Journal of Chromatography, 192 (1980) 173–184

CHROM. 12,576

STIMULATED SEQUENTIAL DEHYDROXY-FLUORINATION: A SAFE, CONVENIENT METHOD FOR GAS CHROMATOGRAPHIC ANALYSIS OF PHOSPHATE DIESTERS WITH POSSIBLE APPLICATION TO HYDROLYSATES OF ORGANOPHOSPHORUS PESTICIDES AND NERVE GASES

WAYNE H. GRIEST* and T. W. MARTIN**

Department of Chemistry, Vanderbilt University, Box 1506, Station B, Nashville, Tenn. 37235 (U.S.A.)

(First received September 10th, 1979; revised manuscript received November 26th, 1979)

SUMMARY

Model studies with di-*n*-butylphosphate show that organic triester derivatives chosen to gain increased volatility by simply minimizing total molecular weight are relatively poorer in gas chromatographic properties than the diesterphosphorofluoridate analogue because both the molecular weight and the overall polarity of the derivative are critical factors. We find the substitution of the hydroxyl group by fluorine is the most successful way at present to derivatize phosphate diesters. Fluorine substitution is effective because it reduces the strong phosphoryl dipole, presumably by decreasing the electron density on the oxygen atom via $p\pi$–$d\pi$ backbonding to the phosphorus. Dehydroxy-fluorination is best carried-out by a sequential reagent process starting with dicyclohexylcarbodiimide to stimulate and direct the dehydroxy-step before adding hydrogen fluoride as the active fluorinating agent. This procedure is safe and convenient and is believed applicable to a wide range of hydroxyphosphates including the hydrolysates of many organophosphorus pesticides and nerve gases at minimal sample sizes.

INTRODUCTION

In a previous paper[1], we demonstrated an unavoidable phosphorus–oxygen bond cleavage reaction which renders trimethylsilyl (TMS) derivatization useless for the quantitation by gas chromatography (GC) of condensed polyphosphates and related nucleotides. Although TMS or methyl ester derivatives of phosphoric acid and its mono- or diesters have often been applied to these simpler phosphate substrates[2–11], derivatization and subsequent GC analysis are not easy or dependable procedures. In fact, reliable reports of sensitive and reproducibly precise GC methods

* Present address: Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830, U.S.A.

** To whom correspondence should be addressed.

for phosphates are practically non-existent, and the few available reports[4,12–17] differ in methods, sensitivity, specificity, and precision.

For a number of years we have sought improvements in the derivatization and GC analysis of certain classes of phosphates by assuming the principal difficulties stem from essentially two interrelated and unavoidable factors: (1) the relatively high molecular weights of all phosphate derivatives and (2) the large inherent polarity of the phosphate group, a complicated function of the normally strong phosphoryl dipole mixed with significant dipolar contributions from the other three P–O bonds. Rather than simply trying to reduce molecular weight, we reasoned that if one could modify the phosphate group's structure in such a way as to reduce its overall polar character such a derivative might have improved GC properties. This paper reports the results of a study to test this "polar hypothesis" using di-*n*-butylphosphate (DBP) as a model monohydroxyphosphate. Such dialkylphosphates are common pesticide hydrolysis products and their determination in physiological fluids[12,13] or water[17] is of continuing importance for public health services and in monitoring environmental pollution.

EXPERIMENTAL

Materials

The principal chemicals and other materials used are listed below by chemical name or formula with any useful acronym in parentheses followed by the supplier's name and address and any special information in square brackets: tetrahydrofuran (THF), pyridine and acetonitrile [Fisher Scientific, Pittsburgh, Pa., U.S.A.; each of these solvents was redistilled, reagent-grade and stored over activated molecular sieve, 13X]; methyl iodide, triethylamine, benzene, methylene chloride, dimethoxypropane, diethylether, *n*-butanol, phosphoryltrichloride, potassium fluoride and sodium fluoride [also from Fisher Scientific]; HF and $COCl_2$ [Matheson Gas Products, East Rutherford, N.J., U.S.A.; in lecture bottles]; AgF and BiF_3 [Alfa Division, Ventron, Danvers, Mass., U.S.A.]; 2,4-dinitrofluorobenzene (DNFB) [Aldrich, Milwaukee, Wisc., U.S.A.]; bis(trimethylsilyl)trifluoroacetamide (BSTFA), hexamethyldisilizane (HMDS), trimethylchlorosilane (TMCS) and dicyclohexylcarbodiimide (DCCI) [Pierce, Rockford, Ill., U.S.A.]; 0.3-ml reaction vials [Regis, Morton, Ill., U.S.A.]; all GC stationary phases, solid supports and syringes [Applied Science Labs., State College, Pa., U.S.A.].

