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Proton magnetic resonance study of the molecular conformation of N-(3-pyridinyl)3-pyridinecarboxamide, N-(3-pyridinyl)acetamide and 3-pyridinecarboxamide

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Abstract

The ^{1}H NMR spectra of N-(3-pyridinyl) 3-pyridinecarboxamide, N-(3-pyridinyl)acetamide and 3-pyridinecarboxamide in different solvents have been assigned utilizing the COSY spectra, chemical shift and coupling constant correlations. The ^{1}H NMR spectra, NOE experiments and MINDO/3 calculations have been utilized for obtaining information on the molecular conformation. The results suggest that in dilute solutions, the 3-pyridyl rings are not coplanar with the amide group, which is in trans orientation in all the three compounds. © 1998 Elsevier Science B.V.

Keywords: NMR spectra; Molecular conformation; Pyridinecarboxamide

1. Introduction

In order to understand the molecular conformation and structure–activity relationships of proteins, it is desirable to undertake a systematic study of the conformations of amides which serve as model systems and note the effect of varying intra- and intermolecular interactions. Pyridyl derivatives of amides show interesting conformational consequences and serve as model compounds for the study of conformation of polypeptides and proteins. In this context, the ^{1}H NMR investigations of N-(3-pyridinyl)-3-pyridinecarboxamide (N3P3PC), 3-pyridinecarboxamide(3PCA) and N-(3-pyridinyl)acetamide (N3PA) are reported (Fig. 1). The latter two compounds also serve as reference for N3P3PC which is reported to be very effective for lowering the blood pressure and possess low toxicity [1]. The ^{1}H NMR spectra of N3P3PC, 3PCA and N3PA in different solvents have been analyzed with the help of COSY spectra. The ^{1}H chemical shifts, NOE enhancements and MINDO/3 calculations have been utilized for determining the molecular conformation. No work on the NMR spectra of N3P3PC has been reported previously. The ^{1}H NMR of N3PA and 3PCA (generally known as nicotinamide) have been reported earlier [2,3]. However, little is known on the conformation present in solution. Long *et al.* [2] have carried out ^{13}C NMR measurements for nicotinamide and its N-alkyl and N,N-dialkyl derivatives and the substituent effects were correlated with dipole moments. Brown *et al.* [3], on the other hand, have investigated the effect of acylation of the 2-amino group in 2-aminopyridines on the ^{1}H NMR spectra.

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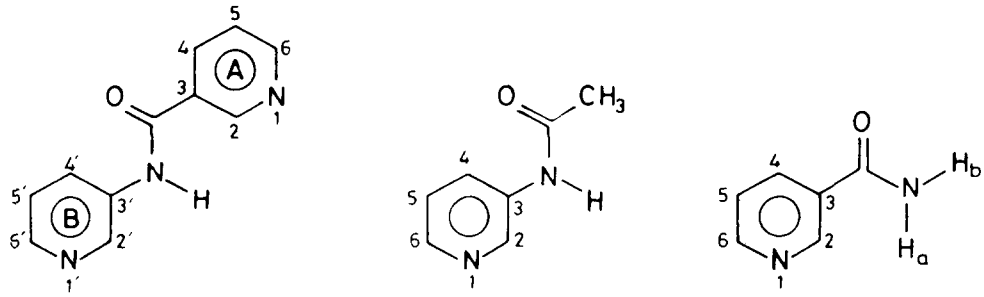

Fig. 1. Molecular geometry and numbering of atoms of N3P3PC, N3PA and 3PCA.

