# 32. Synthesis of 4-{{[(Isopropyloxy)methylphosphoryloxy]imino}methyl}-1-methylpyridinium Iodide and Its Characterisation

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(12.XII.90)

Oximes are used in the therapy of organophosphate poisoning. They form an interaction product with the organophosphate which is claimed to be more toxic than the organophosphate itself. We have synthesized one of these compounds which would be formed by the reaction of pyridine-4-carbaldehyde oxime methiodide with sarin (GB). Thus, reaction of oxime **1** with sarin chloridate (**2**), yielded 4-{{[(isopropyloxy)methylphosphoryloxy]-imino}methyl}-1-methylpyridinium iodide (**4**) which was characterized by NMR spectroscopy and X-ray crystallography.

In the therapy of organophosphorous poisoning, one currently uses a combination of atropine and oximes. According to the generally accepted doctrine, the oxime has the potency to reactivate the blocked active centre of the acetylcholinesterase. But already in 1950, *Hackley et al.* [1] have observed that the oxime reacts directly with the organophosphate by forming a so-called 'complex'. This observation has been confirmed by several other authors [2–6]. To those $O$-phosphonylated oximes which are also formed during the reactivation of the blocked enzyme, one has attributed a higher toxicity than to the organophosphate itself. This conclusion was drawn on the basis of a structural analogy with other quaternary phosphonates [7] [8] and the claimed synthesis of the pyridine-4-carbaldehyde oxime sarin 'complex' [9]. Here we have certain reservations since the only proof of the structure is a not too convincing elemental analysis.

We were interested in the synthesis of this 'complex', because it seems to be illogical to use oximes in therapy, when reactivation only takes place by forming an even more toxic compound. The synthesis was achieved by mixing equimolar amounts of pyridine-4-carbaldehyde oxime (**1**) and Et₃N in CDCl₃ and adding slowly an equimolar amount of sarin chloride ( = isopropyl methylphosphonochloridate; **2**; see *Scheme*). After 10 min, the

*Scheme*
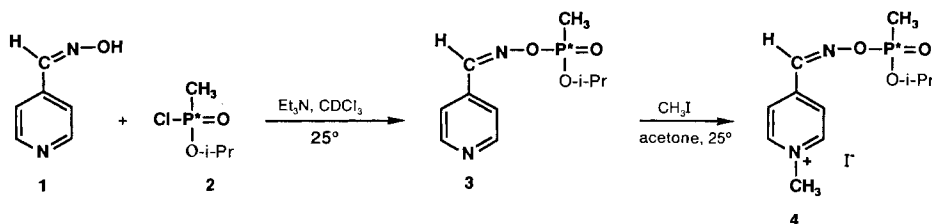
reaction was monitored by $^{31}$P-NMR spectroscopy. The mixture then was concentrated and product **3** purified by chromatography (silica gel). Its structure was established by extensive NMR studies.

A series of preliminary experiments made clear that the safest way to esterify oxime **1** with sarin chloridate (**2**) was to use the pyridinecarbaldehyde oxime rather than the corresponding quaternary salt: a solvent can be used which has no OH groups, hence the susceptibility against hydrolysis is lower. Of all the solvents tried, CHCl$_3$ proved to be best. Moreover, if **2** was not added slowly to the mixture, the formation of diphosphonate was unavoidable.

In the final step, **3** was quaternized with excess MeI in acetone yielding iodide **4**. Inspite of the fact that due to the asymmetrical P-atom, the presence of two enantiomers was expected, **4** could be crystallized. Its structure was established by NMR methods (see below) and finally by a single-crystal X-ray diffraction analysis.

The crystals of **4** for the X-ray analysis were twinned and diffracted weakly. Therefore, the values obtained for the bond lengths and bond angles are not very precise (*Table*) but normal within experimental error. A PLUTO plot [10] is given in *Fig. 1*. The
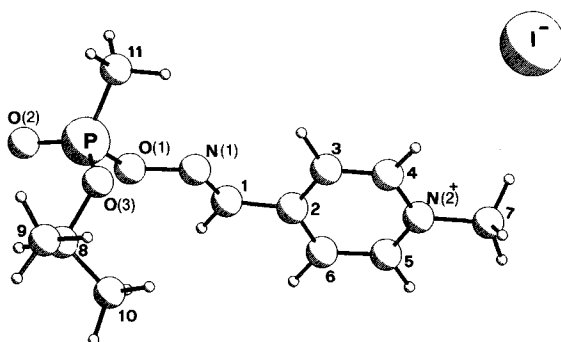


