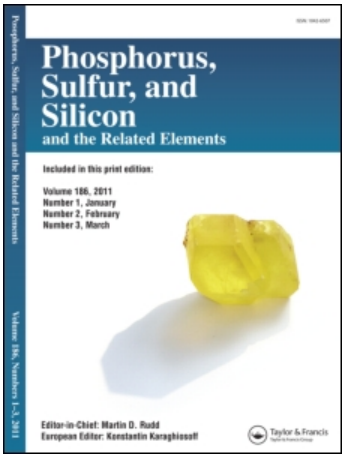

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N-PHOSPHORYL AMINO ACIDS AND PEPTIDES: PART I. THE CRYSTAL AND MOLECULAR STRUCTURE OF *N*-(O,O-DIISOPROPYL PHOSPHORYL)-TRANS-4-HYDROXY-D,L-PROLINE

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N-PHOSPHORYL AMINO ACIDS AND PEPTIDES: PART I. THE CRYSTAL AND MOLECULAR STRUCTURE OF *N*-(O,O-DIISOPROPYL PHOSPHORYL)-TRANS-4-HYDROXY-D,L-PROLINE

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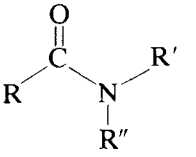
The crystal and molecular structure of *N*-(O,O-diisopropyl phosphoryl)-trans-4-hydroxy-D,L-proline has been determined by X-ray diffraction analysis and is compared with proline or hydroxyproline residue in a peptide chain described in the literature. The compound crystallized in orthorhombic system with space group $P2_12_12_1$, $a = 6.934(2)$, $b = 8.694(3)$, $c = 26.727(7)$ Å, $V = 1611.3(8)$ Å^3, $Z = 4$, $Dx = 1.22$ g/cm^3. The structure was solved by direct method and refined by anisotropic least squares to a discrepancy index $R = 0.072$. In the compound, the nitrogen atom is trigonal and its remaining $2p$ orbital is conjugated with the P=Oπ system; the conformation of the phosphorimidate function is favoured by the trans orientation of the P=O bond with respect to the N-C4 bond. In the pyrrolidine ring moeity, the C2-C1-N-C4 atoms are nearly copolnar and the C3 atom is out of the plane by about 0.47 Å.

INTRODUCTION

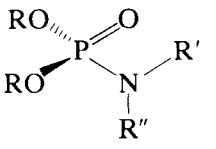
N-phosphorylated amino acids and small peptides have been shown to possess biological activities.[1–3] For example, derivatives of *N*-phosphoaspartic acid and their salts are useful for treating psychic and physic asthenia;[1] *N*-phosphoryl tri-peptides can be used as anti-hypertension drugs.[2] It is likely that the phosphoryl group in these compounds might be, in part, responsible for their peculiar biological functions which are not possessed by other *N*-derivatives of these amino acids and peptides, for instance, *N*-acyl amino acids and peptides.

Phosphoric amides (**2a**) or imides (**2b**) can be considered as close structural analogues of carboxylic amides (**1a**) or imides (**1b**). Both systems are derived



(**1**)

(**1a**): R' = alkyl, R'' = H
(**1b**): $R' = R''$ = alkyl



(**2**)

(**2a**): R' = alkyl, R'' = H
(**2b**): $R' = R''$ = alkyl

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from the primary amine $R'NH_2$ or secondary imine R'R''NH, acylated by carboxyl and phosphoryl groups. However, as far as the bonding characteristics and structural effects are concerned, there are some intrinsical differences between (**1**) and (**2**). For example, the carbonyl center in (**1**) is planar while the phosphoryl center in (**2**) is not planar but tetrahedral. Consequently, the conformational preference of the phosphoramide function in (**2**) might exhibit different behaviour as compared with the carboxamide function in (**1**). In view of this, it is probable that the determination of the molecular structures of *N*-phosphoryl amino acids and peptides will allow to make a comparison of the molecular structures between *N*-phosphoryl and *N*-acyl amino acids and peptides and will be helpful for the understanding of structure-activity relationships of *N*-phosphoryl amino acids and peptides.

As part of our interest in the structure-activity relationships of *N*-phosphoryl amino acids and peptides, we have investigated the conformations of *N*-(O,O-dialkyl phosphoryl)-amino acids and their derivatives in solution by IR and NMR spectroscopy.[4] To help interpret the observed spectroscopic properties in these compounds and to make a comparison of the crystal and molecular structures between *N*-phosphoryl and *N*-acyl amino acids, the crystal and molecular structure of *N*-(O,O-diisopropyl phosphoryl)-trans-4-hydroxy-D,L-proline (*N*-DIPP-Hyp, **3**) has been determined and is presented in this paper.

