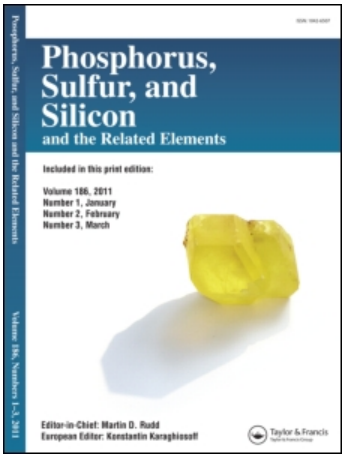

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^{31}P NMR SPECTROSCOPY STUDIES ON THE DIORGANYLPHOSPHOROCHLORIDATE/PYRIDINE PHOSPHORYLATION PROCEDURE

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^{31}P NMR SPECTROSCOPY STUDIES ON THE DIORGANYL PHOSPHOROCHLORIDATE/PYRIDINE PHOSPHORYLATION PROCEDURE

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The interaction of diphenyl phosphorochloridate and three dialkyl phosphorochloridates (alkyl = Et, Me, Bzl) with pyridine was shown by ^{31}P NMR spectroscopy to lead to the formation of a diorganyl phosphoropyridinium intermediate. In the case of diethyl, dimethyl and dibenzyl phosphorochloridate, ^{31}P NMR spectroscopy studies showed that their decomposition in pyridine solution resulted from pyridine-mediated dealkylation of the dialkyl phosphoropyridinium intermediate followed by the generation of diphosphate and triphosphate species. The use of the sterically hindered bases, 2-methylpyridine and 2,6-dimethylpyridine, showed that increased steric hindrance of the tertiary base caused a significant reduction in the extent of decomposition of the dialkyl phosphoro-*N*-pyridinium intermediate.

Key words: Alkyl phosphate cleavage, diorganyl phosphorochloridates, diorganyl phosphorotriesters, mechanism studies, phosphorylation, ^{31}P NMR spectroscopy.

INTRODUCTION

A commonly used method for the preparation of alkyl phosphoromonoesters involves the phosphorylation of an alcohol with a diorganyl phosphorochloridate in the presence of a tertiary base (pyridine, triethylamine, etc) followed by the cleavage of the organyl protecting groups from the resultant diorganyl phosphorotriester.[1,2] While we previously reported that the phosphorylation of protected serine derivatives with diphenyl phosphorochloridate proceeded in 95% yield, the use of diethyl, dimethyl and dibenzyl phosphorochloridate gave variable yields of diorganyl phosphorotriesters, yields of 91, 62 and 40% being obtained respectively.[3,4] In an earlier ^{31}P NMR based study,[5] we found that the low yield of the dibenzyl phosphorotriester was the result of extensive decomposition of dibenzyl phosphorochloridate through its interaction with the tertiary base during the course of the phosphorylation reaction. In this work, we present our studies on the use of ^{31}P NMR spectroscopy to examine the mode of interaction of some commonly used diorganyl phosphorochloridates with pyridine at several temperatures and to evaluate the effect of the organyl group on the decomposition rate.

RESULTS AND DISCUSSION

In the first instance, the mode of interaction between a diorganyl phosphorochloridate and pyridine was examined using diphenyl phosphorochloridate **1**. While

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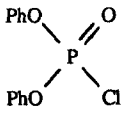

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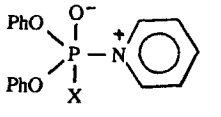
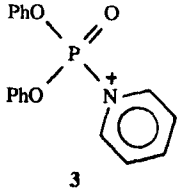
the ^{31}P NMR spectrum of diphenyl phosphorochloridate in THF displayed a single resonance at -7.0 ppm, the addition of pyridine at 4°C produced a mild exothermic reaction with the subsequent ^{31}P NMR spectrum displaying two signals at -6.4 and -25.5 ppm in a 3:1 ratio. The observation of the high field resonance suggested the formation of a pyridine-containing species such as **2** or **3** which is in equilibrium with the diphenyl phosphorochloridate.