2. Experimental

2.1. Materials

3-Aminopyridine, 3-pyridinecarboxylic acid and 3PCA were Aldrich chemicals. For the synthesis of N3P3PC, a mixture of 3-pyridinecarboxylic acid (0.07 mol) and 3-aminopyridine (0.05 mol) was taken in a round bottom flask fitted with an air condenser. It was heated slowly on an oil bath until it melted. The temperature was then raised from 180 to 200° for the liquid to boil smoothly. It was refluxed at this temperature for 4 h. The hot reaction mixture was then poured, while stirring, into a beaker containing 200 ml of water. A brown solid separated out. Excess of saturated aqueous $NaHCO_3$ was added to it with stirring and it was left overnight. It was then filtered, washed with water and purified by recrystallization from ethanol using activated charcoal, from which a nearly colourless filtrate was obtained. The filtrate was concentrated and kept at 0°C, and needle-like crystals separated out after about 12 h. These were dried in air; m.p. 88°C. (Found: C 59.90, H 4.85, N 20.85; Calculated for $C_{11}H_9N_3O$, C 60.27, H 4.52, N 21.08%). For the synthesis of N3PA, a mixture of 3-aminopyridine (0.01 mol) and acetic anhydride (0.011 mol) was refluxed for 3 h. Excess of acetic anhydride was distilled off under reduced pressure. A brown coloured product obtained on cooling was recrystallized from ethanol; m.p. 126°C. (Found: C 61.35, H 6.10, N 20.15: Calculated for $C_7H_8N_2O$, C 61.63, H 5.87, N 20.56%).

2.2. NMR spectroscopy

The ^1H NMR spectra were recorded on Bruker AC 200 MHz and AMX 400 MHz FT spectrometers at 18°C with tetramethylsilane as an internal standard for dilute solutions (1–2 mg/ml) in $CDCl_3$ (Merck isotopes), $(CD_3)_2CO$ and CD_3CN (Aldrich chemicals). The resolution enhanced ^1H NMR spectra were simulated using the program LAOCOON-5 [4]. The COSY spectra were obtained on a Bruker WH 270 MHz FT instrument using standard pulse sequence (P_1, P_2 5.7 μs). The parameters employed were: spectral width, 600–800 Hz; acquisition time, 0.5 s; preparation time, 1–2 s; number of transients, 16 or 32; and number of experiments, 258.

Steady state NOE experiments were carried out using the 200 MHz instrument on degassed samples in $CDCl_3$. The experimental parameters were: pulse width, 5.7 μs; spectral width, 3000 Hz; acquisition time, 1 s; irradiation time, 3–4 s; and relaxation delay, 5 s.

The MINDO/3 calculations were carried out using program no. 308 obtained from Quantum Chemistry Program Exchange [5].

3. Results and discussion

3.1. Assignment of proton resonances

The chemical shifts and coupling constants of N3PA, 3PCA and N3P3PC are given in Table 1 and Table 2, respectively. Assignment of the ring protons

Table 1
^{1}H NMR chemical shifts[a] of 3PCA, N3PA and N3P3PC

Solvent	Compound	Group[b]	δ (ppm)				
			$H_2/H_{2'}$	$H_4/H_{4'}$	$H_5/H_{5'}$	$H_6/H_{6'}$	NH
$CDCl_3$	N3P3PC	3-py	9.13	8.24	7.47	8.80	8.19
	3PCA	3-py	9.03	8.18	7.43	8.76	6.05, 6.24
	N3P3PC	3-py′	8.72	8.29	7.36	8.42	—
	N3PA	3-py′	8.57	8.18	7.26	8.30	9.05
$CDCl_3$+	N3P3PC	3-py	9.20	—	7.46	8.77	9.68
$(CD_3)_2CO$[c]		3-py′	8.86	—	7.34	—	—
	N3PA	3-py′	8.67	8.05	7.26	8.17	—
$(CD_3)_2CO$	N3P3PC	3-py	9.26	—	7.62	8.84	9.97
	3PCA	3-py	9.19	8.33	7.55	8.78	7.08, 7.97
	N3P3PC	3-py′	9.02	—	7.46	—	—
	N3PA	3-py′	8.80	8.21	7.36	8.33	9.47
CD_3CN	N3P3PC	3-py	9.10	8.26	7.51	8.75	9.17
	3PCA	3-py	8.99	8.15	7.44	8.69	6.38, 7.05
	N3P3PC	3-py′	8.85	8.18	7.38	8.35	—
	N3PA	3-py′	8.66	8.03	7.28	8.26	8.47
		py		7.60	7.22	8.65	—

