63,[a] 9·09[b]
(III; R^1 = Pm, R^2 = Me)	2·86		5·13	6·00	8·01	5·58	8·40	6·62,[a] 9·08[b]
(III; R^1 = Tg, R^2 = H)	2·92	4·09	5·60	6·26		4·83	5·86	7·93,[c] 7·98,[d] 4·6—5·5,[e] 6·30[f]
(III; R^1 = Tg, R^2 = Me)	2·96		5·56	6·05	8·02	4·80	5·79	7·92,[c] 7·98,[d] 4·7—5·3,[e] 6·30[f]
(III; R^1 = Bz, R^2 = H)	2·90	4·34	5·33	6·12		5·82		2·79[g]
(III; R^1 = Bz, R^2 = Me)	2·75		5·28	6·17	8·02	5·83		2·70[g]

Assignments: [a] OCH_2, [b] Me, [c] 1 × Ac, [d] 3 × Ac, [e] 2′-, 3′-, and 4′-H, [f] 5′-H, [g] Ph.

TABLE 3

N.m.r. coupling constants (c./sec.)

	$J_{2,4}$	$J_{2,6}$	$J_{5,6}$	$J_{4,5}$	$J_{4,4\text{-Me}}$	$J_{4,6}$
(II; R^1 = Pm, R^2 = H)	0·5	1·0	8·6	4·0		1·7
(II; R^1 = Pm, R^2 = Me)		1·3	8·6	4·9	6·0	
(II; R^1 = Tg, R^2 = H)	1·0	1·5	7·1	2·5		1·3
(II; R^1 = Tg, R^2 = Me)		1·8	8·6	4·3	6·2	
(II; R^1 = Bz, R^2 = H)	0·5	1·5	8·2	3·4		1·7
(II; R^1 = Bz, R^2 = Me)		1·7	7·7	4·6	6·0	
(III; R^1 = Pm, R^2 = H)	1·8	1·5	3·0	8·0		1·6
(III; R^1 = Pm, R^2 = Me)		1·4	4·2			
(III; R^1 = Tg, R^2 = H)	1·6	0·9	3·4	8·6		
(III; R^1 = Tg, R^2 = Me)		1·7	4·2			
(III; R^1 = Bz, R^2 = H)	1·5	1·0	3·9	9·4		
(III; R^1 = Bz, R^2 = Me)		1·3	3·4			

[5] W. L. Meyer, H. R. Mahler, and R. H. Baker, *Biochim. Biophys. Acta*, 1962, **64**, 353; W. C. Caughey and K. A. Schellenberg, *J. Org. Chem.*, 1966, **31**, 1978; J. Biellman and H. Callot, *Tetrahedron Letters*, 1966, 3991; K. S. Choi and S. G. A. Alivisatos, *Biochemistry*, 1968, **7**, 190.

[6] H. Dieckman, G. Englert, and K. Wallenfels, *Tetrahedron*, 1964, **20**, 281.

[7] E. B. Baker, *J. Chem. Phys.*, 1955, **23**, 1981.

coupling constants (0—2 c./sec.), and the relatively large value (7—9 c./sec.) which would be expected from interaction between the 5-proton and the 6-proton is absent.

EXPERIMENTAL

Absorption spectra were recorded with a Unicam SP 800A spectrometer. The temperature was maintained at 35·0° with a Shandon K2 Ultra-Thermostat. Solutions were 10^{-4}M in 95% ethanol and the silica cell path length was 1 cm.

The n.m.r. spectra were examined at 60 Mc./sec. with a Perkin-Elmer R10 spectrometer, to an accuracy of ±0·02 p.p.m. Solutions were 10% w/v in deuteriochloroform; the internal standard was tetramethylsilane. Coupling constants were accurate to ±0·2 c./sec.

M.p.s are corrected. Evaporations were carried out under reduced pressure.

3-Carbamoyl-1-propoxymethylpyridinium Chloride.—Freshly prepared chloromethyl n-propyl ether [8] (15 c.c.) was added to a rapidly stirred solution of nicotinamide (10 g.) in acetone (250 c.c.); after 15 min. the precipitated solid was collected and gave the *pyridinium chloride* (12·8 g., 67%) as colourless plates, m.p. 142—143° (from acetone–methanol) (Found: C, 51·6; H, 6·5; Cl, 15·4; N, 12·3. $C_{10}H_{15}ClN_2O_2$ requires C, 52·1; H, 6·5; Cl, 15·4; N, 12·1%).