X = Cl or Br

2



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In order to obtain supporting evidence for the formation of either of these two postulated intermediates, the more reactive diphenyl phosphorobromidate was then treated with pyridine. In this experiment, the ^{31}P NMR spectrum of diphenyl phosphorobromidate in THF displayed a single resonance at -16.8 ppm which, on addition of pyridine, changed to two signals at -16.8 and -25.5 ppm in a 1:5 ratio. In this later case, the higher concentration of the high-field resonance reflects the better leaving group properties of the bromide group over the chloride group. The observation of the common -25.5 ppm resonance in both experiments excludes intermediate **2** as a possible intermediate and supports species **3** as the likely intermediate. This assignment is further supported by the reported isolation of phosphoropyridinium chloride salts from the treatment of phosphoryl trichloride and phenyl phosphoryl dichloride with pyridine.[6] While the isolation of diorganyl phosphoropyridinium chloride salts have not been reported to date,‡ their role in phosphorylation reactions have often been postulated as a key intermediate.[8,9]

In a following study, the interaction of diethyl, dimethyl and dibenzyl phosphorochloridate **4–6** with pyridine was found to be a marked function of the reaction temperature. In the case of dibenzyl phosphorochloridate (δ + 3.5 ppm in THF), the addition of 1 mmol of reagent to pyridine at 20°C was markedly exothermic with the solution temperature rising to 60°C. The immediate ^{31}P NMR spectrum of the solution displayed a low intensity resonance at +3.5 ppm and numerous polyphosphate-type resonances between -10 and -25 ppm (Figure 1). In contrast, the ^{31}P NMR spectrum of **6** in THF/pyridine at -78°C displayed two resonances at +3.5 and -12.7 ppm in a 1:1 ratio which, on warming to -40°C, resulted in conversion of the +3.5 ppm resonance to the -12.7 ppm resonance followed by loss of the -12.7 ppm resonance and the appearance of numerous polyphosphate-type resonances. The nature of the various phosphorylation species

‡However, Revel and co-workers[7] have reported the isolation of two diorganyl phosphorotrimethylammonium chloride salts from the treatment of 2-bromoethyl ethyl phosphorochloridate and 2-chloroethyl phenyl phosphorochloridate with trimethylamine in benzene.

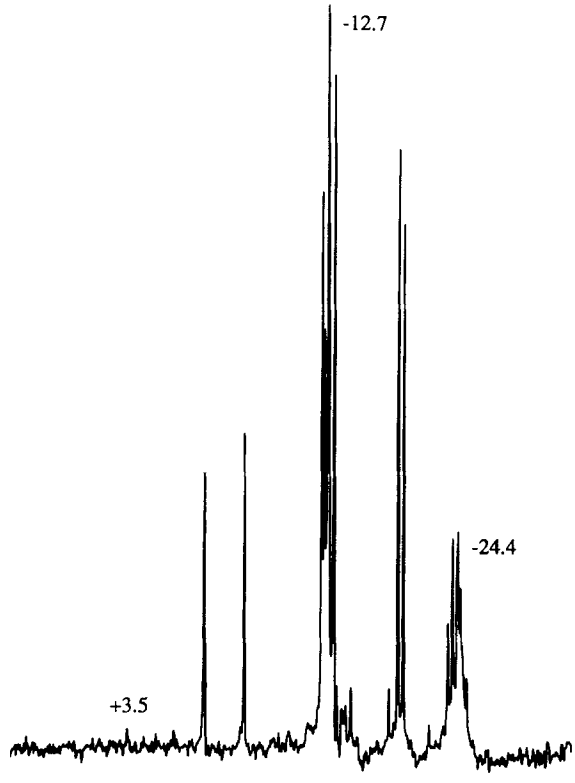


FIGURE 1 ^{31}P NMR spectrum obtained after the rapid addition of dibenzyl phosphorochloridate (1 mmol) to pyridine at 20°C.