[a]Numbering follows from Fig. 1.
[b]3-py denotes 3-pyridyl group and 3-py′ is the 3-pyridyl group bonded to nitrogen.
[c]0.5 M $(CD_3)_2CO$ in $CDCl_3$.

of N3PA and 3PCA was made using COSY spectra and coupling constants. The ^{1}H NMR spectrum of 3PCA measured on 200 MHz instrument in $CDCl_3$ is not a pure first order spectrum. The RE spectrum was simulated which gave a best fit to the experimental spectrum (RMS error 0.015). Owing to the diamagnetic anisotropic effect of the C=O group [6], H_b(N–H) shows a high frequency shift. In dilute

Table 2
^{1}H Coupling constants[a] (Hz) of 3PCA, N3PA and N3P3PC

Solvent	Compound	Group[b]	$^4J_{24}/^4J_{2'4'}$	$^5J_{25}/^5J_{2'5'}$	$^3J_{45}/^4J_{4'5'}$	$^4J_{46}/^4J_{4'6'}$	$^3J_{56}/^3J_{5'6'}$	$^5J_{NH}$
$CDCl_3$	N3P3PC	3-py	2.29	0.79	7.94	1.70	4.85	
	3PCA	3-py	2.30	0.87	7.95	1.74	4.85	
	N3P3PC	3-py′	2.50	0.00	8.35	1.39	4.70	
	N3PA	3-py′	2.52	0.00	8.33	1.48	4.77	
$CDCL_3$ +	N3P3PC	3-py	2.13	0.67	7.85	1.66	4.80	
$(CD_3)_2CO$								
		3-py′	2.47	—	8.32	—	4.76	
	N3PA	3-py′	2.53	0.73	8.34	1.47	4.77	
$(CD_3)_2CO$	N3P3PC	3-py	2.33	0.90	7.97	1.67	4.82	
	3PCA	3-py	2.29	0.89	7.94	1.71	4.81	
	N3P3PC	3-py′	2.59	0.77	8.33	—	4.72	
	N3PA	3-py′	2.59	0.75	8.33	1.53	4.71	0.3
CD_3CN	N3P3PC	3-py	2.34	0.88	7.99	1.67	4.85	
	3PCA	3-py	2.31	0.90	7.96	1.69	4.84	
	N3P3PC	3-py′	2.59	0.77	8.33	1.50	4.75	
	N3PA	3-py′	2.59	0.76	8.33	1.50	4.74	0.3
		py	7.65	1.35	7.65	1.85	4.87	

[a,b,c]As in Table 1.

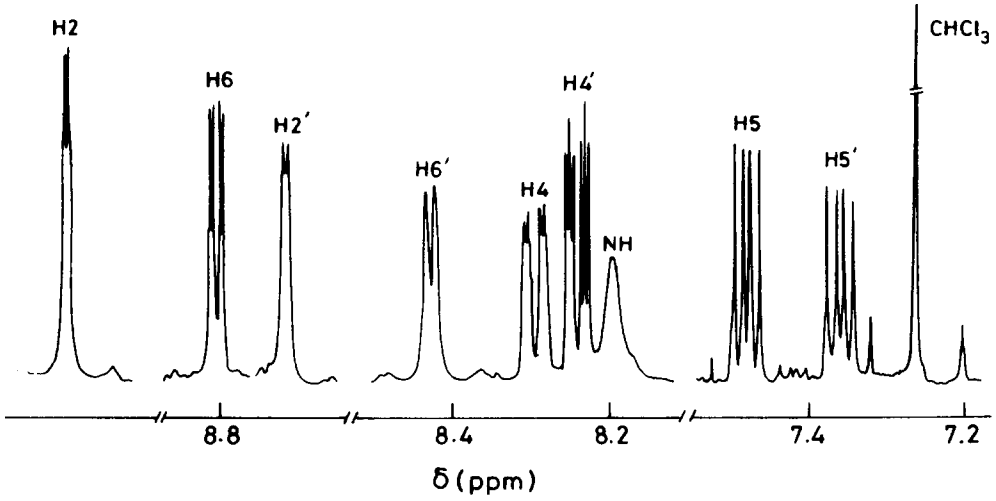

Fig. 2. ^{1}H NMR spectrum of N3P3PC in $CDCl_3$ on 400 MHz spectrometer.

solutions of $(CD_3)_2CO$ and CD_3CN, the NH_2 protons of 3PCA show high frequency shift compared to those in $CDCl_3$ due to intermolecular hydrogen bonding between N–H and the solvent.

