Carbamoylation of B_6 Vitamins

Fabio Ponticelli*

Istituto di Chimica Organica, Università di Siena,
Piano dei Mantellini 44, 53100 Siena, Italy

Enrico Marinello, Roberto Pagani and Lucia Terzuoli

Istituto di Chimica Biologica, Università di Siena,
Piano dei Mantellini 44, 53100 Siena, Italy

Received February 4, 1991

The reaction of B_6 vitamins **1-3** with cyanate, in the presence of equivalent amounts of hydrochloric acid, yields different adducts according to the structure of the starting material. Regiospecific attack on the amino group or the phenolic hydroxy group was found for **2a,b** and **3a,b**, respectively. From the aldehydes **1a,b**, the 2*H*-pyrido[3,4-*e*]-1,3-oxazin-2-ones **7a,b** were obtained through an attack on both the phenolic and aldehyde group.

J. Heterocyclic Chem., **28**, 1225 (1991).

Introduction.

We have recently demonstrated [1] that L-threonine deaminase, a pyridoxal-5-phosphate (PLP) dependant enzyme, is competitively inhibited by carbamoylphosphate (CP) and/or cyanate, which represents the main CP decomposition product in water solution [2]. Enzyme inhibition was attributed to a reaction of CP or cyanate with PLP, with the consequence that the holoenzyme could not be restored.

In order to confirm this hypothesis, we first considered [1] the behaviour towards cyanate, under non enzymatic conditions, of PLP **1b**. On the basis of the spectral data we showed that 2*H*-pyrido[3,4-*e*]-1,3-oxazin-2-one **7b** was formed.

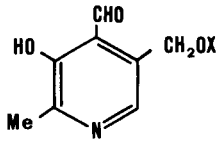
Here we have studied the reactivity of cyanate with the other members of the B_6 vitamin group **1a**, **2a,b** and **3a,b**. Although only PLP is a coenzyme, all these compounds can, in fact, play a role in enzyme regulation, since their facile interconversion under biological conditions is well established [3].

Results and Discussion.

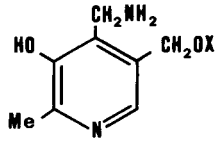
Compounds **1-3** easily reacted with cyanate in the presence of equivalent amounts of hydrochloric acid to give, mainly, a monoaddition product, stabile in neutral or moderately acid solutions. Due to the polifunctional character of the starting materials, the regiochemistry of the cyanate attack was not evident *a priori*. As a consequence, the structure of the adducts was generally identified through careful examination of the spectral properties (Table 2).

Firstly, it is clear that reaction of the amines **2a,b** with cyanate yields the pyridylureas **4a,b**. In fact, compound

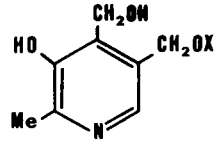
Scheme 1



1 a, b

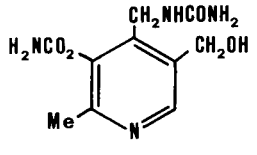


2 a, b

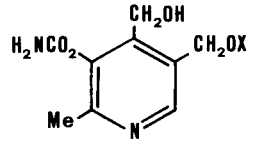


3 a, b

4 a, b



5



6 a, b

a) X= H ; b) X= PO_3H_2

Table 1

Preparation of Compounds 4-7: Yields, mp and Analytical Data

Compound	Yield %	Mp °C	Formula	Elemental Analysis Calcd. (Found) % C	H	N
4a [a]	85	198-200	–	–	–	–
4b	67	198	$C_9H_{14}N_3O_6P \cdot 2\,H_2O$	33.03 (33.06)	5.54 (5.21)	12.84 (12.84)
5	3	149	$C_{10}H_{14}N_4O_4$	47.24 (46.85)	5.55 (5.70)	22.04 (21.68)
6a	19	105	$C_9H_{12}N_2O_4$	50.94 (50.63)	5.70 (5.65)	13.20 (13.22)
6b	– [b]	–	–	–	–	–
7a	90	215 [c]	$C_9H_{10}N_2O_4$	51.43 (51.43)	4.80 (4.94)	13.34 (12.98)

[a] As hydrochloride, lit [4] mp 205-208°, dec. [b] This compound was observed only in the crude reaction mixture. [c] With decomposition.

4a was directly identified by comparison with an authentic sample similarly prepared [4] and **4b** shows an analogous spectral behaviour. That is, in the ir spectrum of both compounds **4a,b**, a strong band was evident in the range 1680-1650 cm^{-1}, attributable to the carbonyl group of an urea moiety.