3-Carbamoyl-4-methyl-1-propoxymethylpyridinium chloride was similarly prepared from 4-methylnicotinamide [9] (4 g.). It formed colourless needles (72%), m.p. 135—136° (from acetone–ether) (Found: C, 54·0; H, 7·1; N, 11·4. $C_{11}H_{17}ClN_2O_2$ requires C, 54·0; H, 7·0; N, 11·4%).

3-Carbamoyl-1,4-dihydro-1-propoxymethylpyridine.—Diethyl ether (500 c.c.) was added to a solution of 3-carbamoyl-1-propoxymethylpyridinium chloride (1 g.) in N-aqueous sodium hydrogen carbonate (300 c.c.). The mixture was cooled to 0°, and air was excluded by the passage of carbon dioxide for 10 min. Sodium dithionite (5 g.) was added in small quantities during 30 min. After 4 hr., the liquid was extracted with ether and the extracts were washed with water and dried (Na_2SO_4). Evaporation gave the *1,4-dihydronicotinamide* (0·7 g., 80%) as pale lemon plates, m.p. 74—75° (from benzene) (Found: C, 61·0; H, 8·1; N, 14·8. $C_{10}H_{16}N_2O_2$ requires C, 61·2; H, 8·2; N, 14·3%). Similarly, 3-carbamoyl-4-methyl-1-propoxymethylpyridinium chloride (1·5 g.) gave *3-carbamoyl-1,4-dihydro-4-methyl-1-propoxymethylpyridine* (0·75 g., 62%) as colourless needles, m.p. 71—72° (from ether) (Found: C, 62·9; H, 8·9; N, 13·5. $C_{11}H_{18}N_2O_2$ requires C, 62·8; H, 8·6; N, 13·3%).

3-Carbamoyl-1,6-dihydro-1-propoxymethylpyridine.—Carbon dioxide was passed through a mixture of ether (200 c.c.) and a solution of 3-carbamoyl-1-propoxymethylpyridinium chloride (1 g.) in water (200 c.c.) at 0°. A mixture of sodium borohydride (0·4 g.) and aqueous N-sodium hydrogen carbonate (100 c.c.) was then slowly added. After the mixture had been stirred for 24 hr., the ether layer was colourless. The mixture was then extracted with ether and the extracts were washed with water, dried (Na_2SO_4), and evaporated. Recrystallisation from methanol–ether gave the *1,6-dihydronicotinamide derivative* (0·7 g., 80%), as colourless crystals, m.p. 92—93° (Found: C, 61·0; H, 8·0; N, 14·1. $C_{10}H_{16}N_2O_2$ requires C, 61·2; H, 8·2; N, 14·3%). Similarly, 3-carbamoyl-4-methyl-1-propoxymethylpyridinium chloride (1 g.) gave *3-carbamoyl-1,6-dihydro-4-methyl-1-propoxymethylpyridine* (0·75 g., 91%), as colourless needles, m.p. 121—122° (Found: C, 63·1; H, 8·6; N, 13·4. $C_{11}H_{18}N_2O_2$ requires C, 62·8; H, 8·6; N, 13·3%).

1-Benzyl-3-carbamoylpyridinium bromide, prepared by the method of Ukita, Mizuno, and Kosaka,[10] was reduced to the 1,4-dihydro-derivative as described by Mauzerall and Westheimer.[11]

1-Benzyl-3-carbamoyl-4-methylpyridinium Bromide.—A solution of 4-methylnicotinamide (1·4 g.) and benzyl bromide (1·7 g.) in acetone (100 c.c.) was heated under reflux for 15 min. at 100°. When the mixture had cooled, the solid was filtered off and gave the *quaternary salt* (2 g., 64%), as colourless needles, m.p. 215—217° (from methanol–ether) (Found: C, 54·7; H, 5·2; Br, 26·0; N, 9·2. $C_{14}H_{15}BrN_2O$ requires C, 54·7; H, 4·9; Br, 26·0; N, 9·1%).

1-Benzyl-3-carbamoyl-1,4-dihydro-4-methylpyridine.—1-Benzyl-3-carbamoyl-4-methylpyridinium bromide (1 g.) was reduced by alkaline sodium dithionite in the same way as the *N*-propoxymethyl salts. The reduction mixture was extracted with chloroform and the extracts were washed with water, dried (Na_2SO_4), and evaporated. Recrystallisation from methanol gave *1-benzyl-3-carbamoyl-1,4-dihydro-4-methylpyridine* (0·65 g., 86%), as colourless needles, m.p. 115—117° (Found: C, 73·2; H, 7·1; N, 11·9. $C_{14}H_{16}N_2O$ requires C, 73·6; H, 7·1; N, 12·3%).