The ^{1}H NMR spectrum of N3P3PC is given in Fig. 2. The ^{1}H chemical shifts and spin–spin coupling constants were obtained by spectral simulation. The protons of the pyridyl rings A and B of N3P3PC were assigned directly from the COSY spectrum given in Fig. 3, where the diagonal peaks due to the protons of the A and B rings are marked "a" and "b" respectively. From a knowledge of the coupling constants in pyridines [6–8], the assignment of H4, H4′, H5 and H5′ was made. The chemical shifts and coupling constants of the two different 3-py groups of N3P3PC are in good agreement with those of 3PCA and N3PA (Tables 1 and 2), which thus serve as reference compounds.

3.2. Molecular conformation

3.2.1. 3PCA and N3PA

The possible molecular conformations of N3PA and 3PCA are shown in Fig. 4. The barrier to rotation of the pyridyl group of 3PCA around the C_3–C bond was calculated by the MINDO/3 method by varying the torsional angle C_4–C_3–C–O from 0 to 360° at intervals of 10° by optimizing the molecular geometry at each step. The total energy was found to be the least for conformation III (− 1545.625 eV) where the 3-py ring is nearly perpendicular to the plane of the amide group. The conformation III is more stable compared to the conformations I and II by 10.5 and 15.1 kJ mol^{-1}, respectively. Steric effect possibly accounts for the non-planarity of the 3-py group from the amide plane [9].

The conformations of N3PA shown in Fig. 4 are planar except for III where the 3-py ring is rotated out of the amide plane. The calculated energies (in kJ mol^{-1}) of the other two trans conformers I and II with reference to III (total energy − 1701.925 eV) are 4.45 and 2.20 kJ mol^{-1}, respectively. As is to be expected, the molecular conformations of N3PA when the –CONH– group is *cis* are less stable than when the secondary amide group has a *trans* conformation (by 15–21 kJ mol^{-1}), possibly due to steric hindrance, and they are not shown in Fig. 4. The calculated total energies indicate the conformers I–III to be almost equally probable.

The NH resonance of N3PA in $CDCl_3$ shows a high frequency shift (0.85 ppm) from N3P3PC suggesting intermolecular hydrogen bonding of N3PA in $CDCl_3$. It is supported by the fact that in very dilute $CDCl_3$ solution, the NH chemical shift of N3PA occurs at 7.59 ppm. In $(CD_3)_2CO$, the NH proton which is hydrogen bonded to acetone oxygen shows a high frequency shift of 1.88 ppm relative to that in dilute $CDCl_3$ solution.