involved in this conversion process was best observed when the diorganyl phosphorochloridate {R = Et **4**, Me **5**, Bzl **6**} was quickly added to a solution of pyridine/THF at 4°C. The comparison of the immediate ^{31}P NMR spectrum showed that similar phosphorus resonances were generated with the use of the three diorganyl phosphorochloridates and that after 30 min at 20°C, the obtained spectra showed that both reagents **5** and **6** had suffered extensive decomposition (Figure 2). The assignment of common resonances to specific intermediates is presented in Table I. While little decomposition was observed in the case of the diethyl phosphorochloridate/pyridine solution at 20°C, extensive decomposition readily occurred when the temperature of the solution was increased at 60°C.

The formation of the above intermediates is considered to be a consequence of pyridine-mediated cleavage of an organyl group from the activated diorganyl phosphoropyridinium specie ***b*** followed by phosphorylation of the resultant species ***c*** by a second molecule of ***b*** to give species ***d***. Instantaneous cleavage of the activated organyl group from ***d*** gives species ***e*** which can then undergo several reactions to generate a variety of polyphosphate products. The observation of a doublet/triplet coupled resonance set amongst the polyphosphate resonances indicates that one of these pathways involves the reaction of species ***e*** with species ***b*** to generate triphosphate intermediate ***f*** (Scheme I). In addition to this pathway, other pathways