Fig. 1. *X-Ray crystal structure of* 4-{{[(isopropyloxy)methylphosphoryloxy]imino}methyl}pyridinium iodide (**4**) *with atom numbering* (arbitrary)

Table. *Final Distances and Bond Angles in* 4-{{[(Isopropyloxy)methylphosphoryloxy]imino}methyl}pyridinium *Iodide. For numbering, see Fig. 1.* Estimated standard deviations are given in parentheses.

| Bond length [Å] | | | | | |
|---|---|---|---|---|---|
| P—O(1) | 1.611(14) | N(1)—C(1) | 1.272(24) | C(2)—C(6) | 1.35(3) |
| P—O(2) | 1.452(14) | N(2)—C(4) | 1.39(3) | C(3)—C(4) | 1.34(4) |
| P—O(3) | 1.543(15) | N(2)—C(5) | 1.35(3) | C(5)—C(6) | 1.39(3) |
| P—C(11) | 1.752(24) | N(2)—C(7) | 1.51(3) | C(8)—C(9) | 1.52(3) |
| O(1)—N(1) | 1.438(20) | C(1)—C(2) | 1.43(3) | C(8)—C(10) | 1.51(3) |
| O(3)—C(8) | 1.526(24) | C(2)—C(3) | 1.38(3) | | |

