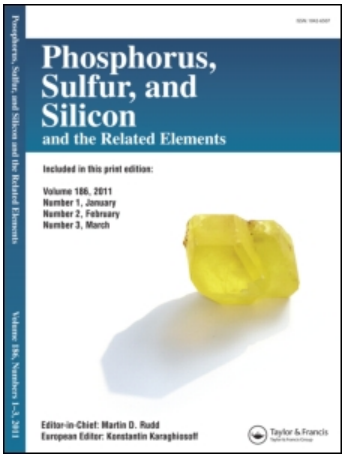

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Margalit, Yair , Amitai, Gavriel and Ashani, Yacov(1977) 'A ^{19}F NMR STUDY OF THE HYDROLYSIS OF ORGANOPHOSPHORUS FLUORIDATES IN AQUEOUS SOLUTION Doubling of the ^{19}F nmr in Diastereoisomeric Mixtures', Phosphorus, Sulfur, and Silicon and the Related Elements, 3: 3, 315 — 319
To link to this Article: DOI: 10.1080/03086647708079940
URL: http://dx.doi.org/10.1080/03086647708079940

Phosphorus and Sulfur
1977, Vol. 3, pp. 315-319

A ^{19}F NMR STUDY OF THE HYDROLYSIS OF ORGANOPHOSPHORUS FLUORIDATES IN AQUEOUS SOLUTION

Doubling of the ^{19}F nmr in Diastereoisomeric Mixtures

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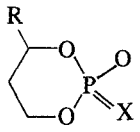
(*Received January 6, 1976; in final form July 2, 1976*)

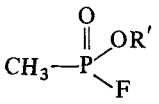
^{1}H and ^{19}F nmr spectra of two series of organophosphorus esters containing a P–F bond were studied. 2-Fluoro-4-methyl 1,3,2-dioxaphosphorinane 2-oxide (**2**) was found to be a mixture of two diastereoisomers in the ratio of *trans*/*cis* = 4.

Stereospecific catalysis of phosphate and maleate anions in the hydrolysis of **2** was observed, leading to enrichment of the non-hydrolysed fluoridates with the *trans* isomer.

The ^{19}F nmr spectra of O-2-butyl methylphosphonofluoridate (**7**) and O-pinacolyl methylphosphonofluoridate (**8**), showed doubling of the ^{19}F nmr spectra, giving rise to two sets of double quartets of equal intensity. 1:1 mixtures of diastereoisomers account for the doubling of the resonance rather than sterically hindered rotation. The applicability of nmr spectroscopy to the study of stereospecific displacement reactions at tetrahedral phosphorus is discussed.

Organophosphorus (OP) esters containing a P–F bond are potential inhibitors or substrates for many esterases.[1,2] The following formulae represent the above compounds:

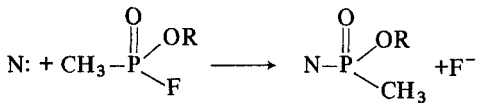




1, R = H X = F **4**, R′ = isoPr **7**, R′ = 2-butyl
2, R = Me X = F **5**, R′ = 3-pentyl **8**, R′ = pinacolyl
3, R = Me X = Cl **6**, R′ = cyclohexyl

It has been established that the reaction between various esterases and chiral OP-esters is stereospecific.[3,4] Esterases are accepted as nucleophiles and there have been many attempts to correlate kinetic data of enzyme-OP interactions with the stability of these esters in the presence of common nucleophiles such as H_2O, OH^- and other neutral or negatively charged species.[5]

We report here a potential application of ^{19}F nmr spectroscopy for the study of the stereospecific reactions between various nucleophiles (including esterases) and mixtures of diastereoisomers of OP fluoridates. The range of ^{19}F chemical shifts is very wide (compared with ^{1}H shifts) and the sensitivity of this nucleus to its electronic environment is high. Thus, one may expect that diastereoisomers of the P–F esters should have distinguishable chemical shifts, which may be useful for the purpose of this study. It has recently been established[6] that ^{19}F nmr spectroscopy is a convenient method for the study of fluorophosphoranes containing an asymmetric centre on the alkyl side chain. However, the facile pseudorotation in these fluorophosphoranes limits the usefulness of the nmr technique to rather low temperatures. The ^{19}F nucleus in nmr spectroscopy may serve as a probe for the study of reactions which follow Scheme 1, where N: represents any nucleophile.



SCHEME 1

Direct measurement in aqueous medium is feasible, provided that the P–F ester is soluble enough to permit reliable ^{19}F nmr measurements.