RESULTS AND DISCUSSION

The final atomic coordinates for nonhydrogen atoms and equivalent isotropic temperature factors are listed in Table I, and the bond lengths and bond angles

TABLE I

Atomic coordinates ($\times 10^4$), isotropic temperature factors ($\times 10^3$) and site occupancy factors

	X	*Y*	*Z*	*U*	S.O.F.
C(1)	5240(18)	3619(17)	2121(6)	57(3)	1.00
C(2)	7165(17)	4173(16)	2337(6)	97(2)	1.00
C(3)	8646(20)	3662(16)	1962(6)	90(3)	1.00
C(4)	7682(20)	3651(21)	1458(6)	84(3)	1.00
C(5)	3735(18)	4894(15)	2173(5)	52(2)	1.00
C(6)	3240(31)	3595(27)	390(9)	117(3)	0.68
C(6')	3717(33)	4788(31)	592(11)	34(3)	0.32
C(7)	4619(28)	4769(26)	80(6)	264(3)	1.00
C(8)	1408(23)	4578(20)	415(7)	132(3)	1.00
C(9)	4881(22)	−614(16)	1207(6)	102(3)	1.00
C(10)	6830(22)	−1388(19)	1151(7)	157(3)	1.00
C(11)	3211(24)	−1288(19)	953(7)	170(3)	1.00
O(1)	3019(12)	4849(11)	2638(4)	72(2)	1.00
O(2)	3336(16)	5812(11)	1869(4)	87(2)	1.00
O(3)	9325(11)	2127(11)	2088(4)	86(2)	1.00
O(4)	2330(12)	2123(11)	1474(3)	80(2)	1.00
O(5)	5200(15)	941(14)	1000(4)	107(2)	1.00
O(6)	4160(25)	3868(22)	811(7)	96(3)	0.61
O(6')	4324(26)	2922(19)	671(7)	61(3)	0.39
N	5689(15)	3254(12)	1608(4)	63(2)	1.00
P	4193(5)	2423(6)	1227(2)	77(1)	1.00

TABLE II

Bond lengths (Å) for *N*-DIPP-Hyp

C(1)–C(2)	1.531(18)	C(6′)–C(7)	1.504(33)
C(1)–C(5)	1.529(19)	C(6′)–C(8)	1.679(28)
C(1)–N	1.442(18)	C(6′)–O(6′)	1.689(31)
C(2)–C(3)	1.501(20)	C(9)–C(10)	1.518(22)
C(3)–C(4)	1.506(22)	C(9)–C(11)	1.465(23)
C(3)–O(3)	1.455(17)	C(9)–O(5)	1.477(18)
C(4)–N	1.480(17)	O(4)–P	1.474(9)
C(5)–O(1)	1.339(17)	O(5)–P	1.587(12)
C(5)–O(2)	1.172(17)	O(6)–P	1.678(20)
C(6)–C(7)	1.625(30)	O(6′)–P	1.550(20)
C(6)–C(8)	1.533(27)	N–P	1.623(11)
C(6)–O(6)	1.315(30)		

are given in Tables II and III, respectively. For the convenience of the following discussion, the selected torsional angles (θ) calculated are shown in Table IV. A perspective view of the molecular structure (the L configuration of (**3**), molecule A) and the numbering scheme are presented in Figure 1, and for clarity a diagrammatic representation of the conformation of molecule A including hydrogen atoms is shown in Figure 2. The open bonds in Figure 1 represent the disorder of one of the two isopropyl groups, which exhibits high flexibility in the crystals. This might affect the accuracy of its parametric determination to some degree, but in general the atomic parameters for other parts of the molecule are accurate and will not affect the following discussion.

