The Molecular Structure of Pyridoxol 5′-Methylphosphonate (1,2)

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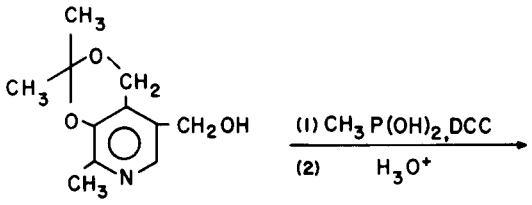
Pyridoxol and pyridoxal 5′-methylphosphonate (II and III respectively) are analogs of the phosphorylated forms of Vitamin B_6 in which the hydroxyl group of the phosphate moiety has been replaced by methyl. Although the syntheses of both compounds were carried out, only pyridoxol 5′-methylphosphonate could be obtained as crystals that were suitable for x-ray diffraction purposes. The results of this crystal structure determination are presented in this communication and are correlated with known biochemical activities of both II and III.

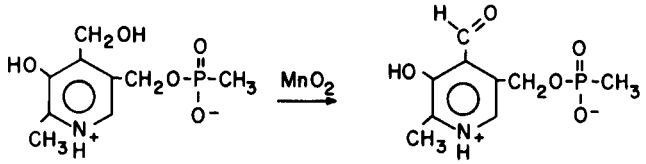
EXPERIMENTAL

Synthesis.

The compounds II and III were synthesized in the following sequence of reactions, the details of which have been recently reported (3).



I



II III

Crystallography.

The crystal data for II are as follows: the space group is $P2_1/c$ a = 7.699 (4), b = 11.913 (7), c = 12.224 (5) and β = 106.61 (7)° at t = 23 ± 2°C. The observed and calculated densities are 1.52 ± 0.02 g. cm^{-3} and 1.51 g. cm^{-3}, respectively. The crystal structure was deduced by direct methods (4) and subsequently refined by least squares techniques using the block-diagonal approximation. The non-hydrogen atoms were assigned anisotropic temperature factors at the stage R = 0.10. At the stage R = 0.08 the hydrogen atoms were located from electron density difference Fourier syntheses, assigned isotropic temperature factors and included in subsequent least squares refinement. The model was refined to R = 0.064. The standard deviations in non-hydrogen bond lengths and angles are less than 0.004 Å and 0.5°, respectively; the standard deviation for those distances and angle involving hydrogen atoms are less than 0.05 Å and 5°, respectively.

Results and Discussion.

The P-C distance of 1.78 Å on the methylphosphonate moiety (see Fig. 1) is significantly shorter than the theoretical sum of the single covalent radii (1.88 Å) of phosphorus and carbon. Other shortened P-C distances of this magnitude have been determined, such as for a cyclic phosphine determined by Townes *et al.* (5). However, the P-C distance found in triethyl phosphine sulfide (6), for example, is in good agreement with theory (1.86 Å). The two shorter P-O distances of 1.48 Å and 1.50 Å and the longer P-O (C) distance of 1.60 Å are similar to the corresponding distances found in other phosphate esters as well as pyridoxol phosphate methylhemiacetal (7) and pyridoxol phosphate oxime (8).

The single negative charge is apparently primarily distributed between O (4) and O (5) of the phosphonate group. This is inferred from the fact that they are the most important H-bond acceptors in the structure.

The atoms H (5) and O (2) are not coplanar with the pyridine ring and are positioned such that they participate in hydrogen bonding with O (4) of another molecule, denoted by O (4)′ having the coordinates 1-x, 0.5 + y, 0.5-z with respect to O (4) (Fig. 2). The O (1)---O (4)′ distance is 2.600 (4) Å and the O (1) -H (5)---O (4)′ angle is 160 (2)°. The values for the O (2)---O (4)′ distance is 2.739 (4) Å and the O (2) -H (8)---O (4)′ angle is 147 (5)°.

The presence of the methylphosphonate group favors the formation of intermolecular hydrogen bonds rather than the intramolecular hydrogen bonds found in the case of pyridoxol HCl and which requires the coplanarity of the C (4′), O (2), O (1) system (9). The C (4), C (4′), N (4′) group in pyridoxamine phosphate is also not coplanar with the pyridine ring (10). The other terminal