Preparation of reagents and substrate

Diazomethane (CH_2N_2). The precursor, N-nitrosomethyl urea, was synthesized by a previous procedure[18]. CH_2N_2 was generated on reaction of the precursor with aqueous sodium hydroxide and was collected by bubbling through diethyl ether in a micro apparatus[19].

DBP. The precursor for this substance, chosen as our model substrate, was dibutylphosphorochloridate (DBPC) which was synthesized from a modification of the published method[20] for the diethyl homologue. In our procedure, a solution of 20 ml of *n*-butanol plus 17.6 ml of pyridine in 80 ml benzene was slowly added over 45 min into a well stirred solution consisting of 10 ml phosphoryl trichloride and 250 ml benzene contained in a 1-l round-bottom flask. The reaction mixture was

stirred 1 h, refluxed 6 h and allowed to stand at room temperature overnight, protected from moisture by a $CaCl_2$-trap, before filtration and vacuum distillation. A 27% yield of DBPC was achieved by this procedure but GC analysis of this product revealed a 10% contamination with tributylphosphate (TBP). However, this impurity was not removed because it served as a convenient internal standard (IS). The DBPC preparation was stored in a round-bottom flask with ground-glass stopper in a desiccator over dry $CaCl_2$. Hydrolysis to DBP was carried out daily on a 3-μl aliquot of a solution containing 120 μl DBPC diluted to 1 ml with THF. This 3-μl aliquot was added to 60 μl of THF and 30 μl of water, and the combined solution was heated at 60 to 90° for 15 min before the solvents were evaporated under a gentle stream of nitrogen. The residue was treated with methylene chloride or dimethoxypropane and re-evaporated to remove traces of water, and was finally redissolved in 250 μl of THF. From this solution, 3-μl aliquots corresponding to 20 nmol of DBP were transferred to reaction vials for derivatization and GC analysis.

HF–THF. Solutions of HF in THF were prepared by bubbling HF from a lecture bottle through dry, redistilled THF contained in a 2-oz. polyethylene jar, using a 0.25-in. O.D. PTFE tube connected to the HF lecture bottle valve. Contact with atmospheric moisture during bubbling was minimized by using a cap on the jar with a hole drilled just large enough to accept the PTFE tubing. This procedure was conducted in a fume hood to avoid exposure to HF. The HF–THF solution was standardized by pipetting a 100-μl aliquot into 15 ml of water, and titrating the HF to the phenolphthalein end point with standard NaOH solution. Generally, HF concentrations of about 1 *M* were prepared, and the tightly capped jar was stored in a desiccator over Drierite for a maximum of two weeks before replacement.

$COCl_2$–THF. A stock solution of $COCl_2$ in THF was prepared by directly bubbling $COCl_2$ into THF contained in a 2-oz. polyethylene jar in a fume hood, similar to the procedure used for preparing the HF–THF solution. The concentration of $COCl_2$ was estimated by pipetting 100 μl of the solution into 15 ml of well-stirred standardized NaOH solution and titrating the excess base with standard HCl solution to the phenolphthalein end point. Generally, a concentration of 0.04 *M* $COCl_2$ was prepared and this $COCl_2$–THF reagent was stored like the HF–THF reagent.

AgF–CH_3CN. Powdered AgF was slowly stirred into CH_3CN at a ratio of 3 mg AgF per ml CH_3CN. The mixture was warmed slightly (30–35°). After cooling the solution, the excess AgF was removed by centrifugation for 3–5 min using a bench-top clinical centrifuge. Gravimetric analysis of the residue obtained from evaporating aliquots of the solution indicated an AgF concentration of 23.0 ± 0.6 m*M*.

Derivatization reactions

Safety precautions. It is to be emphasized that the ability of some dialkylphosphorofluoridates (such as diisopropylphosphorofluoridate) to inhibit neural cholinesterase[21] makes fluorinated DBP derivatives potentially quite hazardous, and extreme care must be exercised in their generation and handling. However, generation and sampling under sealed containment can be accomplished conveniently by the use of 0.3-ml reaction vials with PTFE-lined septum caps. The DBP substrate in these derivatizations was pipetted into the vials, the vials then sealed, and the dehydroxy–fluorination reagents were introduced through the septum with graduated 100-μl Hamilton syringes. Heating was conducted in a metal heating block as previously

described[1]. Samples for GC analysis were withdrawn directly through the septum with a 10-μl Hamilton syringe. After GC analysis of the derivatives, the vials were cleaned and the contents were destroyed by soaking in chromic acid.

The following are the derivatization reactions conducted upon DBP. All but the first three produce a potentially hazardous phosphorofluoridate.

