3-Hydroxy-6-[^{14}C-methyl]pyrid-2-thione (IV). Potassium hydrogen sulphide (400 mg, 5.5 mmoles) was dissolved in propylene glycol (20 ml) by heating, the solution allowed to cool to about 160°, 2-bromo-3-hydroxy-6-[^{14}C-methyl]pyridine (162 mg diluted to 300 mg, 1.6 mmoles) and hydroquinone (20 mg) added. The solution was refluxed under dry nitrogen for 20 h, the propylene glycol distilled off at 90° under reduced pressure, the residue dissolved in water (7 ml), the pH of the solution adjusted to about 5 with acetic acid and the solid precipitate collected by filtration. The filtrate was extracted with ethyl acetate, the extracts dried, evaporated and the solid residue combined with the material isolated above. After drying over P_2O_5, the material was sublimed at 190°/20 mm Hg. The yellow solid obtained was recrystallized from benzene; yield 150 mg, m.p. 166–170°. Chromatography showed that the title compound was slightly contaminated by the corresponding disulphide but was otherwise the same as the 3-hydroxy-6-methylpyrid-2-thione previously prepared.[1] Radioautography (5 days) on TLC plates showed the presence of a third component to the extent of less than 2 %.

Activity: 1 μg of the product gives 70 cpm; sp. activity 0.91×10^{-2} mC/mmole.

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NMR Studies and Alkaline Hydrolysis of 3-Hydroxy-2-oxo-2-phenyl-3,5,5-trimethyl-1,2-oxaphospholane

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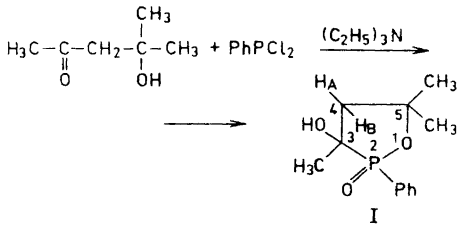
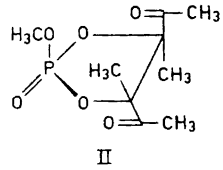
The trivalent phosphorus compounds PCl_6, $PhPCl_2$, and $P(OR)_3$, are known to react with conjugated dienes,[1–5] unsaturated ketones,[6–9] and aliphatic dibromides [10–12] forming derivatives of phospholine, phosphole, oxaphospholine, oxaphospholane, and oxaphosphorinane, respectively. According to this principle of synthesis the 1,2-oxaphospholane I was prepared by the 1,4-addition of dichlorophenylphosphine to diacetone alcohol:



I

The identity of the cyclic compound was established by infrared and proton magnetic resonance spectra, as well as by elementary analysis. The NMR spectrum of the methyl groups in the cyclic compound I consists of four signals (Fig. 1). The signals are found to be solvent dependent, but the separation between two of them remains constant. This is therefore assigned as the phosphorus coupling to the protons of the methyl group in position 3 in I.

It has been observed that the sulfuryl S=O group in cyclic sulfides shows deshielding effect on chemical shifts of protons of ring substituents.[13] Similar effect has also been found for the phosphoryl group as in the racemic cyclic phosphate, 2-methoxy-2-oxy-4,5-diacetyl-1,3,2-dioxaphospholane, II,[14]



II

where the singlet at $\delta = 2.40$ is tentatively assigned to the acetyl group adjacent to the phosphoryl oxygen, while the singlet at $\delta = 2.35$ is assigned to the acetyl group furthest away from this oxygen atom. On the same basis, the singlets at $\delta = 1.57$ and $\delta = 1.47$, are assigned to the CH_3–C groups when methyl is next to, and furthest away from the phosphoryl group, respectively. The methyl groups in position 5 of compound I behaves similarly, the lower signal

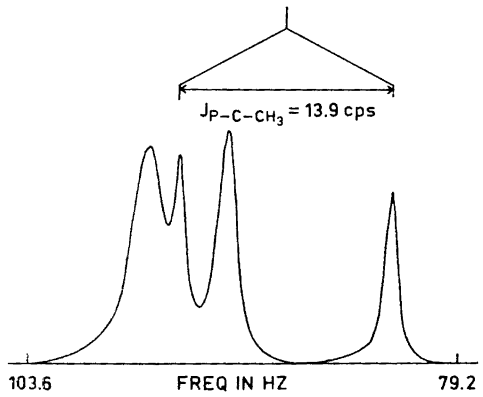


Fig. 1. Spectrum at 60 Mc of the methyl groups in the cyclic compound I in $CDCl_3$ with TMS as internal standard.