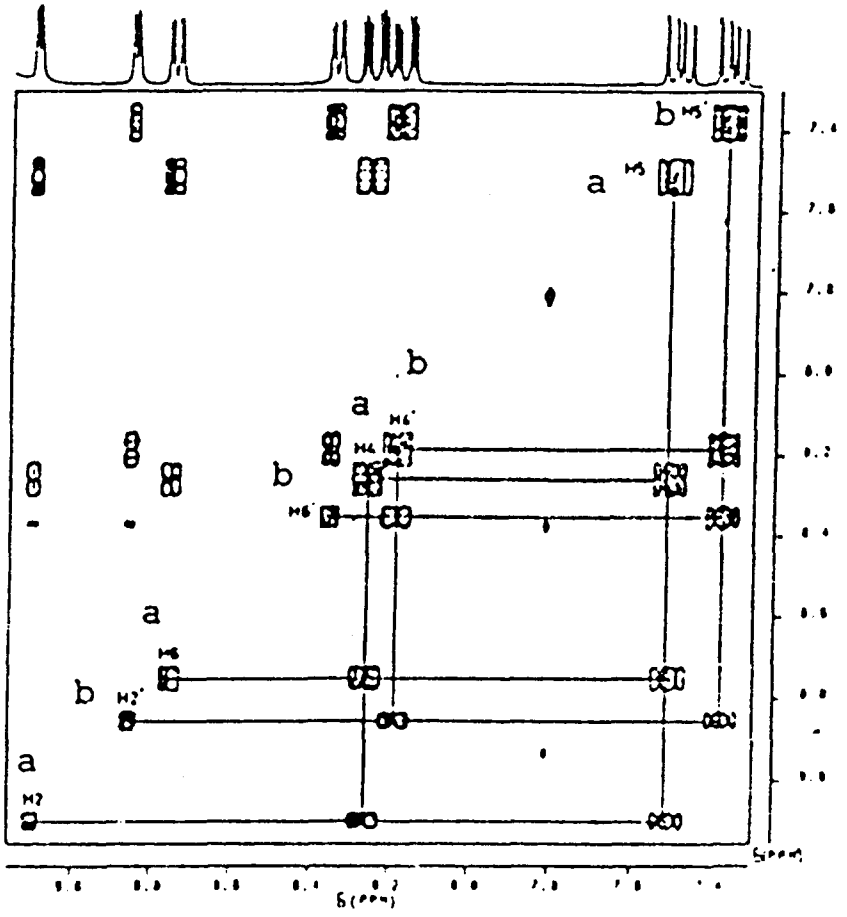

Fig. 3. ^{1}H COSY contour plot of N3P3PC in CD_3CN on 200 MHz instrument.

In dilute $CDCl_3$, when the NH proton of N3PA was saturated, the NOE enhancement observed at H2, H4 and H6 were 6.0, 1.8 and 0.8%, respectively. Using the NOE enhancement at H2, the NOE at H4 was calculated [10,11] taking the distance of the NH proton from H2 (2.23 Å) and from H4 (3.82 Å) from the optimized molecular geometries. From the calculated ratio of f_2(NH) : f_4(NH) 2.9 : 1.0 in good agreement with the observed ratio of 3.0 : 1.0, the torsional angle C_4–C_3–N–C for conformer III was calculated to be nearly 65 ± 5°. Following a similar procedure, in $(CD_3)_2CO$ solution, the torsional angle C_4–C_3–N–C was found to be nearly 84°.

The conformation III of N3PA is apparently sterically more favourable than I and II, where the distance between the amide oxygen and H4 or H2, and that between N–H proton and H2 or H4 would be nearly 2.6 and 2.3 Å, respectively, which are similar to the limiting O···H(2.2–2.4 Å) and N···H(1.9–2.0 Å) distances for short contact [12]. For III, the corresponding distances are greater than the non-bonded O···H and N···H distances for short contact.

3.2.2. N3P3PC

The possible molecular conformations of N3P3PC are shown in Fig. 5. The NOE difference spectrum of N3P3PC in $CDCl_3$ containing 0.5 M $(CD_3)_2CO$ when the N–H proton is irradiated, shows significant NOE enhancements at H2 (4.4%), H2′ (3.6%), H4 (1.9%) and H4′ (2.2%). The distance of the N–H proton from H2 and H2′ is hence expected to be nearly the same, as well as the distance of the N–H proton from H4 and