TMS derivatization. A 20-nmol aliquot of DBP was reacted with a 100-fold molar excess of either BSTFA or HMDS (either reagent in a solution containing 1% of TMCS (v/v) and 100 μl of CH_3CN). The sealed reaction vial was heated at 80° for 15 min, and after cooling was sampled for GC analysis.

Methylation with CH_2N_2. In a fume hood, diazomethane in diethyl ether was added dropwise to 20 nmol of DBP in 100 μl of methanol until a stable yellow color remained. The vial was then sealed and the solution allowed to react at room temperature for 5 min. After this, the top was loosened and excess CH_2N_2 and diethyl ether flushed out by bubbling with a stream of dry nitrogen for a few minutes. Then the remaining methanol solution was sampled for analysis.

Methylation with AgF–CH_3I. A 20-nmol aliquot of DBP was reacted with a 3-fold molar excess of AgF in CH_3CN to precipitate the Ag salt of DBP. The CH_3CN was evaporated with a stream of dry nitrogen, and 100-fold molar excess of CH_3I was added. The sealed vial was heated at 80° for 15 min, and allowed to cool before sampling.

Fluorination with DCCI–HF. A 2.0-μmol aliquot (100-fold molar excess) of DCCI in 100 μl THF was added to 20 nmol of DBP, and the mixture was allowed to react in a sealed vial at room temperature for 2–3 min. A 1000-fold molar excess of HF in 20 μl THF was then added, and the sealed vial was allowed to stand at room temperature for 45 min before sampling.

Fluorination with DCCI–AgF. This derivatization reaction was conducted as described above for the DCCI–HF reagents, except that 26 μl of CH_3CN containing a 300-fold molar excess of AgF was substituted for HF–THF.

Fluorination with DCCI–KF or DCCI–NaF. This derivatization was conducted as described above for the DCCI–HF reagents, except that 2–3 mg of powdered KF or NaF was added instead of HF–THF.

Fluorination with $COCl_2$–AgF. To a 17-nmol aliquot of DBP in 20 μl THF was added a 2000-fold molar excess of $COCl_2$ in 100 μl THF and 2–3 mg powdered AgF. The reaction was allowed to proceed at room temperature for 24 h in a sealed vial before sampling.

Fluorination with HF. A 1000-fold molar excess of HF in 20 μl of THF was added to 20 nmol of DBP in 100 μl of THF and the reaction vial sealed. The reaction was carried out at room temperature for 5 h before analysis.

Fluorination with DNFB. To 20 nmol of DBP in 100 μl THF was added a 1000-fold molar excess of DNFB and a 2700-fold molar excess of triethylamine. The vial was sealed, heated at 95° for 15 min, and cooled before a sample was withdrawn for analysis.

Fluorination with BiF_3. Two to three mg of BiF_3 were added to a 20-nmol aliquot of DBP in 100 μl of THF. The vial was sealed and heated at 40° for 1 h. After the vial was cooled a sample was taken for analysis.

GC analysis

A 2-μl volume of a reaction mixture was withdrawn from the reaction vial through the septum cap using a 10-μl Model 701 Hamilton syringe. This sample was injected into a Hewlett-Packard Model 5750 gas chromatograph. The column used was a 6 ft. $\times$ 0.125 in. O.D. stainless steel coil packed with 10% (w/w) OV-61 coated on 80–100 mesh, acid-washed, dimethyldichlorosilane-treated Chromosorb W prepared by the evaporative method[22]. The column oven temperature was maintained isothermally at 183°, and the injector and detectors were held at 195 and 190°, respectively. Helium carrier gas was flow-regulated at 25 cm^3/min. The stainless steel injection port was lined with PTFE by simply inserting a 6 in. $\times$ 0.0625 in. O.D. PTFE tubing into the injector liner.

TMS derivatives were analyzed with the conventional flame ionization detector (FID), and all others (except methyl esters, when compared with TMS derivatives) were analyzed with the alkali flame ionization detector (AFID), which was installed and adjusted according to factory instructions[23].

Quantitative comparison of DBP derivatives was accomplished conveniently by the method of internal standards[24], letting the unavoidable TBP contamination in the DPBC precursor to the DBP substrate serve as a convenient internal standard (IS).