The values of the P–N–Cl/C4 bond angles (Table III), together with the values of the torsional angles for O4–P–N–C1 and O4–P–N–C4 atoms (Table IV), are in agreement with the coplanarity of the phosphorimidate group. It is thus suggested that the nitrogen atom in (**3**) adopts the configuration of sp^2 hybridization, and the nonbonding electrons of the nitrogen are conjugated with the P=Oπ system. The effect of this conjugation is reflected by the P—N bond

TABLE III

Bond angles (degree) for *N*-DIPP-Hyp

C(2)–C(1)–C(5)	109.4(11)	C(11)–C(9)–O(5)	108.1(12)
C(2)–C(1)–N	103.8(10)	C(9)–O(5)–P	122.2(9)
C(5)–C(1)–N	113.1(11)	C(6)–O(6)–P	116.0(16)
C(1)–C(2)–C(3)	104.6(12)	C(6′)–O(6′)–P	112.0(15)
C(2)–C(3)–C(4)	107.2(11)	C(1)–N–C(4)	114.1(11)
C(2)–C(3)–O(3)	109.8(11)	C(1)–N–P	123.8(9)
C(4)–C(3)–O(3)	110.2(12)	C(4)–N–P	122.0(9)
C(3)–C(4)–N	99.9(11)	O(4)–P–O(5)	114.4(6)
C(1)–C(5)–O(1)	108.4(11)	O(4)–P–O(6)	114.7(7)
C(1)–C(5)–O(2)	126.3(13)	O(5)–P–O(6)	111.1(8)
O(1)–C(5)–O(2)	125.2(12)	O(4)–P–O(6′)	122.0(8)
C(7)–C(6)–C(8)	99.2(16)	O(5)–P–O(6′)	80.5(8)
C(7)–C(6)–O(6)	92.1(16)	O(4)–P–N	111.0(6)
C(8)–C(6)–O(6)	105.3(19)	O(5)–P–N	108.7(6)
C(7)–C(6′)–C(8)	98.0(18)	O(6)–P–N	95.2(8)
C(7)–C(6′)–O(6′)	90.0(17)	O(6′)–P–N	116.0(8)
C(8)–C(6′)–O(6′)	99.7(16)		
C(10)–C(9′)–C(11)	118.7(13)		
C(10)–C(9′)–O(5)	108.1(12)		

TABLE IV

Selected torsional angles (degree) for *N*-DIPP-Hyp

C(5)–C(1)–C(2)–C(3)	−136.2	C(7)–C(6)–O(6)–P	−148.6
N–C(1)–C(2)–C(3)	−14.9	C(8)–C(6)–O(6)–P	109.0
C(2)–C(1)–N–C(4)	−5.4	C(10)–C(9)–O(5)–P	−143.9
C(2)–C(1)–N–P	172.6	C(11)–C(9)–O(5)–P	90.7
C(5)–C(1)–N–C(4)	113.6	C(9)–O(5)–P–N	97.4
C(5)–C(1)–N–P	−68.4	C(6)–O(6)–P–N	170.2
C(1)–C(2)–C(3)–C(4)	30.0	C(1)–N–P–O(4)	0.6
C(1)–C(2)–C(3)–O(3)	−90.1	C(4)–N–P–O(4)	178.5
C(2)–C(3)–C(4)–N	−32.0	N–C(1)–C(5)–O(1)	162.6
O(3)–C(3)–C(4)–N	87.5	N–C(1)–C(5)–O(2)	−21.4
C(3)–C(4)–N–C(1)	23.1	C(1)–N–P–O(5)	−126.1
C(3)–C(4)–N–P	−155.0	C(1)–N–P–O(6)	119.5

length (1.62 Å) which is shorter than the typical P—N single bond length (1.78 Å) in the dipolar molecule of phosphoramidic acid H_3N^+—PO_3H^-,[5] but the resonance interaction in (**3**) is less strong than in cyclotriphosphazenes as can be seen from their P—N bond lengths 1.52–1.60 Å which are thought to be typical for a strong pπ–dπ delocalization along the P—N bond.[6] The phosphoryl P=O bond length (1.47 Å) lies within the normal range of 1.46–1.48 Å observed for analogous phosphoramidate compounds.[7] In the pyrrolidine ring moeity, the C2–C1–N–C4 atoms are nearly coplanar ($\theta = -5.4°$) and the C3 atom is out of the plane by about 0.47 Å in the direction to place this atom on the opposite side of the plane from the carboxyl group and thus to move the hydroxyl group even further from the plane. This result is consistent with that obtained from peptides containing a hydroxyproline or proline residue.[8] The N—C1 bond length (1.44 Å) is shorter than the N—C4 bond length (1.48 Å). This is in contrast with *N*-tosyl-prolyl-hydroxyproline (**4**) where the N—C1 (corresponding to the numbering scheme in (**3**)) bond length (1.45 Å) is longer than the N—C4 bond length

