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DERIVATIZATION OF ALKYL HALIDES AND EPOXIDES WITH PICRIC ACID SALTS FOR IMPROVED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETECTION*

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SUMMARY

The use of picric acid (2,4,6-trinitrophenol) salts present in solution or on a solid support for use as labelling reagents for alkyl halides and epoxides is discussed. These derivatization reactions were performed off-line before the chromatographic separation. Labelled standards were prepared in solution, and characterized by melting point, IR, NMR, mass spectrometry and elemental analysis. These were then used as external standards to quantitate the percent derivatization under a variety of reaction conditions. The labelled derivatives can be monitored with a UV detector set at 220 nm, an electrochemical detector in the reductive mode, or with an electrochemical detector in the oxidative mode after post-column photolysis.

INTRODUCTION

In recent years, it has been recognized that environmental and food samples often are contaminated with mutagenic and carcinogenic substances. Alkyl halides, epoxides and other electrophiles are potential health hazards and have been found in a variety of food and environmental samples[1,2]. These compounds are very important industrial chemicals considering their many uses, the large amounts produced each year and the potential harm these chemicals can cause. Halogenated compounds are used extensively as solvents, aerosol propellents, degreasing agents, dry-cleaning agents, refrigerants, flame retardants, synthetic feed stocks, monomers in the production of textiles and plastics, and so on[2]. The ability to constantly monitor these chemicals is very important, and several analytical techniques to detect and/or confirm the presence of these chemicals are needed.

Gas chromatography (GC) using flame ionization detection (FID) or electron-

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capture detection (ECD) can be used for the analysis of epoxides and alkyl halides respectively. There has been little work done in the area of liquid chromatographic analyses of these compounds, because of their lack of a good chromophore or fluorophore. For those laboratories that lack a gas chromatograph, or those which need a method other than GC to confirm the presence of a suspected analyte, a liquid chromatographic method to analyze for these compounds is needed.

One liquid chromatographic analysis method for halogenated organics involved decomposition of the parent compound over a Pd catalyst or with sodium fumes followed by ion chromatographic analysis of the products[3,4]. Reports dealing with derivatization reactions of alkyl halides and epoxides to be used in conjunction with liquid chromatography have been limited to two. Both reports label (or tag) the electrophile with a chromophoric or fluorophoric nucleophile via substitution reactions. The first report used 4-nitrothiophenol (NTP) as the nucleophilic chromophore[5]. The authors report a limit of detection of 25 ppt* in a water sample after 40 000-fold preconcentration (which corresponds to a 1 ppm spike level). The second report used β-naphthol as a chromophore and fluorophore[6]. The alkyl halides were reacted by refluxing with naphthol in basic ethanol for 30 min. Primary iodinated and brominated compounds gave near quantitative yields using this procedure, but chlorinated compounds were found to be unreactive. The authors report that this method is applicable to concentration levels in the ppm range for UV detection, and in the ppb* range using fluorescence detection.

In this study, we have chosen picrate as the nucleophilic reagent to label alkyl halides and epoxides. The picryl ethers formed can be monitored using three different liquid chromatographic detectors. These detectors include a UV detector set at 220 nm, a reductive electrochemical detector, or an oxidative electrochemical detector after post-column photolysis[7–10]. In addition to its versatility as a labelling reagent, the reactivity of picrate and other anionic nucleophiles can be increased by complexing the counter ion in a crown ether or macrobicyclic cryptand[11–13]. Reactivity of these reactions can also be increased by intensifying the pull of the halogen away from the carbon being attacked. Mercury(II) and silver(I) are very electrophilic towards halides (except F^-), and have been shown to dramatically increase the reaction rate due to the anchimeric assistance of the heavy metal ions[14–16]. This work using picrate as the label is similar to, and has the same advantages as other labelling reactions using nitroaromatic reagents[17–22].

The picrate labelling reactions can be carried out either in solution or on a solid support. Today, almost all of the routine derivatizations performed for high-performance liquid chromatography (HPLC) involve the use of homogeneous reactions, in which the reagent is present in solution, and is mixed with a solution of the substrate of interest[23–35]. The synthetic organic literature is replete with examples of the use of supported and polymeric reagents for a variety of reasons, but until recently, there has been only a small amount of work involving the use of solid phase reagents for derivatizations to be used in conjunction with HPLC[36–46].