The envelope conformation of 1-chloro-1,3,2-dioxaphospholane [15] is also confirmed by X-ray analysis of 2-methoxy-2-oxo-1,3,2-dioxaphospholane,[16] where the atoms C_4, C_5, O_3, and O_1 are nearly in the same plane, while phosphorus is out of this plane. In compound I, the geminal protons at position 4 show clearly an AB part of a ABX system (Fig. 2), where phosphorus

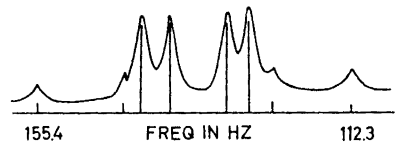


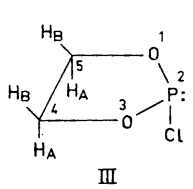
Fig. 2. The AB spectrum of the ring protons in position 4 in I. *Upper:* observed spectrum in $CDCl_3$ at 60 Mc. *Lower:* Calculated line spectrum.

(Fig. 1) is assigned to the methyl group *cis* to the phosphoryl group. No detectable coupling between the phosphorus nucleus and these methyl protons has been observed.

In six-membered ring phosphites and phosphates [21–23] and in stereoidal phosphonates,[24] the dependences of the P—O—C—H and P—C—C—H coupling constants on the dihedral angles are found to be:

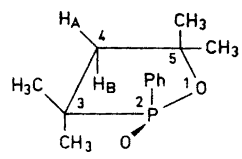
Phosphites: J_{gauche} (60°) 2 cps, J_{trans} (180°) 7 cps
Phosphates J_{gauche} (60°) 6 cps, J_{trans} (180°) 20 cps
Phosphonates J_{gauche} (60°) 10, J_{trans} (180°) 35 cps J (90°) 2 cps

In accordance with this, the different coupling constants, $J_{PH_A}=2$ cps and $J_{PH_B}=9$ cps, from the phosphorus nucleus to the ring protons, A and B, in 2-chloro-1,3,2-dioxaphospholane, III,[15] indicate different dihedral angles in the two P—O—C—H systems, θ(P—O—C—H_A): 90°, and θ(P—O—C—H_B): 180°.



III

is the X part. These protons, however, are found to give nearly the same coupling to the phosphorus atom, 10.9 cps and 11.7 cps., respectively. This indicates that the dihedral angles in the two P—C—C—H systems are nearly the same. A reasonable explanation is that the carbon atom C_4 in compound I is very close to the plane through the phosphorus atom and the two carbon atoms C_3 and C_5, while the oxygen atom O_1 is out of this plane:



A similar structure is suggested for 1-methyl-1-phenyl-phospholanium iodide.[17]

The AB signals are unusually broad, probably due to unresolved coupling to the methyl groups. Chemical shifts and coupling constants are given in Table 1.

The alkaline hydrolysis of the cyclic compound I occurs with ring cleavage at the P—O bond. Rate data together with activation parameters for the hydrolysis are recorded in Table 2. The rate of the alkaline hydrolysis of I is about ten times slower than that of 2-ethoxy-2-oxo-1,2-

Table 1. Chemical shifts and coupling constant for I.