| Bond angles [°] | | | | | |
|---|---|---|---|---|---|
| O(1)—P—O(2) | 104.3(8) | O(1)—N(1)—C(1) | 112.8(15) | C(2)—C(3)—C(4) | 121.7(22) |
| O(1)—P—O(3) | 105.5(9) | C(4)—N(2)—C(5) | 120.1(18) | N(2)—C(4)—C(3) | 118.6(20) |
| O(1)—P—C(11) | 107.4(9) | C(4)—N(2)—C(7) | 119.4(18) | N(2)—C(5)—C(6) | 120.3(19) |
| O(2)—P—O(3) | 117.0(9) | C(5)—N(2)—C(7) | 120.5(17) | C(2)—C(6)—C(5) | 119.3(20) |
| O(2)—P—C(11) | 118.1(9) | N(1)—C(1)—C(2) | 120.4(17) | O(3)—C(8)—C(9) | 105.2(16) |
| O(3)—P—C(11) | 103.5(10) | C(1)—C(2)—C(3) | 122.3(18) | O(3)—C(8)—C(10) | 103.7(16) |
| P—O(1)—N(1) | 114.7(10) | C(1)—C(2)—C(6) | 117.9(17) | C(9)—C(8)—C(10) | 111.2(21) |
| P—O(3)—C(8) | 122.5(14) | C(3)—C(2)—C(6) | 119.6(2) | | |
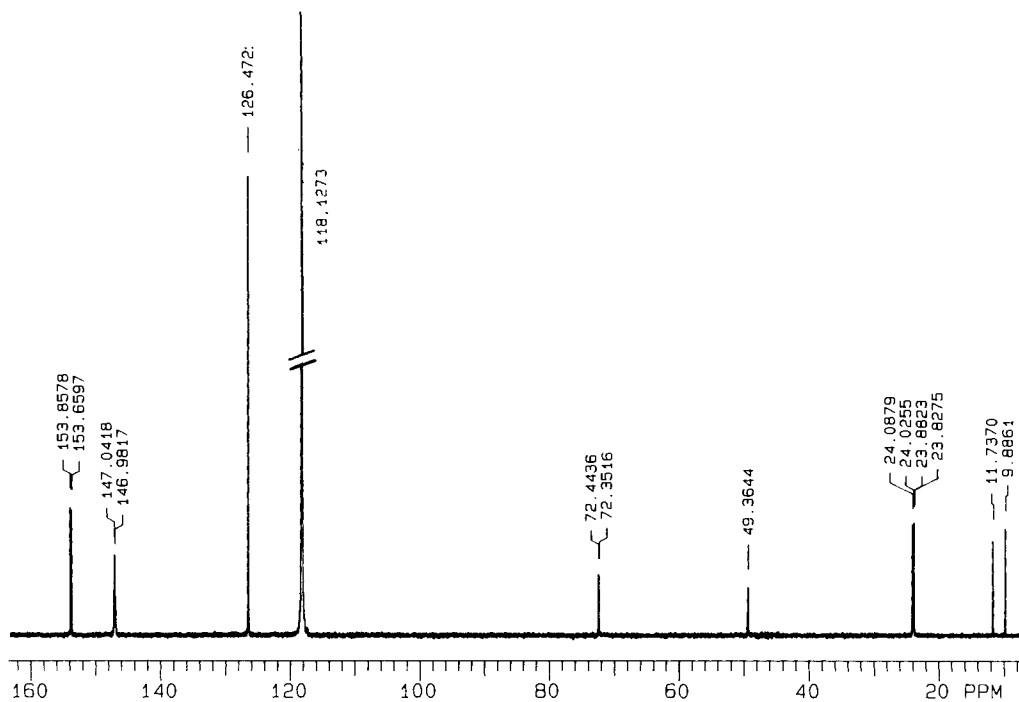
Fig. 2. *${}^{13}C$-NMR spectrum* (CD$_3$CN, 75.5 MHz, 24°) *of 4-{{[(isopropyloxy)methylphosphoryloxy]imino}methyl}-pyridinium iodide* (**4**)
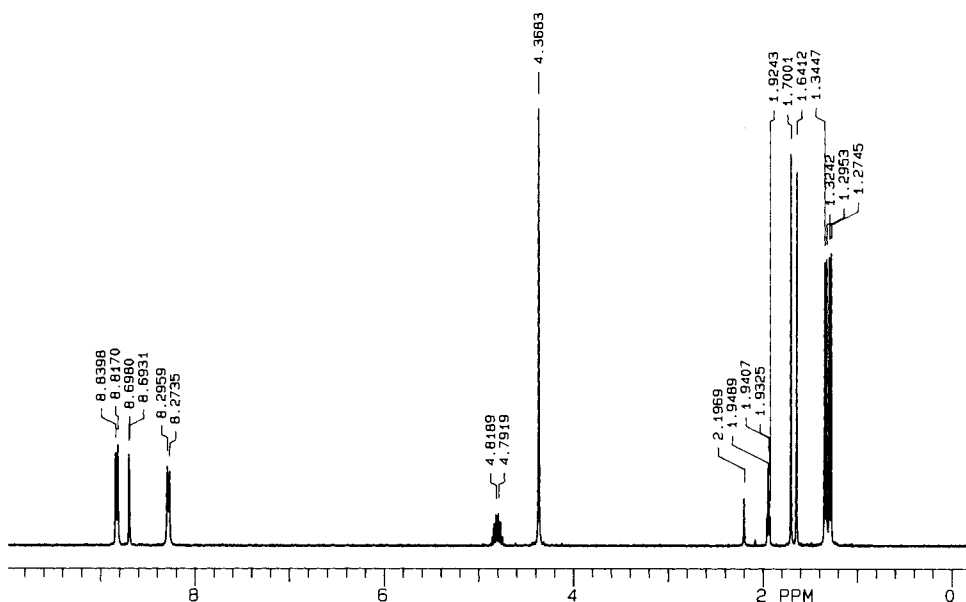
Fig. 3. *${}^{1}H$-NMR spectrum* (CD$_3$CN, 300 MHz, 24°) *of 4-{{[(isopropyloxy)methylphosphoryloxy]imino}methyl}-pyridinium iodide* (**4**)

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unit O–N=CH–C(Py) is planar to within 0.01 Å and inclined by 14.2° to the best plane through the pyridine ring (planar to within 0.03 Å). In the crystal structure, there are no short ( < 3.2 Å) intermolecular contacts between non-H-atoms. The shortest I· · ·N(2) distance is 3.81(2) Å.

The proceeding of the reaction $\mathbf{1} + \mathbf{2} \rightarrow \mathbf{3}$ was monitored by comparing the relative intensities of the 2 $\Delta$ at 56.4 and 52.0 ppm of **2** and **3**, respectively, in the $^1$H-decoupled $^{31}$P-NMR spectrum (CDCl$_3$). The end of the reaction was assumed when the signal of **2** had disappeared. Two peaks at 39.2 and 38.7 ppm were tentatively attributed to the diphosphonate corresponding to a by-product formed by the reaction of $\mathbf{2} + \mathbf{3}$, and being present as a pair of diastereoisomers due to the asymmetric P-atoms.