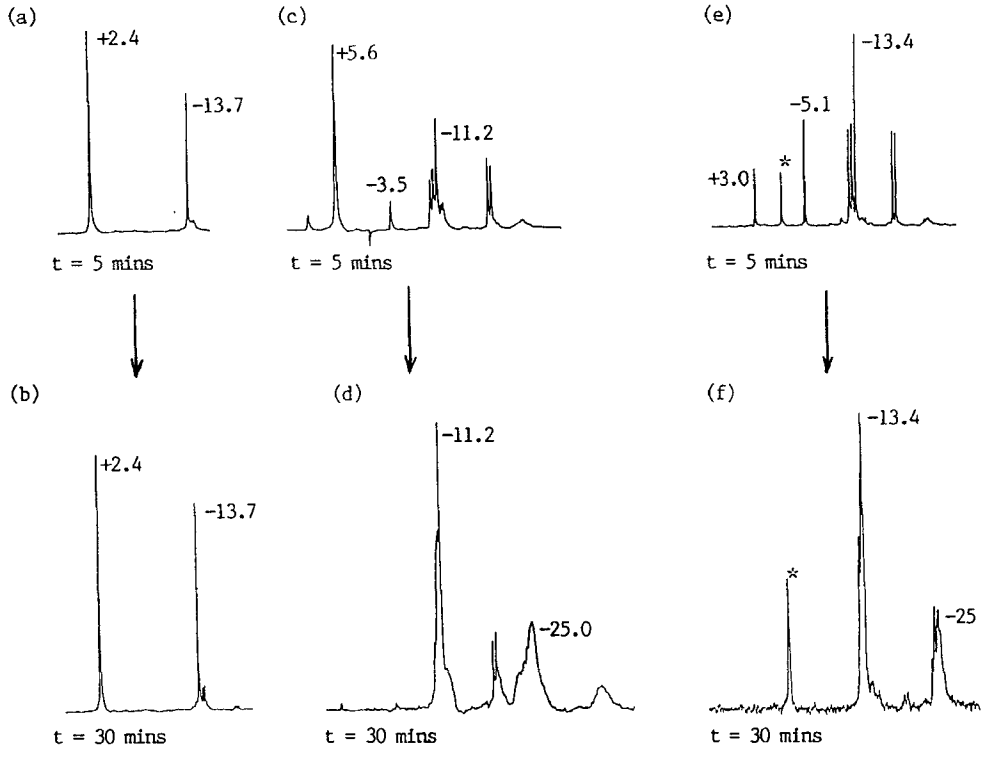


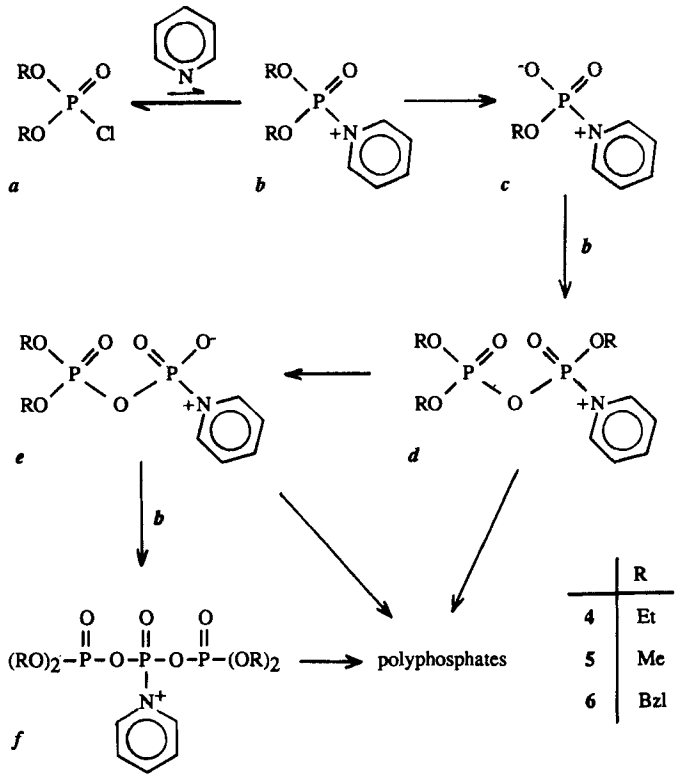
FIGURE 2 ^{31}P NMR spectra obtained from the addition of diethyl phosphorochloridate to pyridine/THF at 0°C after (a) t = 5 min and (b) t = 30 min, the addition of dimethyl phosphorochloridate to pyridine/THF at 0°C after (c) t = 5 min and (d) t = 30 min, and the addition of dibenzyl phosphorochloridate to pyridine/THF at 0°C after (e) t = 5 min and (f) t = 30 min (* = inert impurity in the commercial dibenzyl hydrogen phosphonate which was used in the preparation of dibenzyl phosphorochloridate).

TABLE I

Assignment of ^{31}P NMR chemical shifts to proposed intermediates

R	*a*	*b*	*c*	*e*	*f*
	(s)	(s)	(s)	(d,d 21.9 Hz)	(d,t 17.1 Hz)
Et	+2.4	-13.7	-5.4	-12.8/-19.8	-13.1/-25.7
Me	+5.6	-11.2	-3.5	-10.4/-19.8	-10.8/-24.8
Bzl	+3.0	-13.4	-5.1	-12.6/-19.8	-12.8/-24.7

are likely to involve pyridine-mediated cleavage of the organyl substituent from the diphosphate and triphosphate intermediates and their subsequent re-phosphorylation. While only one doublet of doublet resonance set was observed in the ^{31}P NMR spectrum, this resonance set was assigned to intermediate *e* on the basis of the similar chemical shift values of the high-field doublet resonance and that the presence of an organyl group has a marked influence on the observed chemical



SCHEME I

shift (see Table I). The non-observation of species ***d*** indicates that the cleavage of an organyl group from this intermediate is extremely rapid and is attributed to extensive activation of the organyl group by the additive effect of the electron withdrawing diorganyl phosphoro moiety and the pyridinium group. Thus, under the above reaction conditions, the failure to observe ***d*** is due to the rate of its deorganylation being considerably faster than its rate of formation. Thus, on the basis of the ^{31}P NMR data, the rate of decomposition of the diorganyl phosphorochloridates was found to be dependent on the rate of cleavage of the organyl group from the diorganyl phosphoropyridinium intermediate; this being more pronounced for the electropositive benzyl and methyl groups.