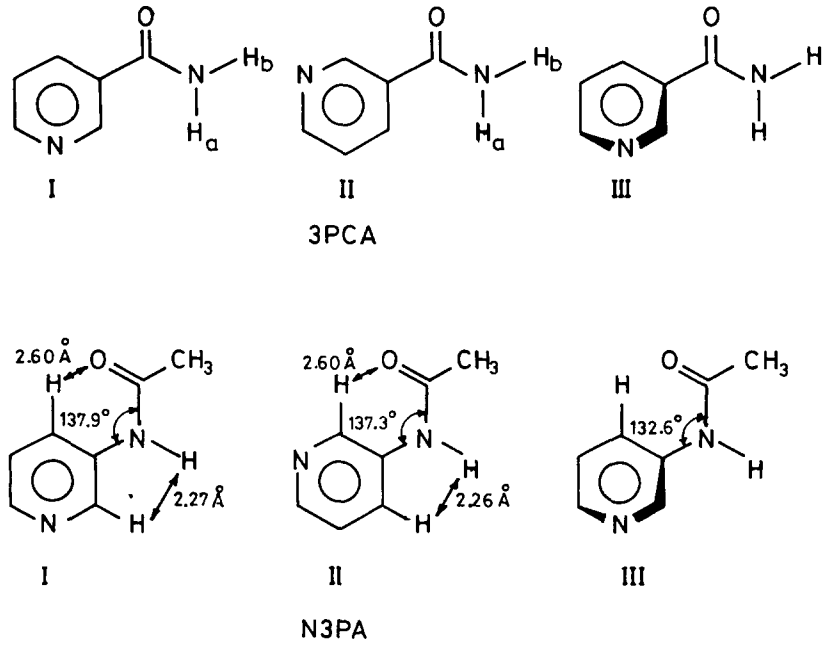

Fig. 4. Molecular conformation of 3PCA and N3PA.

H4′. These distances reveal that the amide group exists in the *trans* orientation as shown in Fig. 5, where the distances obtained from the MINDO/3 optimized molecular geometry are given. Using the NOE enhancements, the distances of H2 and H4 (and similarly of H2′ and H4′) from N–H were estimated [10,11]. The results indicate the A- and B-rings are nearly perpendicular to the amide plane. For free rotation of the rings A and B, the two ratios $f_2(NH) : f_4(NH)$ and $f_{2'}(NH) : f_{4'}(NH)$ were calculated to be 1.02 : 1.0 and 1.04 : 1.0, respectively, which differ from observed values 2.32 : 1.0 and 1.64 : 1.0, respectively. The torsional angles C_4–C_3–C–O and $C_{4'}$–$C_{3'}$–N–C were calculated to be nearly 75 and 85°, respectively, utilizing the optimized molecular geometry of I and the observed NOEs at H2, H4, H2′ and H4′. It is hence unlikely that the rings A and B rotate freely around the N–C_3 and C–$C_{3'}$ bonds, respectively. The MINDO/3 calculation for N3P3PC also favours I as the most stable rotamer (see Fig. 5) compared to the other conformers by 20–30 kJ/mol. Thus, at ambient temperature, conformer I is expected.

The pi-electron delocalization apparently should favour the two py rings in the amide plane. The distance of H4 and H4′ of N3P3PC from the carbonyl oxygen (about 2.6 Å) for the planar conformers II–V is almost the same distance as the non-bonded limiting O···H distance for short contact [12]. Similarly the distance of H2 and H2′ from the NH proton (2.3–2.0 Å) is nearly the same as the non-bonded H···H distance for short contact [12]. On the other hand, these distances from N–H proton (3.14–3.48 Å) and from the carbonyl oxygen (3.5 Å) for I are larger than the non-bonded O···H and H···H distances for short contact [12]. For planar conformations II–V, the protons H2 and H2′ or H4 and H4′ should show high frequency shift due to the diamagnetic anisotropic effect of the amide group, which was not observed. This implies that N3P3PC adopts a less sterically hindered conformation with the pyridyl groups out of the amide plane. Fasone *et al.* [13] have studied the electronic absorption spectra and dipole moments of aromatic amides of the type R_1CONHR_2 where R_1 and R_2 denote pyridyl or phenyl groups. The compounds were inferred to exist in S-*trans* form from the calculated dipole moments from simple Huckel molecular orbital calculations which were compared with the experiment. These results are obviously not reliable. It is of interest to note here that in acetanilide and