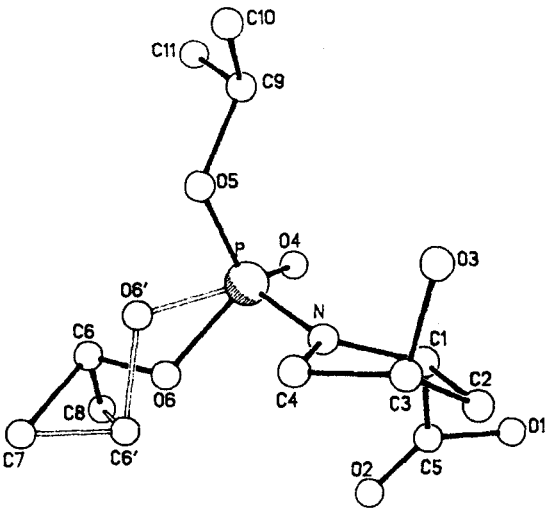


FIGURE 1 A perspective view of the molecular structure (the L configuration, Molecule A) of (**3**).

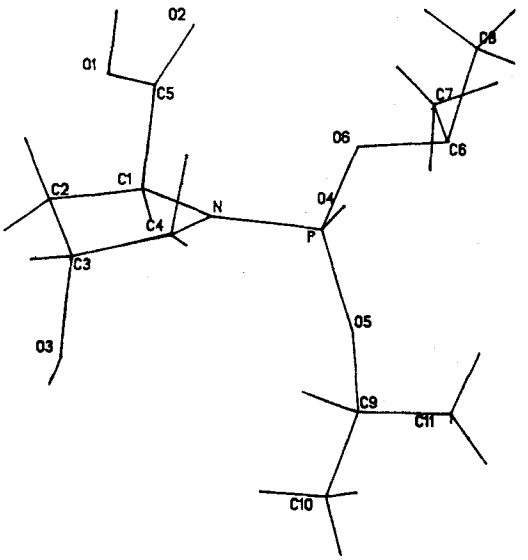
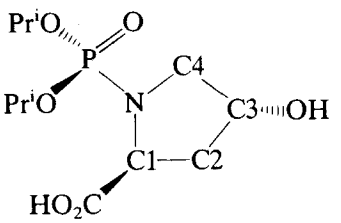


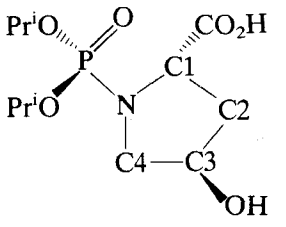
FIGURE 2 A diagrammatic representation of the conformation including hydrogen atoms for Molecule A of (**3**).

(1.43 Å).[9] It is interesting that the elongation of the N—C4 bond length in (**3**) is compensated by the shortening of the C2—C3 and C3—C4 bond lengths (both 1.50 Å) as compared to the bond lengths of these two corresponding bonds (both 1.54 Å) in (**4**). The P–N–C1 and P–N–C4 bond angles (123.8° and 122.0° respectively) in (**3**) are also different compared to the corresponding bond angles of C′–N–C1 and C′–N–C4 (114.8° and 125.4° respectively) in (**4**). The widening of the C′–N–C4 bond angle as compared to the C′–N–C1 bond angle in (**4**), where the hydroxyproline residue exists in the trans conformation, is probably due to the steric repulsion between the R and C4 groups as can be seen in (**5b–6b**). In (**3**), the trans conformation, with the projection of the N–C4 bond bisecting the RO–P–OR angle, should not suffer such steric effect as can be seen from Figure 1 and Figure 2, and as a result the P–N–C1 and P–N–C4 bond angles in (**3**) share similar values.

Since the phosphorimidate group in (**3**) is coplanar, there are two possibilities for the orientation of the P=O bond with respect to the N—C4 bond, namely, the cis orientation (cis conformation, **3a**) and the trans orientation (trans conformation, **3b**). It is found that the phosphorimidate function of (**3**) exists

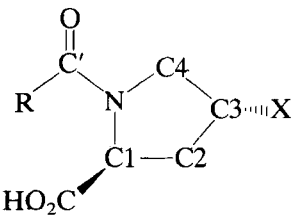


(**3a**)