In the $^1$H-decoupled $^{13}$C-NMR spectrum (CD$_3$CN) of **4**, a $d$ at 153.8 ppm ($J = 14.8$ Hz) is observed (*Fig. 2*), indicating a coupling of the aldoxime C-atom with the P-nucleus as expected for such a phosphonate. All other peaks are in agreement with the proposed structure: The reduced height of the signal at 147.0 ppm (C(2), C(4), C(5); numbering according to *Fig. 1*) can be explained by line broadening due to the neighbourhood of the quaternary N-atom. The $CH_3$ signal of the i-PrO group of the sarin moiety is split in 2 $d$, a consequence of the magnetic nonequivalence due to the asymmetry of the P-nucleus. Analogous effects are observed in the $^1$H-NMR spectrum (CD$_3$CN) of **4** (*Fig. 3*): The aldoxime proton at 8.70 ppm suffers a long-range coupling with the P-atom ($d, J = 1.5$ Hz), and the $CH_3$ signal of the i-PrO group is split into 2 $d$ because of magnetic nonequivalence. The signals at ca. 1.94 and at 2.20 ppm are due to CD$_3$CN and traces of H$_2$O contained therein. The $^1$H-decoupled $^{31}$P-NMR spectrum (CD$_3$CN) of **4** shows 1 $s$ at 51.3 ppm. In the $^1$H-coupled spectrum, the P-signal appears with the expected symmetrical fine-structure, a $qdd$ with $^2J(\text{P}, CH_3) = 17.7$, $^3J(\text{P}, (CH_3)_2CH) = 8.1$, and $^4J(\text{P}, CH=\text{NOP}) = 1.5$ Hz). Selective irradiation of the signal of $CH=$NOP simplified the P-signal to a $qd$.

## Experimental Part

*General.* NMR spectra: *Varian VXR-300* with a broad bandwidth probe tunable for $^1$H (300 MHz), $^{13}$C (75 MHz), and $^{31}$P (121 MHz); TMS for $^1$H and $^{13}$C and PO(OPh)$_3$ for $^{31}$P as reference (usually external); measurements at r.t.

*4-{{[(Isopropyloxy)methylphosphoryloxy]imino}methyl}pyridine* (**3**). To a soln. of 300 mg (2.46 mmol) of pyridine-4-carbaldehyde oxime (*p.a., Jansen*; **1**) and 249 mg (2.46 mmol) of Et$_3$N (*purum, Fluka*) in 5 ml of CDCl$_3$ (*Merck*), 350 µl (2.46 mmol) of sarin chloridate (synthesised in house; *ca.* 95% pure; **2**) are added slowly with stirring within *ca.* 10 min to prevent side-reactions. A sample (750 µl) of this mixture is withdrawn and analyzed by $^{31}$P-NMR (see *General Part*). The mixture is concentrated to *ca.* 2 ml in a rotatory evaporator (→white precipitate of Et$_3$NHCl) and then separated by column chromatography (1.6 × 16 cm; silica gel *60* (*Merck*, 230–400 mesh); column prepared with anh. Et$_2$O (*Merck*, dried over CaSO$_4$); elution with 150 ml of anh. Et$_2$O, then with dry acetone (*Fluka*), 5-ml fractions. *Fractions 5–12* contain pure **3**. TLC (silica gel *60*, 254 nm): $R_f$ 0.00 (Et$_2$O), 0.50 (acetone), 0.17 (Et$_2$O/MeOH 1 :20).

In TLC, all substances with exception of sarin chloridate (**2**) can be detected by their quenching of fluorescence. For the detection of **2** and **3**, an enzyme-inhibition assay is used. The plates are first sprayed with a soln. of enzyme (150 units of human acetylcholinesterase (*Biozyme*)) per 100 ml of phosphate buffer (1.9 g Na$_2$HPO$_4$· 12 H$_2$O; 0.18 g KH$_2$PO$_4$), pH 7.4. After 5 min, they are sprayed with a freshly prepared 4:1 mixture of soln. A and soln. B (soln. A, 400 mg of 'Fast Blue B salt' (*Serva*, Heidelberg) in 160 ml of H$_2$O; soln. B, 250 mg of naphth-1-yl acetate (*Jansen*) in 100 ml of EtOH: blocked enzyme is unable to hydrolyze the dye and the spot stays white, uninhibited enzyme produces a violet color.