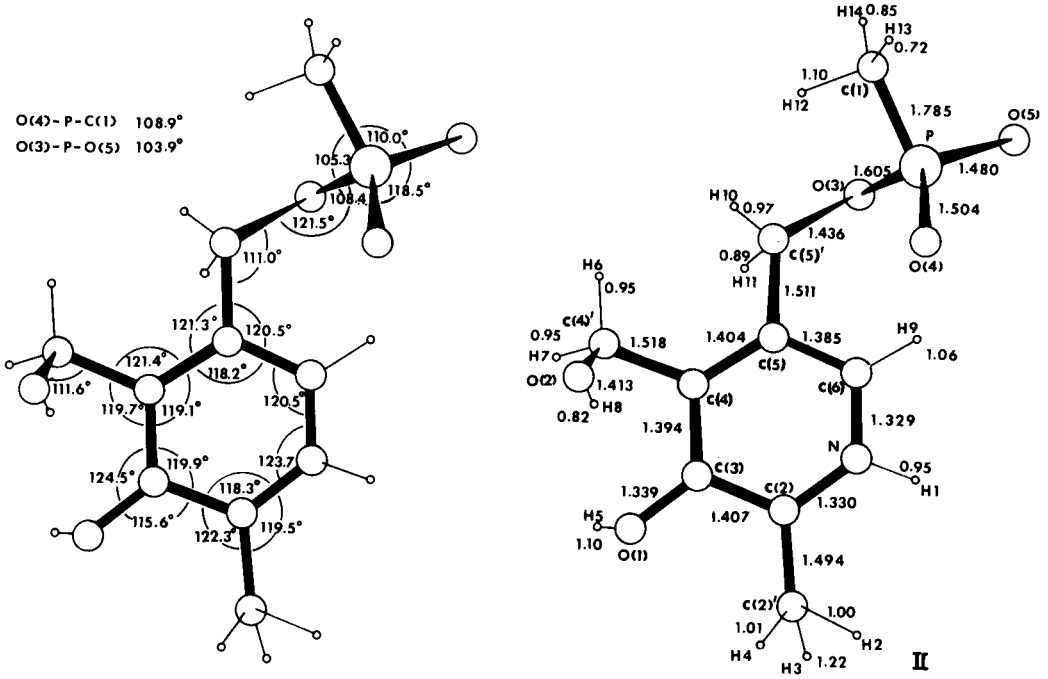


Figure 1. Molecular Dimensions.

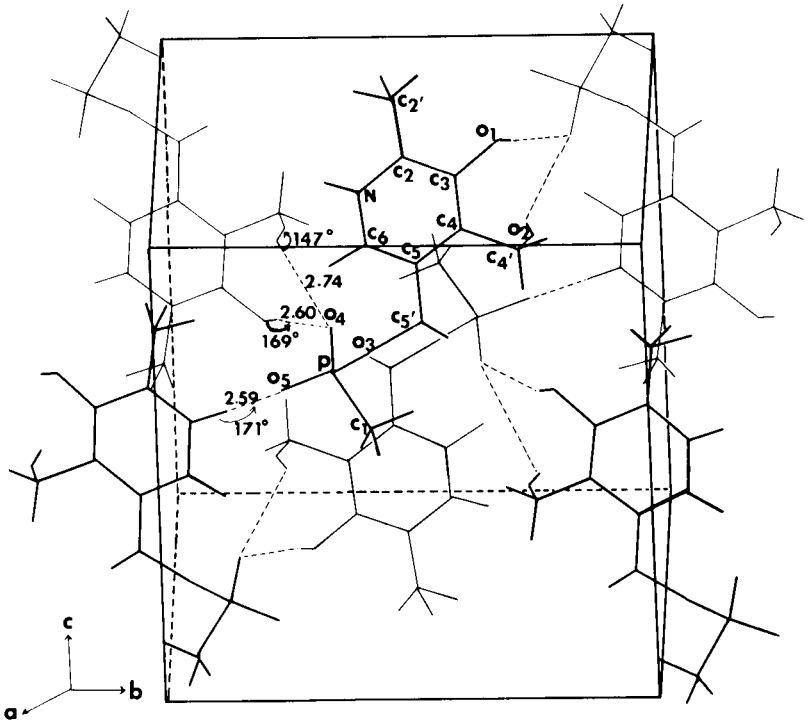


Figure 2. Hydrogen bonding and molecular packing.

oxygen, O (5), of the methylphosphinic group is very strongly H-bonded with N, as indicated by the extremely short N^{+}-H---O (5′) distance of 2.59 Å. In this structure, the shortest non-hydrogen bonded intermolecular contact is 3.21 Å long and exists between C (5) of the reference molecule and O (2) in the molecule with coordinates 1-x, 0.5 + y, 0.5-z.

Pyridoxol 5′-methylphosphonate (II) is inactive as the substrate of pyridoxine-P-oxidase (3). The aldehyde (III) however, binds to the cofactor site of aspartate amino

transferase (11) and to D-serine dehydratase (12), but does not activate these enzymes. It should be noted that in all of the phosphorylated vitamin B_6 derivatives cited above whose molecular structures have been elucidated, the three oxygen atoms of the phosphate group participate in strong hydrogen bonding and electrostatic interactions with neighboring molecules. A replacement of an ionized hydroxyl group by methyl, in the present case, has reduced the number of oxygens available for the above intermolecular interactions. Furthermore, the effective van der Waals radius of a methyl group is at least twice as large as that of hydroxyl. Thus a smaller electronegative hydrophilic group has been replaced by a larger hydrophobic species. These observations suggest that the replacement of one of the ionizable hydroxyl groups by a methyl has a dramatic effect on the biological properties of the phosphorylated forms of vitamin B_6, and that the unmodified phosphate group has an important role in binding to the active site, or to the cofactor site, whichever it may be.

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