RESULTS AND DISCUSSION

Our contention that simply increasing the volatility of an organic phosphate triester by minimizing molecular weight alone is not sufficient to produce a superior derivative for GC analysis is supported by a comparison of the properties of trimethylsilyl dibutylphosphate (TMS-DBP) and methyl dibutylphosphate (CH_3-DBP) prepared by the TMS derivatization and the methyl esterification methods described above. Using the FID technique because the AFID loses sensitivity when exposed to TMS compounds[25], we found that the response of the TMS-DBP produced with the BSTFA reagent was approximately the same as that of the CH_3-DBP produced with either CH_2N_2 or AgF–CH_3I. However, the response of TMS-DBP produced with the HMDS reagent was approximately 8% less than that produced with the BSTFA reagent, consistent with our previous observations[1] of their relative potencies as TMS-donors. Although CH_3-DBP was somewhat more volatile than TMS-DBP (GC retention times of 3.5 and 3.7 min, respectively), the chromatographic peaks of both derivatives exhibited noticeable tailing, suggesting that the column and/or packing material were not sufficiently inert toward these derivatives. Hence, we believe only a reduction in the net polarity of the phosphate group will improve the GC response of these compounds.

The obvious means of diminishing the phosphate group polarity is by a reduction of the phosphoryl dipole strength, which entails either a chemical reduction of the formal oxidation state of the phosphorus atom (such as from 5+ in phosphate to 3+ in phosphite) or by replacement of the hydroxyl group with a more electronegative substituent such as flourine. The lack of literature precedent, plus the many difficulties we experienced[26] in attempting to carry-out controlled chemical reduction of phosphate to a stable lower oxidation state, forced us to discard actual chemical reduction as a possible route to improved GC derivatives. In contrast, the general stability of P–F bonds[27,28] and the many experimental successes for the chlorination

of phosphates by $COCl_2$ [29–31], the fluorination of organic compounds by COF_2 [32], and the fluorination of phosphates by HF[33,34] or by DNFB[35] convinced us that a systematic study of the fluorination of DBP as a model diester might be a promising way to test whether decreasing the relative polarity of the phosphate moiety by dehydroxy-fluorination might yield a better derivative in support of our introductory "polar hypothesis".

Initial investigation revealed that a number of reagents can be employed to convert DBP to dibutylphosphorofluoridate (DBPF). Normalized DBPF yields for the reagents studied are listed in Table I. The $COCl_2$–AgF reagent combination was unsatisfactory because it produced mixed products of both DBPF and DBPC. Also, it is seen in Table I that most reagents gave relatively low DBPF yields except for the DCCI–HF sequential method which is so unique that it deserves more extensive discussion.

TABLE I

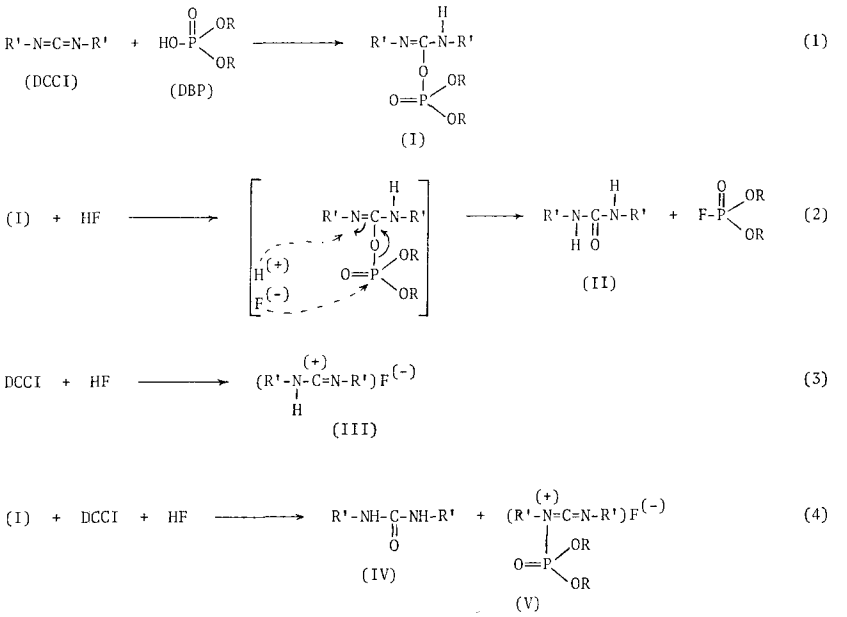
RELATIVE YIELDS OF DBPF IN THE DEHYDROXY-FLUORINATION OF DBP

Reagents	*Relative yield (%)*
DCCI–HF	100*
$COCl_2$–AgF	35
DCCI–AgF	25
HF	23
DNFB–$(C_2H_5)_3N$	17
BiF_3	3.0
DCCI–KF or DCCI–NaF	1.0

* Assumed to be quantitative.