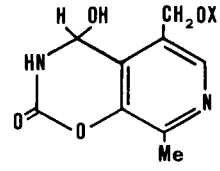
In the case of **2a**, a bisadduct was also obtained. The structure **5** was assigned to this compound on the basis of the presence in the ir spectrum of two stretching bands at 1730 and 1650 cm^{-1}, attributable to the *-COO-* and *-CON-* groups, respectively, and of the signals of the CH_2OH system in pmr spectrum (a doublet at 4.62 and an exchangeable triplet at 5.37). Mass spectra of compounds **4a** and **5** were identical, showing that, under electron impact, loss of the 3-carbamoyl group from **5** is a very facile process.

Starting from the pyridoxine **3a**, the carbamate **6a** was obtained, as demonstrated by the carbonyl absorption at 1720 cm^{-1} and by the presence, in the pmr spectrum, of signals of two CH_2OH groups (two doublets at 4.42 and 4.64 and two exchangeable triplets at 5.03 and 5.25). Here too, the 3-carbamoyl group disappeared completely under electron impact: the mass spectrum of **6a** was identical to that of the starting material **3a**.

Compound **3b** reacted with cyanate presumably to give, as we may infer on the basis of ir and pmr spectra of the crude reaction mixture, the corresponding monoadduct **6b**; however, hygroscopicity and higher instability of this compound did not allow for complete purification and characterization.

As to the product of the aldehyde **1a** with cyanate, pmr clearly indicated that the CH_2OH group was adjacent to a chiral center. In fact, the methylene hydrogens resonated as an ABX system. In addition, mass spectrum (FAB) evidences a $[M+1]^{+\cdot}$ signal at m/z 211, corresponding to the molecular formula $C_9H_{10}N_2O_4$. On this basis we can attribute to the adduct the 3,4-dihydro-2*H*-pyrido[3,4-*e*]-1,3-oxazin-2-one **7a** structure, analogous to that which we previously found for compound arising from PLP and cyanate [1].

Scheme 2



7a,b

Table 2

Spectral Data of Compounds 4-7

No.	IR (cm^{-1})	PMR δ (multiplicity, J, assignment)
4a [a]	3380, 3250, 3100-2100, 1675	2.60 (s, Me), 4.33 (d, J = 6.5, 4'-CH_2), 4.76 (s, 5'-CH_2), 6.43 (s [b], NH_2), 7.59 (t [b], J = 6.5, NH), 8.12 (s, H_6)
4b	3410, 3180, 2800-2000, 1650	2.35 (s, Me), 4.16 (d, J = 3.4, 4'-CH_2), 4.50 (br s [b], NH_2/OH), 4.82 (d, J = 5.0, 5'-CH_2), 6.28 (br s [b], NH/OH), 7.36 (br s [b], OH/NH)
5	3440, 3400, 3305, 3205, 1730, 1650	2.34 (s, Me), 4.17 (d [c], J = 5.4, CH_2NH), 4.62 (d [c], J = 5.4, CH_2OH), 5.37 (t [b], J = 5.4, OH), 5.58 (s [b], NH_2), 6.00 (t [a], J = 5.4, NH), 7.20 (s [b], NH_2), 8.32 (s, H_6)
6a	3380, 3270, 3190, 3130-2500, 1720	2.29 (s, Me), 4.42 (d, J = 4.7, 5-CH_2), 4.64 (d, J = 4.7, 4-CH_2), 5.03 (t, J = 4.7, 5-CH_2OH), 5.25 (t, J = 4.7, 4-CH_2OH), 7.01, 7.38 (2 br s [b], NH_2), 8.30 (s, H_6)
6b	3420, 3350, 3260, 3160, 1715	2.62 (s, Me), 5.13 (d, J = 7.2, 5-CH_2), 5.31 (s, 4-CH_2), 8.13 (s, H_6) [d]
7a	3420, 3320, 3170, 3100-2400, 1740	2.41 (s, Me), 4.62 (ABX, J_{AB} = 13.0, J_{AX} = J_{BX} = 4.8, 5-CH_2OH), 5.35 (t [b], J = 4.8, 5-CH_2OH), 5.83 (dd [c], J_{CHOH} = 8.6, J_{CHNH} = 3.1, 4-CH), 6.76 (d [b], J_{CHOH} = 8.6, 4-CHO*H*), 8.24 (s, H_6), 8.96 (d, J_{CHNH} = 3.1, 4-CHN*H*)

[a] As the hydrochloride. [b] Exchangeable with deuterium oxide. [c] Singlet with deuterium oxide. [d] In deuterium oxide.

Coupling between CH in 4-position and two exchangeable hydrogens (NH and OH) was observed in the case of **7a**, whereas for **7b** there was coupling with a NH group only [1]. However, it is well known that OH-CH couplings are observed only in absence of rapid exchange processes, such as those occurring when the molecule contains an H_2PO_3 moiety. In fact, OH-CH coupling also disappears in the spectrum of compound **7a** when a small amount of hydrochloric acid is added to the pmr solution. Regarding the structure of compounds **7a,b**, it is to be noted that similar covalently hydrated form had been previously observed for 2*H*-benzo-1,3-oxazin-2-ones [5], strictly correlated to **7a,b**. A facile loss of water to a $[M-18]^{+\cdot}$ ion was evident in mass spectrum (EI) of **7** (Experimental).