The ^{1}H and ^{19}F nmr spectra of selected esters were examined; relevant data are summarized in Table I. The spectral patterns clearly indicate that

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TABLE I

^1H and ^{19}F nmr data for some organophosphorus fluoridates†

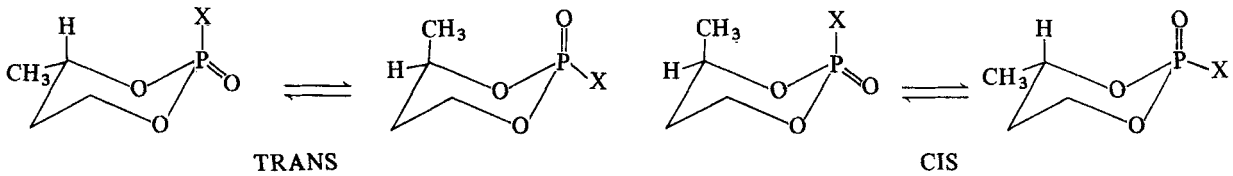
Compound		δCH_3(ppm)	$^4J_{CH_3-P}$ (Hz)	δF(ppm)	J_{F-P} (Hz)	$^3J_{CH_3-F}$ (Hz)
1		–	–	+8.8	998	–
2	TRANS	1.41	3.0	+8.7	1005	–
	CIS	1.42	0.8	–7.0	990	–
3	TRANS	1.44	3.0	–	–	–
	CIS	1.57	1.1			
4		1.60 (CH_3–P)	19	–19.2	1045	6.0
5		1.58 (CH_3–P)	17.5	–22.0	1095	6.0
6		1.64 (CH_3–P)	19	–20.0	1040	6.0
7		1.60 (CH_3–P)	19	a = –20.8 b = –21.8	a=b=1012	6.0
8		1.62 (CH_3–P)	19	a = –18.7 b = –21.5	a=b=1045	6.0

† ^1H and ^{19}F chemical shifts were measured with Me_4S and CF_3COOH as internal and external standards, respectively.

only the diastereoisomeric mixtures **2**, **7** and **8** exhibit doubling of the resonance, which enables one to measure the relative quantity of each isomer in a given mixture. For example, we have found (Figure 1) the ratio 4 for mixture **2** (*trans/cis*).

This finding confirms the observations of Stec and Mykolaijczyk[7] concerning the predominant *trans* isomer content of mixture **3**. Assuming that by fluorination of **3** to obtain **2** an S_N2 nucleophilic displacement[5] takes place, one might expect inversion of the configuration at P. However, as can be judged from the ^1H and ^{19}F nmr spectra, the major constituents in both **2** and **3** are the *trans* isomers. The determination of the isomer distribution is based on the higher $^4J_{P-CH_3}$ values for the *trans* isomer.[7] We suggest that a double inversion has occurred under the fluorination conditions, leading to enrichment of the mixture with the more stable *trans* isomer. We note that the epimerization of *cis*-**3** to *trans*-**3** may be explained by the presence of chloride ions in the reaction mixture.[7] In this context it should be mentioned that the stereochemistry of nucleophilic substitution at phosphorus is highly dependent on the solvent nature and the added salt. Inversion as well as retention could be detected under various experimental conditions.[8]

Of greater interest is the conformation analysis of the two isomers of **2**. Both isomers can theoretically exist in two chair conformations as shown in Scheme 2. Free energy differences (minimization of steric interactions between axial substituents such as P=O and