*4-{{[(Isopropyloxy)methylphosphoryloxy]imino}methyl}pyridinium Iodide* (**4**). The fractions containing pure **3** are evaporated. After addition of 2 ml of dry acetone, **3** is quaternized by adding a 10-fold excess (1.5 ml, 24.1 mmol) of MeI (*Fluka*). After 3 days standing at r.t., the soln. has the color of brandy. Hexane (*p.a., Merck*) is added until a slight turbidity is discernible (*ca.* 1 ml). After further standing, the crystals are filtered off and washed with acetone/hexane 1:1: 230 mg (24.3%) of **4**. Bright yellow crystals. M.p. 121–123°. TLC (details as above): pure. $^1$H-NMR (300 MHz, CD$_3$CN, 24°; numbering, see *Fig. 1*): 8.83 ($d, J = 6.7$, H–C(4), H–C(5)); 8.70 ($d, J = 1.5$, H–C(1)); 8.28 ($d, J = 6.7$, H–C(3), H–C(6)); 4.8 ($m$, H–C(8)); 4.37 ($s$, CH$_3$(7)); 1.67 ($d, J = 17.6$, CH$_3$(11)); 1.33, 1.28 ($2d, J = 6.2$, CH$_3$(9), CH$_3$(10)); *Fig. 3*. $^{13}$C-NMR (75.5 MHz, CD$_3$CN, 24°; numbering, see *Fig. 1*): 153.8 ($dd$, $^3J(\text{C,P}) = 14.8$, C(1)); 147.0 ($s, d$, C(2), C(4), C(5)); 126.5 ($d$, C(3), C(6)); 72.4 ($dd$, $^2J(\text{C,P}) = 6.9$, C(8)); 49.4 ($q$, C(7)); 24.1, 23.9 ($2 dd$, $^3J(\text{C,P}) = 4.5$, C(9), C(10)); 10.8 ($qd$, $^1J(\text{C,P}) = 139.5$, C(11)); *Fig. 2*.

*X-Ray Crystallography.* A pale yellow transparent crystal of **4** was sealed in a glass capillary. Crystal data: $C_{11}H_{18}N_2O_3PI$, $M_r$ 384.2, space group $P2_1/n, a = 14.611$ (4) Å, $b = 8.082$ (2) Å, $c = 15.372$ (4) Å, $\beta = 117.17$ (4)°, $V = 1614.9$ Å$^3$, $F(000) = 712$, $Z = 4$, $D_c = 1.580$ g/cm$^3$, Mo$K_\alpha$, $\lambda = 0.71073$ Å, $\mu = 20.6$ cm$^{-1}$. A crystal of dimensions $0.68 \times 0.30 \times 0.15$ mm was used for data collection. Intensity data with index limits $h -14$ to 12, $k$ 0 to 7, $l$ 0 to 14, and $\Theta_{max} = 20°$ were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated Mo$K_\alpha$ radiation) using the $\omega/\Theta$ scan mode. There was less than 2% intensity variation for five standard reflections measured every h. Of the 1353 unique reflections, 1160 were considered observed with $I_{net} > 2.5\sigma$ $(I_{net})$. Cell parameters from $\pm\omega$ values of nine reflections and their equivalents in the range $20° < 2\Theta < 25°$. Preliminary *Weissenberg* and precession photographs had indicated the crystals to be monoclinic with space group $P2_1/n$, but they were also twinned about a diagonal. Because of this and because the crystals were enclosed in a capillary tube, it was not possible to make an absorption correction. The structure was solved using the program SHELXS-86 [11]. The program NRCVAX [12] was used for all further calculations. The H-atoms were included in idealized positions ($U_{iso} = U_{eq}$ (attached atom) $+0.01$ Å$^2$ and distance C–H $= 1.08$ Å, their positions were renormalized after every second cycle of refinement). Weighted anisotropic full-matrix least-squares refinement for 1160 reflections converged at $R = 0.117$, $wR = 0.177$. Maximum shift to sigma ratio was 0.005. The rather high $R$ factor obtained can be explained by the fact that the crystal was twinned and the diffraction intensity fell rapidly beyond $30°$ in 2 $\Theta$ and the fact that it was not possible to correct for absorption. Heights in the final difference map were 4.20 (max) and $-1.15$ (min) e/Å$^3$. The residual density was found in ripples surrounding the I-atom in the $xy$-plane which probably results from termination errors. Final positional and equivalent isotropic thermal parameters have been deposited with the *Cambridge Crystallographic Data Centre*, Lensfield Road, Cambridge (UK).

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