We are convinced that the DCCI–HF sequential system is the best known method for carrying-out the dehydroxy-fluorination of phosphate diesters. A comprehensive review[36] indicates the few methods available for the direct conversion of a dialkyl phosphate to a dialkylphosphorofluoridate. Judging from the relative yields of DBPF from the DCCI–HF sequential combination as compared to that achieved by HF alone, there can be no question that DCCI stimulates the dehydroxy-fluorination process in a remarkably way. Since DCCI has been used for years to promote the synthesis of pyrophosphates[37–40], presumably by activating one phosphate for nucleophilic attack by another, we envision by analogy in Scheme I a reasonable mechanism for converting DBP to DBPF in accord with our results and which explains the possible role of DCCI in the dehydroxy-fluorination process.

Reaction 1 can be viewed as a rather fast acid–base addition reaction between DBP and DCCI. The result is intermediate complex I which is the first necessary stage for stimulated dehydroxylation and a directed activation of the phosphate group. Reaction 2 completes the dehydroxy-fluorination process by a double attack of both the proton electrophile and the fluoride nucleophile. We have no information to decide whether reactions 1 and 2 proceed in time as distinguishable steps or in a more concerted or simultaneous fashion; but the sequential use of DCCI and HF is crucial to maximize yields of DBPF, so we postulate that there are at least two stages in the process along the lines suggested by reactions 1 and 2.



Scheme I. R = butyl and R′ = cyclohexyl.

The time course for DBPF production, determined with a 100-fold molar excess of DCCI followed by a 1000-fold molar excess of HF is shown in Fig. 1A. Fluorination proceeds rapidly at room temperature and appears to reach a maximum after 30–40 min. Thus, a reaction period of at least 45 min was chosen as standard protocol. The 100-fold molar excess of DCCI forces a close to quantitative yield of intermediate complex I and inhibits possible pyrophosphate formation. The latter process is possible whenever phosphate anions are free to complete as nucleophiles in this system.

To find the optimum ratio of HF–DCCI, we fixed one reagent while the other was systematically varied. The results of these experiments are shown in Fig. 1B and C. The optimum level of DCCI appeared to be about a 100-fold molar excess relative to DBP. We interpret the gradual decline in DBPF yield above this level as evidence for possible competing side reactions induced by excess DCCI which either inhibit the attack by HF or catalyze the rearrangement of intermediate complex I. Reactions 3 and 4 are reasonable examples of such competing processes. Product III ties up HF in an ionic salt-like complex, reducing its activity as a fluorinating agent. Reaction 4 is a suggested HF catalyzed rearrangement of intermediate I to a stable urea derivative (IV) and a quarternary salt (V). Somewhat analogous rearrangements have been observed between 2′- and 3′-nucleotides and DCCI[37,38]. It is reasonable that a stable quarternary salt such as V might not be as susceptible to nucleophilic attack by F^- as I, explaining how the yield of DBPF could fall off as observed.

Extending the pre-incubation of DCCI with DBP to 16 h before adding HF decreased the yield of DBPF to 86%. This also is evidence for a possible rearrangement process such as reaction 4. Interestingly, an even lesser yield of DBPF (49%) was achieved by premixing the HF and DCCI before adding DBP. This effect may

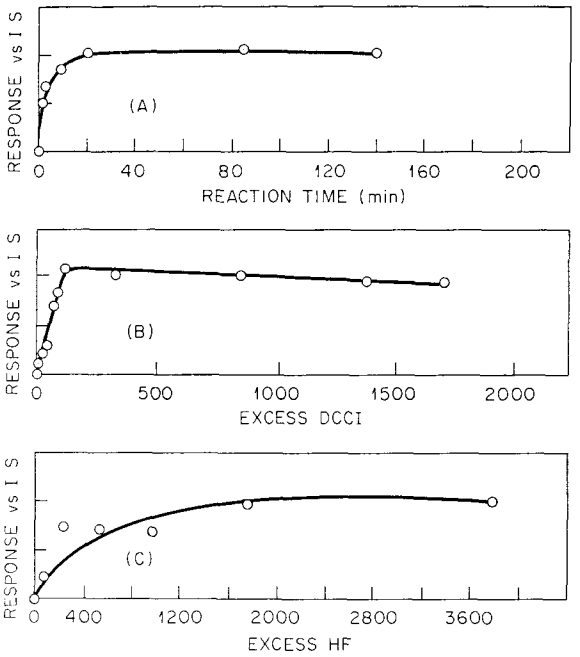


Fig. 1. Effects of (A), reaction time at room temperature; (B), concentration of DCCI and (C), concentration of HF on DBPF yield.

have resulted from the formation of a salt-like addition compound III via reaction 3, but it demonstrates dramatically why our reagent sequence method is a necessary protocol for maximizing DBPF yields.