TRANS CIS

X = F, **2a** **2a′** **2b** **2b′**

X = Cl, **3a** **3a′** **3b** **3b′**

SCHEME 2

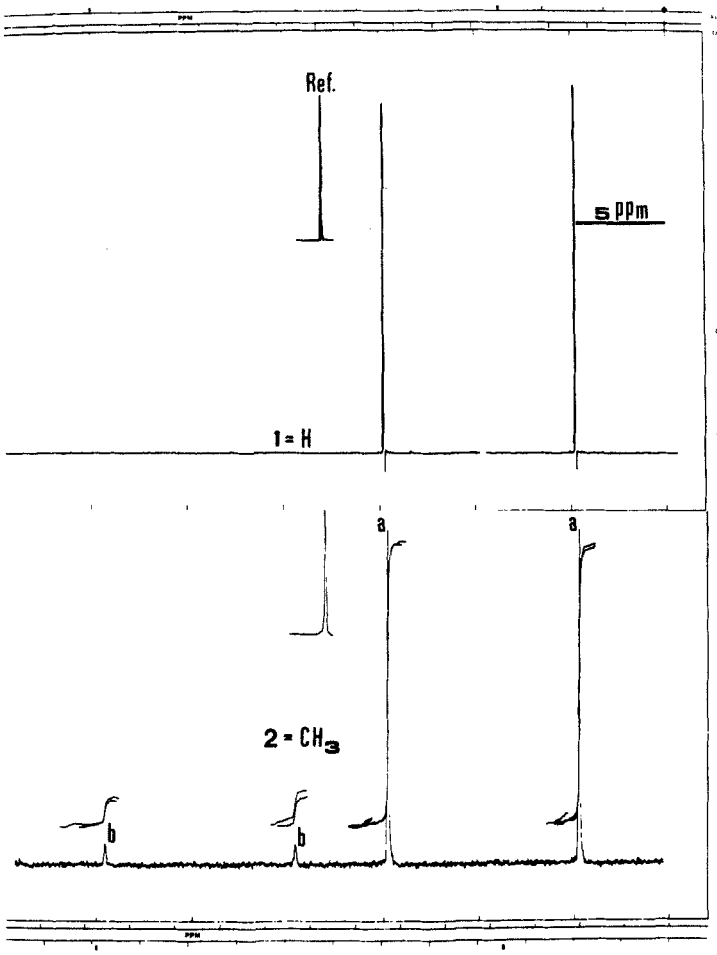


FIGURE 1 ^{19}F nmr spectra of cpds **1** and **2**.

C–CH_3) favours conformers **2a** and **3a** as the more stable species in the *trans* configuration. On the other hand, $^4J_{P-CH_3}$ values for the *cis* isomers (0.8 Hz) indicate that a mobile equilibrium might result in conformational time averaging. This interpretation rests on the assumption that $^4J_{P-CH_3}$ for conformer **2b** is likely to be zero.[9] This assignment implies that the *cis* isomers share at room temperature considerable fractions of equatorial P–F bonds *ca.* 25% (**2b'**)[9], in contrast to the exclusive axial orientation of the P–F bond in the *trans* isomer (**2a**). The difference in the ^{19}F chemical shifts between the two isomers and the higher field assignment to the ^{19}F chemical shift of the *trans* isomer are in a good agreement with other observations pertaining to these systems.[10, 11] Nevertheless, it should be mentioned here that a large difference in ^{19}F chemical shift between two isomers in a similar system where the fluorine is believed to occupy the axial position in both cases, was reported very recently in the literature.[12] Perhaps electronic and steric effects imposed by the axial 4-methyl on the axial fluorine can account for this. The unsubstituted cyclic analogue **1** has the same ^{19}F nmr characteristics as *trans* **2a** suggesting similar conformations. Thus, it is not illogical to predict one stable chair conformer for **1**, where the P=O bond is in the equatorial position.

In order to demonstrate the applicability of this technique for the study of stereospecific interactions involving P–F esters and nucleophiles in aqueous media, we studied the hydrolysis of **2** in buffered solutions at pH 7.0. The extraction procedure did not change the isomer ratio (experiments 2 and 5, Table II). Stereospecific hydrolysis was observed. The selec-

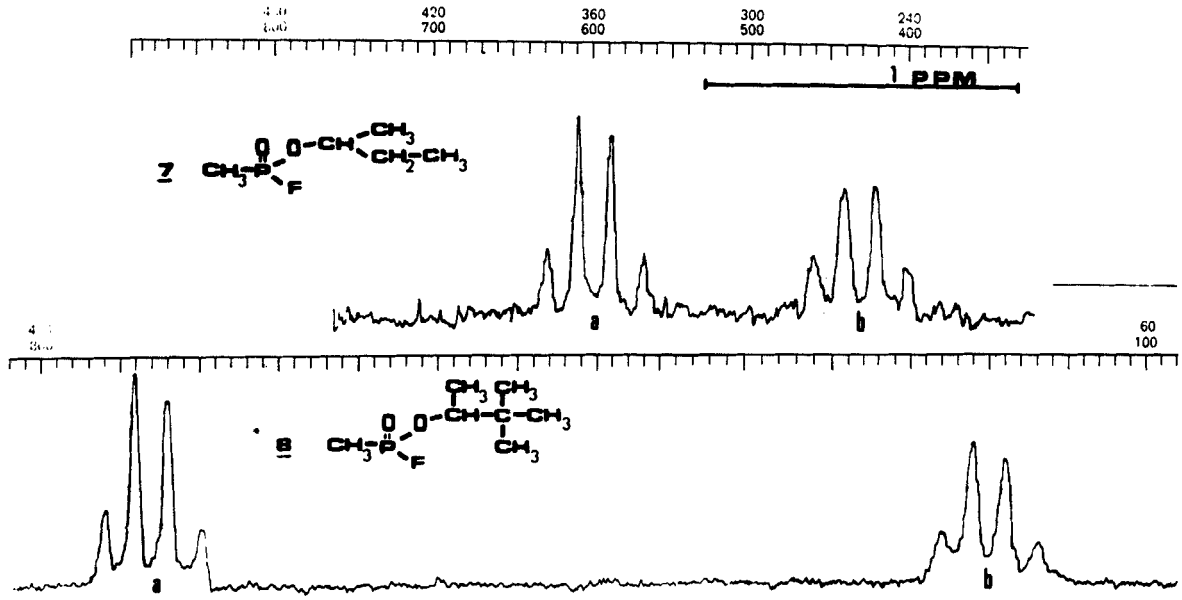
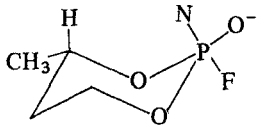
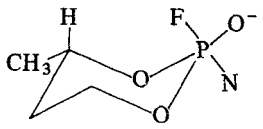