1-Benzyl-3-carbamoyl-1,6-dihydro-4-methylpyridine.—A mixture of sodium borohydride (0·8 g.) and sodium carbonate (4 g.) in water (200 c.c.) was added to a solution of 1-benzyl-3-carbamoyl-4-methylpyridinium bromide (2 g.) in water (100 c.c.) at 0°. After 1 hr., the mixture was extracted with chloroform and the extracts were washed with water, dried (Na_2SO_4), and evaporated. Recrystallisation from methanol–ether gave the *1,6-dihydronicotinamide derivative* (1 g., 66%), as colourless hairs, m.p. 114—115° (Found: C, 73·3; H, 6·9; N, 12·3%). Similarly, 1-benzyl-3-carbamoylpyridinium bromide (1 g.) gave *1-benzyl-3-carbamoyl-1,6-dihydropyridine* (0·6 g., 60%), as pale yellow needles, m.p. 110—111° (Found: C, 73·1; H, 6·8; N, 13·0. $C_{13}H_{14}N_2O$ requires C, 72·9; H, 6·6; N, 13·1%).

3-Carbamoyl-1-(tetra-acetyl-β-D-glucopyranosyl)pyridinium bromide and 3-carbamoyl-1,4-dihydro-1-(tetra-acetyl-β-D-glucopyranosyl)pyridine were prepared by the method of Haynes and Todd.[12]

3-Carbamoyl-4-methyl-1-(tetra-acetyl-β-D-glucopyranosyl)-pyridinium Bromide.—A solution of acetobromo-β-D-glucose [12] (8 g.) in acetonitrile (100 c.c.) was added to a solution of 4-methylnicotinamide (1·4 g.) in acetonitrile (700 c.c.) and the mixture was filtered. After 5 days at room temperature in the absence of light, the 4-methylnicotinamide hydrobromide (0·6 g.) was filtered off. The filtrate was evaporated (bath temperature 20°) to a small volume and passed down a column (5 cm. × 1 cm. diam.) of alumina (Spence type H). Elution with methanol (2 l.), afforded the *nucleoside* (0·8 g., 14%), which formed colourless plates, m.p. 220—221° (from methanol–ethyl acetate) (Found: C, 46·1; H, 4·6; N, 5·5. $C_{21}H_{27}BrN_2O_{10}$ requires C, 45·8; H, 5·0; N, 5·1%).

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3-*Carbamoyl*-1,4-*dihydro*-4-*methyl*-1-(*tetra-acetyl*-β-D-*glucopyranosyl*)*pyridine*.— 3-Carbamoyl-4-methyl-1-(tetra-acetyl-β-D-glucopyranosyl)pyridinium bromide (0·25 g.) was reduced by the method of Haynes and Todd [12] to yield the 1,4-*dihydronicotinamide derivative* (0·1 g., 45%), as colourless plates, m.p. 180—182° (Found: C, 53·3; H, 6·3; N, 6·2. $C_{21}H_{28}N_2O_{10}$ requires C, 53·8; H, 6·0; N, 6·0%).

3-*Carbamoyl*-1,6-*dihydro*-4-*methyl*-1-(*tetra-acetyl*-β-D-*glucopyranosyl*)*pyridine*.—A mixture of sodium borohydride (0·1 g.), sodium carbonate (2 g.), and sodium hydrogen carbonate (2 g.) in water (100 c.c.) was added to a solution of 3-carbamoyl-4-methyl-1-(tetra-acetyl-β-D-glucopyranosyl)pyridinium bromide (0·25 g.) in water (100 c.c.). After 10 min. the yellow precipitate was collected and gave **3-*carbamoyl*-1,6-*dihydro*-4-*methyl*-1-(*tetra-acetyl*-β-D-*glucopyranosyl*)*pyridine*** (0·1 g., 45%), as pale yellow needles, m.p. 165—166° (from ether–ethanol) (Found: C, 54·3; H, 5·9; N, 6·0%). In the same way, 3-carbamoyl-1-(tetra-acetyl-β-D-glucopyranosyl)pyridinium bromide (5 g.) gave **3-*carbamoyl*-1,6-*dihydro*-1-(*tetra-acetyl*-β-D-*glucopyranosyl*)-*pyridine*** (2·6 g., 59%), as bright yellow needles, m.p. 147—148° (Found: C, 53·1; H, 5·4; N, 6·2. $C_{20}H_{26}N_2O_{10}$ requires C, 52·9; H, 5·8; N, 6·2%).

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