Further work is necessary in order to verify the biological role of the products thus obtained; however, since the reactions between CP and B_6 vitamins **1-3** occur easily, also under physiological concentration of the reagents (*p*H 7, 37°) [1], it is possible that such reactions occur also in the cell. Consequently, they might regulate both the intracellular concentration of vitamin B_6 derivatives and the activity of vitamin B_6 dependent enzymes.

EXPERIMENTAL

Melting points were taken on a Köfler micro hot stage and are uncorrected. Infrared spectra were measured for potassium bromide dispersion on a Perkin Elmer 782 grating spectrometer. The pmr spectra were recorded, unless otherwise stated, for solution in hexadeuteriodimethyl sulfoxide on a Varian XL-200 instrument; chemical shifts (J in Hz) are reported in ppm downfield from TMS as internal standard. The EI (70 eV) or FAB (CsI) mass spectra were recorded on a VG 70-250 S spectrometer for pure solid compounds or dispersion in glycerol, respectively. Yields, mp, analytical data, ir and pmr spectral data of compounds prepared in this paper are reported in Tables 1 and 2.

[3-Hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridyl]methyl Urea **4a** and [3-Carbamoyloxy-5-hydroxymethyl-2-methyl-4-pyridyl]methyl Urea **5**.

To a solution of **2a** (as the hydrochloride) (0.61 g, 3 mmoles) in water (5 ml) potassium cyanate (0.49 g, 6 mmoles) was added in portions. Final solution was acidified (*p*H 4) with 1*N* hydrochloric acid and, on standing overnight in the refrigerator, compound **5** was separated as white crystals. After concentration *in vacuo* to 2 ml, the hydrochloride of compound **4a** was collected by filtration as a white powder and washed with cold water (2 ml); ms: m/z 211 (M^+, 51), 194 (19), 176 (18), 151 (97), 139 (42), 123 (55), 106 (47), 94 (100). When this reaction was carried out using a 1:1 molar ratio between **2a** and cyanate, pmr analysis of the crude reaction mixture indicated that both compounds **2a** and **5** were obtained with some unreacted starting material present.

[3-Hydroxy-2-methyl-4-(ureidomethyl)-5-pyridinemethyloxy] Phosphonic Acid **4b**.

Operating as above from the hydrochloride of **2b** (0.71 g, 2.5 mmoles) and potassium cyanate (0.4 g, 4 mmoles) we obtained compound **4b** which separated slowly, on cooling, into white crystals.

4,5-Dihydroxymethyl-2-methyl-3-pyridyl Carbamate **6a**.

Repeating the same procedure for compound **3a** (as the hydrochloride) (1.03 g, 5 mmoles) and potassium cyanate (0.41 g, 5 mmoles), compound **6a** was obtained, as a white powder, after standing.

4-Hydroxy-8-methyl-5-hydroxymethyl-3,4-dihydro-2*H*-pyrido-[3,4-*e*]-1,3-oxazin-2-one **7a**.

With the same procedure, using pyridoxal hydrochloride (0.6 g, 3 mmoles) and potassium cyanate (0.4 g, 4.9 mmoles), compound **7a** was obtained as white crystals after standing in the refrigerator; ms: m/z 192 ($M-H_2O$, 78), 167 (51), 150 (100), 135 (55).

(3-Carbamoyloxy-4-hydroxymethyl-2-methyl-5-pyridinemethyloxy) Phosphonic Acid **6b**.

To a solution of compound **3b** (0.75 g, 3 mmoles) in water (5 ml), 1*N* hydrochloric acid (3 ml) and potassium cyanate (0.25 g, 3 mmoles) were added. Solvent was carefully removed *in vacuo* at room temperature and the hygroscopic residue identified as **6b** by ir and pmr analysis (Table 2).

Acknowledgment.

We thank the Centro di Analisi e Determinazioni Strutturali, University of Siena, Italy, for making available the mass spectrometer.

REFERENCES AND NOTES

[1] R. Pagani, F. Ponticelli, L. Terzuoli, R. Leoncini and E. Marinello, *Biochim. Biophys. Acta*, in press.

[2] C. M. Allen and M. E. Jones, *Biochemistry*, **3**, 1238 (1964).

[3] H. Wada and E. E. Snell, *J. Biol. Chem.*, **236**, 2089 (1961).

[4] G. E. McCasland, E. Blauz, Jr. and A. Furst, *J. Org. Chem.*, **24**, 1000 (1959).

[5] R. E. Strube and F. A. Mackellar, *Rec. Trav. Chim.*, **83**, 1191 (1964).