FIGURE 2 The right-hand side half ^{19}F nmr spectra of the diastereoisomers mixtures, 7 and 8.

tive enrichment of the *trans* isomer, as judged by ^{19}F nmr spectroscopy, can be explained in terms of differences in activation energy of the two isomers.[13] Thus, the P–F equatorial (**2b'**) transition state energy level may be lower than the axial transition state energy level (**2a**):



P–F eq. transition state (**2b'**)



P–F ax. transition state (**2a**)

Steric interference differences in the approach of the nucleophile may also account for the stereospecific hydrolysis.

^{19}F nmr analysis of **7** and **8** gives rise to two sets of double quartets of equal intensity (see Figure 2, where *only* the half spectra are presented). No changes in the spectrum were observed, even when the temperature was raised to 80°C. Sterically hindered rotation as an explanation for the doubling of the resonance may be ruled out, as can be judged from the spectral data obtained for **5**. Although **5** has a relatively bulky alkyl group, no doubling was observed in its ^{19}F nmr spectrum. Compounds **1**, **4**, **5** and **6** bear only one chirality centre (P) and do not show doubling of the resonance.

TABLE II

Data on the reactions of nucleophiles with **2** at pH 7.0 and at 25°C

Experiment No.	Nucleophile	Incubation[a] time (min)	pH, at the[b] end of the reaction	TRANS/CIS[c] (± 10%)
1	Neat	–	–	4.0
2	H_2O	60	3.5	4.0
3	Maleate (0.5 M)	60	6.9	9.0
4	Phosphate (1.0 M)	60	6.7	9.0
5	Phosphate (1.0 M)	5	7.0	4.5

[a] Time till extraction with ether.
[b] Measured before extraction.
[c] Measured by ^{19}F nmr spectra (see Figure 1).

During the preparation of this manuscript, Jennings also described doubling of ^{19}F nmr in steroidal phosphorofluoridates. However, no definite interpretation of this observation was made.[14]

We suggest the application of this technique to the study of stereospecific enzymatic reactions with P–F esters. The phosphoryl enzyme intermediate should be unstable enough to ensure reliable interpretation with reasonable times of incubation in buffered solutions.

EXPERIMENTAL

Warning: Cpds **4–8** are powerful cholinesterase inhibitors and are extremely toxic. Inhalation and skin contamination hazards should be taken into account.

Cpds **1** and **3** were prepared according to the procedures described in refs. 15 and 7 respectively. **2** was prepared according to the procedures described by Fukuto and Metcalf for the preparation of **1**. Cpds **4–8** were prepared according to standard procedures.[16,17] All cpds were at least 98% pure. Hydrolysis experiments were conducted at pH 7.0 and 25°C. Initial concentration of **2** was 8%. As a result of the low solubility of **2** in water, we were not able to follow the ^{19}F nmr changes using the nmr tube directly. The non-hydrolysed fluoridate was extracted from the hydrolysis medium into ether and the spectra were recorded after removal of the ether. ^{1}H and ^{19}F nmr spectra were recorded both with a Jeol C60 HL spectrometer, and varian XL100.

ACKNOWLEDGEMENT

The authors thank Mrs. L. Gradovsky and B. Manistersky for their excellent technical assistance.

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