	Chemical shifts ppm from TMS	Coupling constants cps
3-Methyl	1.48	$^3J_{PH}$ = 13.9
5,5-Dimethyl	1.54	
	1.62	
4-Protons	A: 2.14	$^4J_{PH_A}$ = 10.88
		J_{HH} = 14.10
	B: 2.32	$^4J_{PH_B}$ = 11.71

oxaphospholane.[11] This is due to the increasing stability of the ring by the substituents. The same observation has also been made in the case of ring substituted 2-alkoxy-2-thiono-1,3,2-dioxaphospholanes.[18]

Experimental. The NMR spectra were measured at 60 Mc (JEOL C-60H) in 20 % solution of the compound in $CDCl_3$. Spectra were calibrated relative to an internal standard of tetramethylsilane. Line positions were obtained by averaging the results of four spectra. The spectrum was analyzed according to previous description[19,20] using the iterative least squares computer program, LAOCOON III.

3-Hydroxy-2-oxo-2-phenyl-3,5,5-trimethyl-1,2-oxaphospholane (I). A solution of phenylphosphine dichloride (20 g) in 100 ml sodium-dried diethyl ether was added dropwise with stirring at room temperature to a solution of diacetone alcohol (20 g) in 100 ml diethyl ether and 75 ml triethylamine. The triethylamine hydrochloride was filtered off *in vacuo*. The white solid reaction product was washed four times with sodium-dried benzene. After three recrystallizations from anhydrous benzene-ether 15 g (35 %) of white needles was obtained, m.p. 176°C. (Found: C 60.16; H 7.15; P 12.86. Calc. for $C_{12}H_{17}O_3P$: C 60.10; H 7.08; P 12.91).

Kinetic measurements were performed on a recording pH-stat, Radiometer TTTlc, at various pH-values. The hydrolysis was studied as a pseudo first-order reaction, and the method of Guggenheim [13] was used for evaluation of the rate constants. Measurements were made at three temperatures, each of which was kept constant within ±0.01°C. The average error of the calculated rate constants is estimated to ±2 %.

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Table 2. Rate constants and activation parameters for the alkaline hydrolysis of I; solvent water.

pH	Rate constants l mole^{-1} sec^{-1}			*E* kcal/mole	log *A*	*ΔS* *
	30°	40°	50°			
10.0	1.94	3.86	7.71			
10.5	1.96	3.83	7.69			
				13.4±0.5	9.9	−15.3
11.0	1.92	3.87	7.78			

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Cell-free C-Methylation in Relation to Aromatic Biosynthesis

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Feeding experiments with methyl-labelled methionine have demonstrated that methyl groups attached to aromatic nuclei in mould products originate from C-1 metabolism in many cases. Lederer and his collaborators are studying extensively the mechanism of C-methylation, and they have recently been able to synthesize menaquinone by methylation of demethylmenaquinone in a cell-free system using *S*-adenosylmethionine as the methyl donor.[1] So far, however, the enzymic introduction of a C-methyl group into an aromatic structure has not been reported.

The mould *Aspergillus flaviceps* produces a series of aromatic compounds,[2,3] *e.g.* 5-methylorsellinic acid, flavipin, and, in low yield, orsellinic acid. A conceivable biological relation between these compounds would be the following sequence: orsellinic acid ⟶ 5-methylorsellinic acid ⟶ flavipin.

In order to study the C-methylation of orsellinic acid on an enzymatic basis, a protein fraction was prepared from *A. flaviceps*. The mould was grown for 24 h at 28° on a shake table in Czapek-Dox medium. The mycelial growth was collected and washed with buffer solution (tris 0.2 M, Na phosphate 0.05 M, NaCl 0.2 M, pH 7.8) before homogenization in an X-press. From the supernatant obtained after centrifugation of the homogenate for 40 min at 20 000 *g* and +2°, a protein fraction was precipitated by ammonium sulfate (up to 35 % of saturation) and discarded. Continued precipitation with ammonium sulfate (60 % of saturation) yield the protein fraction used in this investigation.

This protein fraction, dissolved in buffer solution at pH 7.8, was incubated with *S*-adenosylmethionine-$^{14}CH_3$ with and without added orsellinic acid in the presence of dithiothreitol. Although no significant difference in radioactivity was observed in ether extracts from acidified incubation mixtures with and without added orsellinic acid, they were