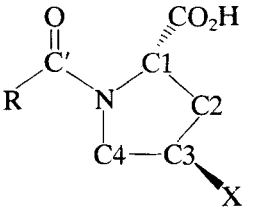


(**3b**)

uniformly as the trans conformation (**3b**) in the crystals as can be seen from Figure 1 and Figure 2. The preference of the trans conformer (**3b**) over the cis conformer (**3a**) is probably due to the greater stability of (**3b**) than (**3a**), because the cis orientation of the P=O and N—C4 groups in (**3a**) results in the steric crowding between the carboxyl group and one of the isopropyloxy groups which lie within a plane, while in (**3b**) the carboxyl group is far away from the isopropyloxy groups and hence there is no steric crowding between these two groups. This situation is in contrast to that in *N*-acyl proline (**5**) and *N*-acyl hydroxyproline (**6**). In these two compounds, the cis conformation (**5a–6a**) and the trans conformation (**5b–6b**) have similar stability,[10] since the cis conformer in which the carboxyl group and the R group do not lie within a plane should not suffer such destabilizing effect as the analogous conformer (**3a**). Consequently, the cis or the trans conformations can occur in a peptide chain containing a proline or a hydroxyproline residue.[10–11]



(**5a–6a**)



(**5b–6b**)

EXPERIMENTAL

Synthesis of compound (**3**). Trans-4-hydroxy-D,L-proline (1.3 g, 10 mmol) was dissolved in water-ethanol-triethylamine (1:1:1, 15 ml) under stirring and the solution was cooled to below 0°C in an ice bath. To this solution was added diisopropyl phosphite (1.8 g, 11 mmol) in carbon tetrachloride (4 ml). After stirring at low temperature for 2 hours and then at room temperature for 8 hours, the reaction mixture was diluted with water (20 ml) and extracted with ethyl ether three times. The aqueous solution was acidified with 1N NCl at 0°C and was extracted with the mixed solvent of ethyl acetate-tert-butanol (1:1) four times. The extracts were washed with water three times, dried over anhydrous Na_2SO_4 and evaporated to dryness to give a white powder. Recrystallization from chloroform/petroleum ether gave pure product (**3**) 2.5 g (85%), m.p. 55–56°C. FAB-MS: m/z 296 (MH^+). Anal. Calcd. for $C_{11}H_{22}NO_6P$: C, 44.8; H, 7.5; N, 4.7. Found: C, 45.1; H, 7.4; N, 4.7. ^{31}P NMR ($CDCl_3$): δ 4.45 ppm. ^{13}C NMR ($CDCl_3$): δ 175.1 (C5, $J < 1$ Hz); δ 72.0, 71.9 (C6 and C9, $J = 6.3$, 5.5 Hz); δ 71.0 (C3, $J = 6.3$ Hz); δ 59.0 (C1, $J = 5.4$ Hz); δ 54.8 (C4, $J = 3.5$ Hz); δ 39.1 (C2, $J = 8.8$ Hz); δ 23.8, 23.7, 23.6, 23.5 (C7, C8, C10, C11, $J = 4.0$, 5.2, 4.2, 5.3 Hz) [ppm].

The ^{31}P NMR spectrum was measured on a Jeol JNM-FX 100 FT NMR spectrometer using 85% H_3PO_4 as external reference. The ^{13}C NMR data were taken on a Varian XL 200 spectrometer at 50.309 MHz and referenced to $CDCl_3$ at 77.0 ppm.

Crystallographic data to compound (**3**). Single crystals of good quality for X-ray analysis were obtained by crystallization from the mixed solvent system of chloroform + ethyl ether + *n*-hexane. The crystals belong to orthorhombic system, space group $P2_12_12_1$, $a = 6.934(2)$, $b = 8.694(3)$, $c = 26.727(7)$ Å, $V = 1611.3(8)$ Å^3, $Z = 4$, $Dx = 1.22$ g/cm^3.

Intensity data were measured under ambient condition on a Nicolet R3m/E diffractometer with graphite-monochromated MoKα rediation in a ω scan mode with 2θ up to 45°. 1290 reflections were collected, of which 653 with $I \geq 2\sigma(I)$ were considered to be observed and corrected for LP factors, but no absorption correction was made.

The structure was solved by direct method. Refinements with positional and anisotropic thermal parameters were carried out by blocked-cascade least-squares. The site occupancy factors of the disordered O6–O6′ and C6–C6′ atoms were bound to 1. Only partial hydrogen atoms were located on

difference Fourier maps and theoretical positions were given for the rest. On convergence of the refinements, the final $R = 0.072$, and $Rw = 0.072$ with unit weights. All calculations were conducted by using SHELXTL program system on a Eclipse/S140 computer.

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