The effect of HF on DBPF yield was more scattered at lower concentrations but became steady and optimal at about 1000-fold mol excess of substrate. Replacement of HF with AgF decreased the DBPF yield to about 25%; substituting solid NaF or KF for HF likewise in each trial gave poorer yields. A summary of the various methods and relative yields is found in Table I. It is clear that our sequential dehydroxy-fluorination method with DCCI–HF (see recommended procedure in Experimental) is by far the best reagent system tried.

A derivatization reaction must also be quantitative to be of value in an analytical method. Because no authenic DBPF standards were available commercially, we resorted to two independent, indirect procedures for evidence of the completeness of the sequential DCCI–HF dehydroxy-fluorination reaction. The first procedure consisted of a comparison of GC responses of DBPF and CH_3-DBP. Comparison of the GC peaks for DBPF produced from our sequential DCCI–HF system on DBP, and for CH_3-DBP from the reaction of CH_2N_2 on the same amount of DBP, showed a 47% greater GC response (peak area relative to TBP as the IS) for DBPF. Although this result suggests a significantly greater derivatization yield of DBPF *versus* CH_3-DBP, we note that it may also indicate a substantial enhancement of the AFID response to phosphorus compounds by F-substitution. It is reported[41] that halogens can enhance the AFID response to some phosphorus compounds; hence, it is not unreasonable to suppose that direct fluorine to phosphorus bonding

can explain most of the increased response of DBPF *versus* CH_3-DBP. The possibility that the TBP impurity (used as IS) might react with HF, as reported for triphenylphosphate[42], and thus be a second source of DBPF increasing its relative response is discounted because TBP is too inert and too dilute to account for the DBPF enhancement observed.

More convincing indirect evidence for the completeness of the sequential process was obtained by a post-fluorination derivatization of the DCCI–HF–DBP reaction solution. We found that subsequent treatment of this mixture with a gross excess of CH_2N_2 produced no detectable CH_3-DBP, proving conclusively that no free DBP was present. In a previous experiment using this technique, we did recover some CH_3-DBP from aged HF–THF–DBP reaction mixtures. This demonstrated that unreacted DBP could be derivatized and detected by this post-fluorination technique. Therefore, we are confident that the sequential DCCI–HF reaction is capable of quantitative conversion of DBP to DBPF when fresh reagents are employed.

The general GC properties of the fluorinated phosphate derivative in comparison to other derivatives are illustrated by the chromatogram shown in Fig. 2. The relative retention data in Table II indicate the relative volatilities of these species. Not only is the fluoride species much more volatile, but it also exhibits considerably less GC peak tailing than the other ester derivatives. We were pleasantly surprised to note that DBPF is even more volatile than dibutyl phosphite, an ester with phosphorus in the 3+ oxidation state. This greater volatility of DBPF, despite the greater molecular weight of F- *versus* H-substitution, is convincing evidence in support of our "polar hypothesis" proving that substitution of hydroxyl by a more electronegative group, such as fluorine, can reduce the net polar character of the phosphate moiety and give a better GC derivative. We interpret the greater volatility and sharper peak shape of DBPF over triesters to be due primarily to an inductive effect of the very electronegative fluorine substituent on the phosphorus atom. This induction forces an increased $p\pi$–$d\pi$ back-bonding from the phosphoryl oxygen and a general shifting of electron density from all oxygens toward the central phosphorus. This shift of electron density strengthens the phosphorus to oxygen bonds and decreases the magnitude of the P–O dipoles, especially that of the phosphoryl dipole. This general interpretation is corroborated by the infra-red spectral shifts of selected phosphorus compounds[43].

Previous experience[1] with the cleavage of di- and triphosphates by nitrogen-containing reagents, and the ability of HF to cleave condensed phosphates[42] strongly indicates that our DCCI–HF reagent combination will not be suitable for the derivatization and GC analysis of inorganic pyrophosphates and substituted condensed phosphates of biological interest; unfortunately, these species still remain inaccessible to quantitative GC analysis.

In conclusion, we have discovered that the sequential DCCI–HF reagent system reported here is particularly well suited for the dehydroxy-fluorination of phosphate diesters and that the substitution of hydroxyl by fluorine leads to advantageous changes in the GC properties of this stable and volatile phosphate derivative. By itself, this looks like a rather esoteric and very limited piece of scientific information until one realizes that there are many known commercial and military chemicals which are quite similar to the phosphate diester structure, namely, pesticides and nerve gases. Most known nerve gases are related to methylphosphonic acid, $CH_3P(O)(OH)_2$,