The above spectroscopic observations allow a re-interpretation of earlier literature reports involving the phosphorylation of protected serine derivatives and seryl-containing peptides. In their studies, Avaeva and coworkers[10] reported the isolation of minor quantities of *O*-diphosphoseryl- and *O*-triphosphoseryl-containing peptides from the phosphorylation of protected seryl-containing peptides with dibenzyl phosphorochloridate in pyridine at 4°C. The formation of these by-products was attributed by the authors to result from pyridine-mediated debenzylation of the dibenzyl phosphorotriester followed by rephosphorylation of the benzyl phosphorodiester by excess dibenzyl phosphorochloridate. However, on the basis of the above spectroscopic findings and our earlier observations[5] where we found

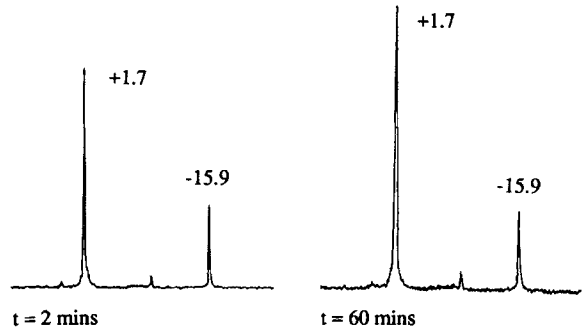


FIGURE 3 ^{31}P NMR spectra obtained for di-2,2,2-trichloroethyl phosphorochloridate in pyridine.

that pyridine was unable to effect the debenzylation of Boc-Ser(PO_3Bzl_2)-ONBzl at 20°C, we propose that the by-products isolated by Avaeva *et al.*[10] are more likely the result of phosphorylation of the seryl residue by the generation of reactive species ***e*** and ***f*** during the course of the phosphorylation.

In addition to electronegativity factors, steric factors also play a role in the diorganyl phosphorochloridate/pyridine interaction since no high-field resonance was observed for the dissolution of di-*t*-butyl phosphorochloridate in pyridine. In this case, the nonformation of the phosphoropyridinium species is attributed to the steric influence of the bulky *t*-butyl groups on the P—Cl linkage and is consistent with the known failure of di-*t*-butyl phosphorochloridate for effecting the phosphorylation of alcohols. Alternatively, electronic factors also play a role in the stability of dialkyl phosphoro-*N*-pyridinium intermediates since the dissolution of di-2,2,2-trichloroethyl phosphorochloridate (+1.7 ppm) in pyridine gave rise to a high-field resonance at −15.9 ppm (2:1 ratio) and, as in the ethyl case, showed no decomposition after 60 min at 20°C (Figure 3).

Phosphorylation Studies

On the basis of the above spectroscopic observations, we considered that the use of lower phosphorylation temperatures would minimize the extent of decomposition of the dialkyl phosphoropyridinium specie and thereby, maximize the yield of the desired dialkyl phosphorotriester. In order to test this proposition, a series of phosphorylations using Boc-Ser-ONBzl were performed using dibenzyl, dimethyl and diethyl phosphorochloridate at reaction temperatures of 0°, −10° and −40°C. A comparison of the phosphorotriester yields showed that there was a marked increase in yield with decreasing reaction temperature (Table II), this effect being most pronounced in the case of the benzyl derivative.

In the case of the phosphorylations performed using dimethyl and dibenzyl phosphorochloridate at −40°C, the analysis of the product mixtures by ^{13}C NMR spectroscopy showed that the phosphorylations were incomplete and contained between 6–10% of Boc-Ser-ONBzl. As the incomplete phosphorylations were attributed to the steady loss of the reactive dialkyl phosphoropyridinium intermediate during the course of the reaction period, this situation was overcome by increasing the

TABLE II

Comparison of isolated yields of Boc-Ser(PO_3R_2)-ONBzl (R = Et, Me, Bzl) derivatives using various reaction temperatures

R	0°/15h[a]	-10°/15h[a]	-40°/15h[a]	-40°/6h[b]
Bzl	40%	65%	84%[c]	94%
Me	62%	80%	86%[c]	96%
Et	91%	96%	98%	

[a] performed using 1.2 equiv. of the dialkyl phosphorochloridate

[b] performed using 1.5 equiv. of the dialkyl phosphorochloridate followed a second addition of 0.75 equiv. of the dialkyl phosphorochloridate after 3 h.