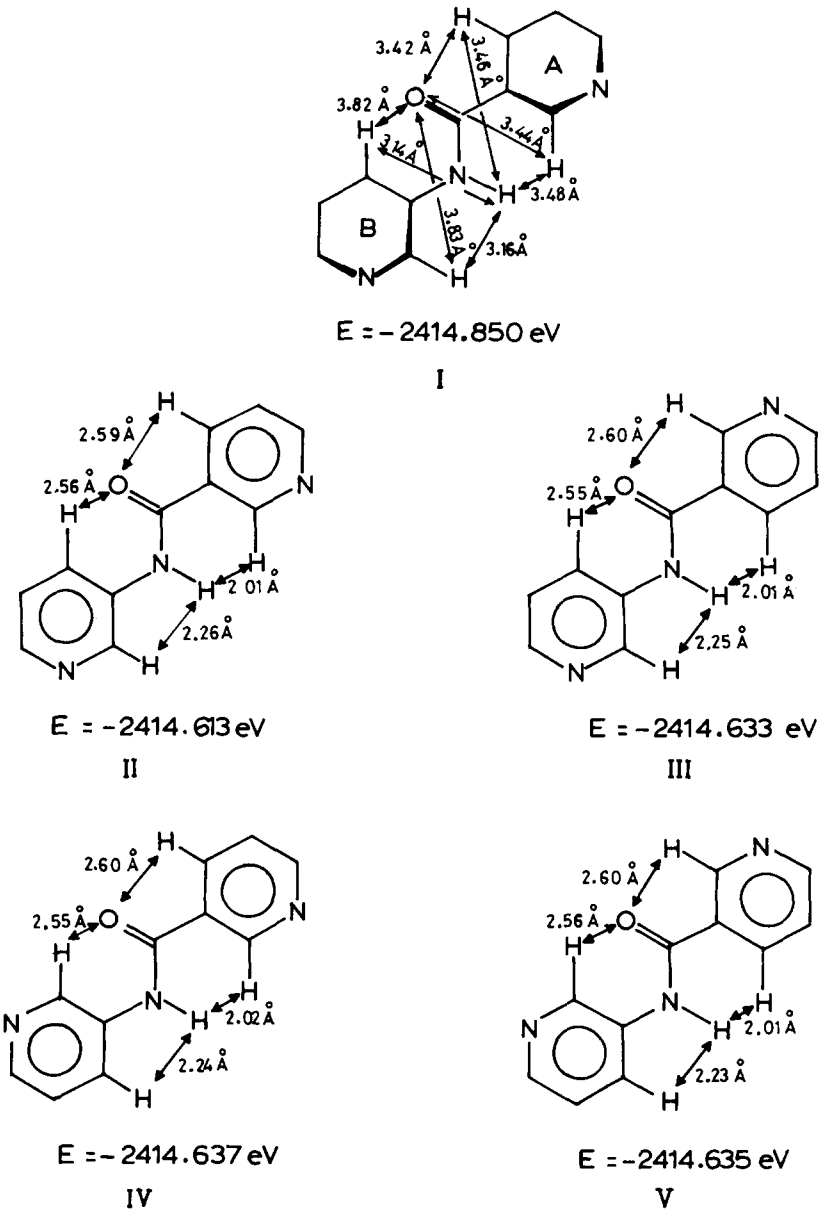

Fig. 5. Molecular conformations of N3P3PC with amide group in the *trans* orientation.

N-methyl acetanilide which are structurally similar to N3PA, the phenyl ring is out of the plane of the amide group by 38 and 90°, respectively [14,15]. Similarly the X-ray structure of N-(3-phenyl-2-propyl)N-(methyl) benzenecarboxamide has shown that the aromatic ring is perpendicular to the amide group [16].

The chemical shifts of the ring protons are nearly the same in all the three solvents (Table 1), showing that the molecular conformation of N3P3PC in these solvents is nearly the same. In $(CD_3)_2CO$ and CD_3CN, the N–H proton is hydrogen bonded to the solvent molecules and shows high frequency shift of 1.78

and 0.98 ppm in $(CD_3)_2CO$ and CD_3CN, respectively, relative to that in $CDCl_3$.

Acknowledgements

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