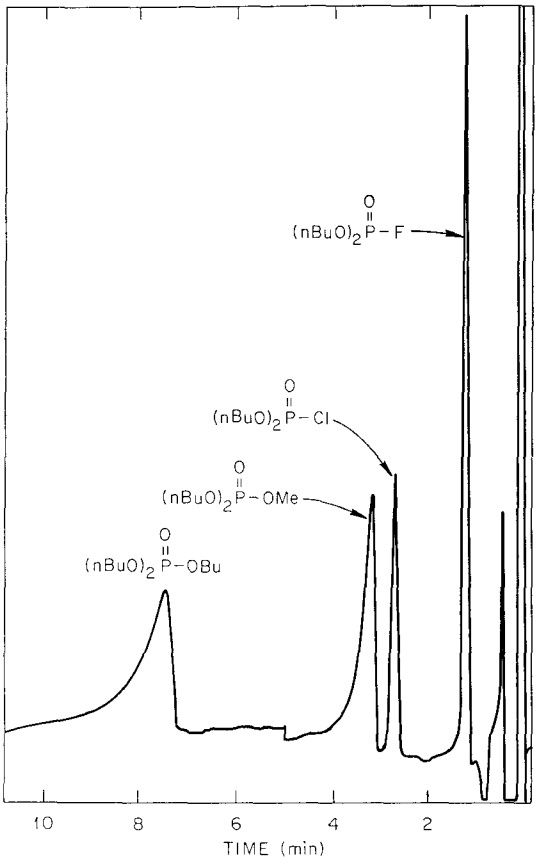


Fig. 2. GC resolution of DBP derivatives. (See Table II for RRT data). Bu = C_4H_9; Me = CH_3.

whereas many commercial organophosphorus pesticides are derivatives of phosphoric acid, $HOP(O)(OH)_2$ [44]. It is not surprising that such nerve gases and pesticides form a variety of hydroxylated substances on hydrolysis. Hydrolysates such as dialkylphosphates, methylphosphonic acid, and phosphoric acid are commonly converted into more volatile compounds for GC analysis by treatment with a diazoalkane in methanol or diethyl ether solution[12,13,17,45], or by co-injection with quaternary

TABLE II

RELATIVE RETENTION TIMES (RRT) FOR MODEL DERIVATIVES OF DBP

$(BuO)_2P(O)X$	RRT*
X = F	0.20
X = H	0.32
X = Cl	0.38
X = OCH_3	0.44
X = OTMS	0.46
X = OC_4H_9	1.00

* Normalized to retention time of TBP (8.0 min).

ammonium salts[15,16]. We believe our DCCI–HF procedure is a safer and more convenient method than these techniques and that it is applicable to a wide range of hydroxyphosphates at minimal sample sizes. Also, since fluoro-organic chemistry is making an increasing impact on the biomedical and health-related sciences, we suggest that this method be tried in any situation where dehydroxy-fluorination might be useful to synthesize fluoro-analogues of important biochemical agents[46]. However, once again we warn that certain fluoro compounds, especially of the fluorophosphorus type, can be deadly poisons[47]. Hence, extreme caution must be exercised in preparing them above the nmol level used in this work.

ACKNOWLEDGEMENTS

This work was supported in part by the Atomic Energy Commission (contract no. AT-(40-1)-2825), the National Science Foundation (grant GP-2671) and Vanderbilt University. W.H.G. is also grateful for fellowship support from the E. I. du Pont de Nemours & Co., the Eastman-Kodak Co., and Vanderbilt University as well as for educational assistance from the Veterans Administration. Lastly, we owe special thanks to our colleague, Professor R. E. Rummel, for the many ways he has assisted this research.