[c] incomplete phosphorylation, contained 6-10% of Boc-Ser-ONBzl.

initial amount of dimethyl or dibenzyl phosphorochloridate from 1.2 to 1.5 equivalents and performing a second addition of 0.75 equivalents of the dialkyl phosphorochloridate after 3 h. Under these conditions, ^{13}C NMR analysis of the product mixture showed complete phosphorylation had occurred with the final yields of Boc-Ser(PO_3Bzl_2)-ONBzl and Boc-Ser(PO_3Me_2)-ONBzl being 94 and 96% respectively.

Effect of Sterically Hindered Pyridines on the Rate of Decomposition

In consideration that past workers[11] have employed the use of the sterically hindered pyridine bases, 2-methylpyridine and 2,6-dimethylpyridine, in phosphorylation studies, we therefore decided to investigate the effect of these sterically hindered bases on the rate of decomposition of dibenzyl phosphorochloridate. While initial ^{31}P NMR monitoring of a dibenzyl phosphorochloridate/2-methylpyridine solution showed less decomposition of the phosphopyridinium species occurred in comparison to the use of pyridine (compare Figure 4c with Figure 4a), the observation of high intensity polyphosphate resonances after 30 min (Figure 4d) indicated that the introduction of the methyl group to the pyridine base did not provide significant steric hindrance and that 2-methylpyridine was as effective as pyridine for effecting extensive base-mediated debenzylation of the dibenzyl phosphoro-*N*-2-methylpyridinium intermediate. In the case of 2,6-dimethylpyridine though, the use of this base caused a marked reduction in the generation of polyphosphate products (Figure 4f) and indicated that the introduction of two methyl groups to the pyridine base caused significant steric hindrance and was effective for decreasing the rate of debenzylation from the phosphoro-*N*-2,6-dimethylpyridinium species. A more detailed comparison of decomposition rates by integration of relative peak areas was not possible due to the variable precipitation of a brown gum from each solution. The failure to observe any resonance signals corresponding to intermediates ***e*** and ***f*** with the use of either 2-methylpyridine or 2,6-dimethylpyridine suggests that the rate of formation of these particular intermediates is considerably slower than their rate of consumption and, as a consequence, are not observed on the spectroscopic time scale.