REFERENCES

1 W. H. Griest and T. W. Martin, *J. Chromatogr.*, 148 (1978) 405.
2 M. Zinbo and W. R. Sherman, *Tetrahedron Lett.*, (1969) 2811.
3 W. C. Butts and W. T. Rainey, Jr., *Anal. Chem.*, 43 (1971) 538.
4 D. R. Matthews, W. D. Shults, M. R. Guerin and J. A. Dean, *Anal. Chem.*, 43 (1971) 1582.
5 T. Hashizume and Y. Sasaki, *Anal. Biochem.*, 15 (1966) 199.
6 T. Hashizume and Y. Sasaki, *Anal. Biochem.*, 15 (1966) 346.
7 T. Hashizume and Y. Sasaki, *Anal. Biochem.*, 21 (1967) 316.
8 T. Hashizume and Y. Sasaki, *Anal. Biochem.*, 24 (1968) 232.
9 D. J. Harvey and M. G. Horning, *J. Chromatogr.*, 76 (1973) 51.
10 A. D. Hortin, *J. Chromatogr. Sci.*, 10 (1972) 125.
11 W. W. Wells, in H. S. Kroman and S. R. Bender (Editors), *Theory and Application of Gas Chromatography in Medicine and Industry*, Grune and Stratton, New York, 1968, pp. 169–181.
12 D. Blair and H. R. Roderick, *J. Agr. Food Chem.*, 24 (1976) 1221.
13 C. G. Daugherton, D. G. Crosby, R. L. Garnas and D. P. H. Hsieh, *J. Agr. Food Chem.*, 24 (1976) 236.
14 F. C. Wright, *J. Agr. Food Chem.*, 23 (1975) 820.
15 F. C. Churchill, D. N. Ku and J. W. Miles, *J. Agr. Food Chem.*, 26 (1978) 1108.
16 J. W. Miles and W. C. Dale, *J. Agr. Food Chem.*, 26 (1978) 480.
17 E. M. Loves and D. E. Bradway, *J. Agr. Food Chem.*, 25 (1977) 75.
18 A. I. Vogel, *A Textbook of Practical Organic Chemistry*, Wiley, New York, 1956, pp. 969–970.
19 H. Schlenk and J. L. Gellerman, *Anal. Chem.*, 32 (1960) 1412.
20 T. W. Mastin, G. R. Norman and E. A. Weilmuenster, *J. Amer. Chem. Soc.*, 67 (1945) 1662.
21 H. McCombie and B. C. Saunders, *Nature (London)*, 157 (1946) 287, 776.
22 W. R. Supina, *The Packed Column in Gas Chromatography*, Supelco, Bellefonte, Pa., 1974, pp. 91–94.
23 *Operating Note, Phosphorus Detector Model 15150-S*, Hewlett-Packard, Böblingen/Württemberg, G.F.R., 1970.
24 I. G. Young, *Amer. Lab.*, 7 (1975) 11.
25 R. F. Coward and P. Smith, *J. Chromatogr.*, 61 (1971) 329.
26 W. H. Griest, *A Study of the Derivatization and Analysis of Nucleotides by Gas–Liquid Chromatography, Ph.D. Thesis*, Vanderbilt University, Nashville, Tenn., 1975, pp. 109–121.

27 B. C. Saunders and G. J. Stacey, *J. Chem. Soc., London*, (1948) 695.
28 S. B. Hartley, W. S. Holmes, J. K. Jacques, M. F. Mole and J. C. McCoubrey, *Quart. Rev., Chem. Soc.*, 17 (1963) 204.
29 J. J. G. Cadogan, *J. Chem. Soc., London*, (1961) 3067.
30 A. Deutsch and O. Fernö, *Nature (London)*, 156 (1945) 604.
31 H. S. Aaron, R. T. Uyeda, H. F. Frack and J. I. Miller, *J. Amer. Chem. Soc.*, 84 (1962) 617.
32 F. S. Fawcett, C. W. Tullock and D. D. Coffman, *J. Amer. Chem. Soc.*, 84 (1962) 4275.
33 W. Lange and R. Livingston, *J. Amer. Chem. Soc.*, 69 (1947) 1073.
34 V. W. Lange, *Z. Anorg. Chem.*, 214 (1933) 44.
35 R. Whittman, *Angew. Chem., Int. Ed. Engl.*, 1 (1962) 213.
36 E. Cherbuliez, in G. M. Kosolapoff and L. Maier (Editors), *Organic Phosphorus Compounds*, Vol. 15, Wiley-Interscience, New York, 1973, p. 211.
37 C. A. Dekker and H. G. Khorana, *J. Amer. Chem. Soc.*, 76 (1954) 3522.
38 M. Smith, J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, 80 (1958) 6204.
39 H. G. Khorana, *Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest*, Wiley, New York, 1961, p. 128ff.
40 H. G. Khorana and A. R. Todd, *J. Chem. Soc., London*, (1953) 2257.
41 A. Karmen, *J. Chromatogr. Sci.*, 7 (1969) 541.
42 A. Hood and W. Lange, *J. Amer. Chem. Soc.*, 72 (1950) 4956.
43 D. E. C. Corbridge, in M. Grayson and E. J. Griffith (Editors), *Topics in Phosphorus Chemistry*, Vol. 6, Interscience, New York, 1969, pp. 260, 261, 337.
44 A. Verweij, H. L. Boter and C. E. A. M. Degenhardt, *Science*, 204 (1979) 616.
45 A. I. Vogel, *Practical Organic Chemistry*, Longmans Green, London, 3rd ed., 1970.
46 T. B. Patrick, *J. Chem. Educ.*, 56 (1979) 228.
47 H. E. Christensen, E. J. Fairchild, B. S. Carroll and R. J. Lewis (Editors), *Registry of Toxic Effects of Chemical Substances*, U.S. Department of Health, Education and Welfare, Rockville, Md., June 1976.