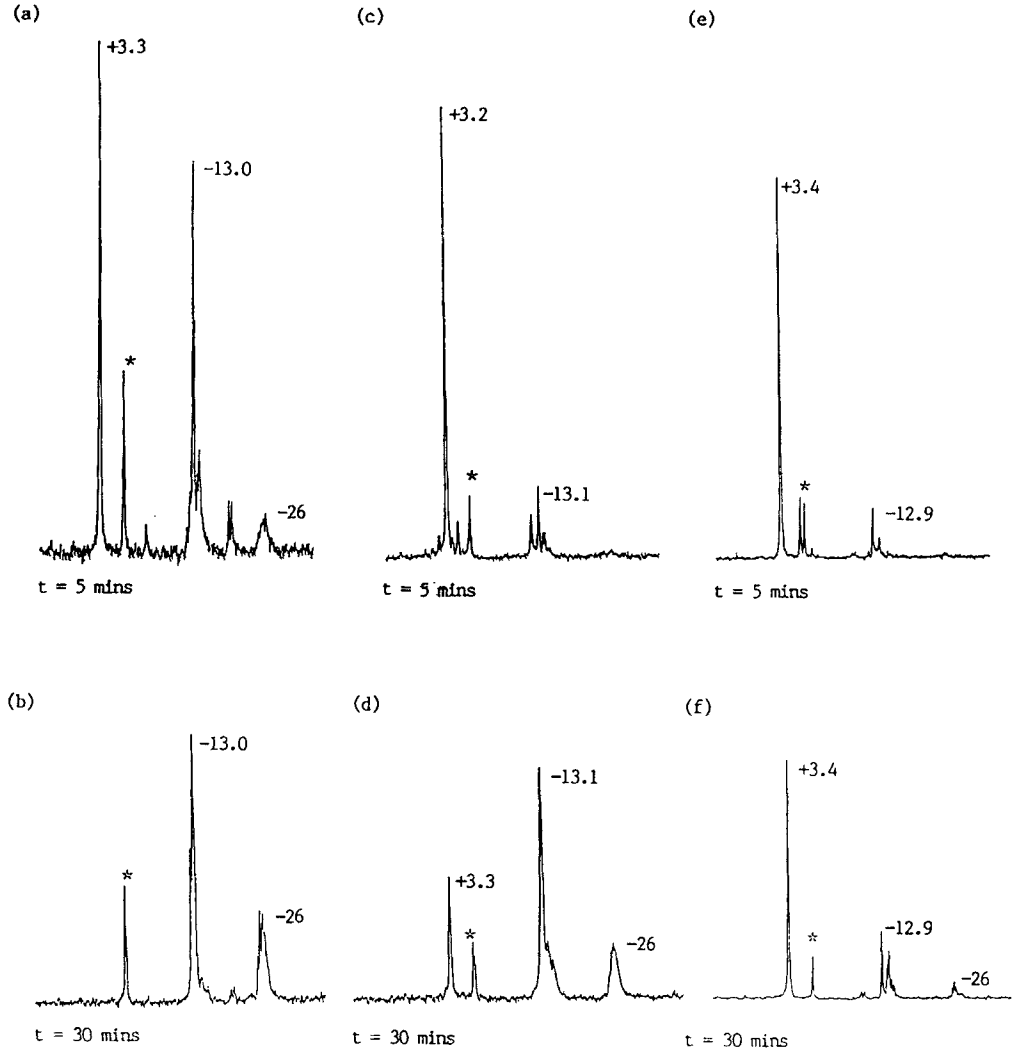


FIGURE 4 ^{31}P NMR spectra obtained after the addition of dibenzyl phosphorochloridate to pyridine/THF at (a) t = 5 min and (b) t = 30 min, after its addition to 2-methylpyridine/THF at (c) t = 5 min and (d) t = 30 min, and after its addition to 2,6-dimethylpyridine/THF at (e) t = 5 min and (f) t = 30 min (all additions performed at 0°C with the solution then allowed to rise to 20°C) (* = inert impurity in the commercial dibenzyl hydrogen phosphonate which was used in the preparation of dibenzyl phosphorochloridate).

On the basis of the above spectroscopic data, we are able to conclude that, despite the increased steric bulk of 2,6-dimethylpyridine, this tertiary base is able to generate a dibenzyl phosphoro-2,6-dimethylpyridinium intermediate and that this intermediate is subject to slow decomposition since the rate of base-mediated debenzylation is decreased due to the poor nucleophilicity of the sterically hindered 2,6-dimethylpyridine. However, since Todd and coworkers[10] reported that 2,6-dimethylpyridine gave lower yields of phosphorylated products in comparison to the use of pyridine, this suggests that the increased stability of the dibenzyl phos-

phoro-2,6-dimethylpyridinium intermediate in the 2,6-dimethylpyridine/THF solution is negated by the marked reduction in its reactivity due to increased steric hindrance about the P—N linkage. Alternatively, while the use of steric-free pyridine generates a more reactive dibenzyl phosphoro-*N*-pyridinium intermediate, this greater reactivity is counterbalanced by the greater susceptibility of the benzyl phosphate group to undergo base-mediated cleavage in the pyridine/THF solution at temperatures above −40°C.

In the case of dimethyl phosphorochloridate, ^{31}P NMR studies showed that the rate of decomposition of this reagent in pyridine, 2-methylpyridine and 2,6-dimethylpyridine was also a function of the steric bulk of the tertiary base with 2,6-dimethylpyridine causing the least decomposition. For diethyl phosphorochloridate though, the ^{31}P NMR spectra of this reagent in the three pyridine bases showed low intensity high-field resonances at −13.7, −13.6 and −13.5 ppm respectively with very minor decomposition resonances being observed after 60 min at 20°C; the marked stability of these phosphoropyridinium intermediates being due to the low susceptibility of the ethyl phosphate group to undergo nucleophilic cleavage. While both the tertiary base and chloride ion are available to effect benzyl and methyl cleavage from the dialkyl phosphoro-*N*-pyridinium intermediates (alkyl = benzyl and methyl), the marked variation in decomposition rates observed with the three tertiary bases indicates that the pyridine base is the more likely nucleophile involved in the decomposition process.

CONCLUSION

From the above spectroscopic studies, it was found that the dissolution of a dialkyl phosphorochloridate (alkyl = Et, Me, Bzl) in pyridine resulted in the generation of a dialkyl phosphoropyridinium intermediate which was in equilibrium with the dialkyl phosphorochloridate. While the ethyl groups of the dialkyl phosphoropyridinium intermediate were resistant to base-mediated cleavage below 20°C, both the methyl and benzyl groups underwent rapid cleavage at 4°C with the rate of organyl cleavage reduced at lower temperatures. While the use of sterically hindered pyridines did not prevent the formation of the dialkyl phosphoropyridinium intermediate, the use of 2,6-dimethylpyridine caused reduced organyl cleavage.

EXPERIMENTAL

Diphenyl, diethyl, dimethyl and dibenzyl hydrogen phosphonate were obtained from Aldrich, dried over 4 Å sieves and distilled. Diphenyl phosphorochloridate was obtained from Sigma and diphenyl phosphorobromidate was obtained from the treatment of diphenyl hydrogen phosphonate with *N*-bromosuccinimide in benzene. Diethyl, dimethyl and dibenzyl phosphorochloridate were obtained from the treatment of the corresponding diorganyl hydrogen phosphonate with *N*-chlorosuccinimide in benzene.[12] *N*-Chlorosuccinimide and *N*-bromosuccinimide were obtained from Aldrich. Di-2,2,2-trichloroethyl phosphorochloridate was obtained from Aldrich and di-*t*-butyl phosphorochloridate was prepared from di-*t*-butyl hydrogen phosphonate.[13] Pyridine, 2-methylpyridine and 2,6-dimethylpyridine were dried over 4 Å sieves and distilled. Tetrahydrofuran was distilled from the potassium ketyl of benzophenone prior to use. ^{31}P NMR spectra were obtained on a JEOL FX-100 instrument and referenced to external (capillary) H_3PO_4.

Phosphorylation procedure: A solution of dialkyl phosphorochloridate (1.5 mmol) in THF (1 mL) was added to a stirred solution of Boc-Ser-ONBzl (1.00 mmol) in pyridine (1 mL) such that the temperature was maintained at −40°C. After 3 h, a second solution of the dialkyl phosphorochloridate (0.75 mmol) in THF (0.5 mmol) was added to the reaction solution at −40°C. Water (1 mL) was then added and the solution stirred at 20° for 10 min. Diethyl ether (50 mL) was then added and the solution washed with 1 M HCl (3 × 25 mL), 5% $NaHCO_3$ (2 × 20 mL), dried (Na_2SO_4) and filtered. Evaporation of the organic solvent under reduced pressure gave Boc-Ser(PO_3Me_2)-ONBzl in 96% yield and Boc-Ser(PO_3Bzl_2)-ONBzl in 94% yield.

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