There are several reported approaches utilizing polymeric reagents for derivatizing electrophiles in S_N2 type reactions, but these have never, to our knowledge,

* Throughout this article the American trillion (10^{12}) and billion (10^9) are meant.

been used in conjunction with HPLC[47–55]. Solid phase reagents (SPRs) may be used either on-line or off-line. On-line reactions are more convenient, but, often the best solvent for the derivatization is not compatible with the mobile phase needed for the separation, the supported reagent is not stable in the mobile phase, or long reaction times are required. In situations such as these, it is necessary to perform the reaction off-line. Off-line reactions can be performed either in solution or on a solid support, but there are several advantages that might be achieved using supported reagents including: (1) reagents can become more stable after immobilization on a support; (2) derivatizations can be more convenient, reagent solutions of known concentration do not need to be made each time; it is possible to perform derivatizations on the support in reaction vials; (3) reactions not possible in solution because of lack of solubility of one or more of the reagents can be carried out in high effective reagent concentrations on a support; (4) reagents that are dangerous (toxic or explosive) can be diluted with the support; and (5) reactions on solid supports often are more selective and give fewer side products.

EXPERIMENTAL

Chemicals

HPLC solvents were obtained from Waters Chromatography Division, Millipore (Milford, MA, U.S.A.) as their HPLC grade, distilled in glass, or from MCB Manufacturing Chemists (Gibbstown, NJ, U.S.A.) as their Omnisolv brand HPLC solvent. All solvents were used as received after filtering through a 0.45-μm solvent filter and degassing with stirring under vacuum.

The chemicals used throughout this study were obtained from a variety of commercial suppliers including: Aldrich (Milwaukee, WI, U.S.A.), Chem. Service (West Chester, PA, U.S.A.), J. T. Baker (Phillipsburg, NJ, U.S.A.), Fisher Scientific (Fair Lawn, NJ, U.S.A.), MCB Manufacturing Chemists (Gibbstown, NJ, U.S.A.) and Fluka (Hauppauge, NY, U.S.A.). These chemicals generally were of the highest purity available when necessary, and were used as received without further purification.

Apparatus

The HPLC system used for these studies consisted of a Waters Model 6000A solvent delivery system, a Waters Model U6K syringe loading injection valve, and a Linear dual pen recorder. The detectors used were a Waters Model 480 variable-wavelength UV detector, a Bioanalytical Systems (West Lafayette, IN, U.S.A.) Model LC-4A, or Model LC-4B amperometric electrochemical detector, a glassy carbon working electrode, an Ag/AgCl reference electrode, and a stainless-steel counter electrode. At times the BAS electrochemical detector was used in conjunction with a post-column photolysis unit. This instrumental arrangement has been discussed elsewhere[7–10]. Chromatographic columns used were a μBondapak CN reversed-phase column, 30 × 0.39 cm I.D., and 30 × 0.78 cm I.D. (semipreparative). At times an Alltech 600 CN reversed-phase column, 25 × 0.46 cm, was used (Alltech, Deerfield, IL, U.S.A.). In some applications, a Rheodyne (Cotati, CA, U.S.A.) Model 7030 switching valve was used. The instrumentation used to characterize the labelled picryl ethers consisted of a Varian T-60 NMR spectrometer (Varian, Palo Alto, CA,

U.S.A.), a Perkin Elmer 599B infrared spectrophotometer (Perkin Elmer, Norwalk, CT, U.S.A.), a Nuclide magnetic sector mass spectrometer (Nuclide, State College, PA, U.S.A.), a Thomas Hoover capillary melting point apparatus (Arthur H. Thomas, Philadelphia, PA, U.S.A.), and a Bausch and Lomb Spectronic 600 UV/Vis spectrophotometer (Bausch and Lomb, Rochester, NY, U.S.A.).

Preparation, isolation and characterization of labelled picryl ethers

Silver picrate. Silver picrate was used to react with the various substrates to form the picryl ethers. This was prepared by adding roughly equal molar amounts of either silver nitrate or silver acetate with picric acid in water and removing the acid (nitric or acetic) and the water by rotoevaporation. Picric acid, when completely dry is explosive, and therefore it is stored with approximately 10% water in a tightly sealed bottle. The exact amount of water is not known, therefore, the amount of picric acid was approximated in the initial preparations of silver picrate. Later, a solution of picric acid was made in a volumetric flask which was then titrated with a standardized sodium hydroxide solution. An exact equivalent of silver acetate could then be added to the picric acid solution. The dry silver picrate was then dried at room temperature in a drying pistol over phosphorous pentoxide.

The initial work of this study used silver picrate prepared from silver nitrate. The nitrate should be removed as nitric acid in the synthesis. An ion chromatographic analysis of the silver picrate using a previously reported method[56], showed that the majority of the nitrate was still present, and therefore, the silver ion may be complexed with nitrate instead of picrate. The silver picrate prepared from silver nitrate was used to prepare the labelled ether compounds later to be used as external standards. It also was used to determine the best solvent for the derivatization, and to show that the percentage of derivatization was independent of substrate concentration. Silver picrate prepared from silver acetate was found to be more reactive and was used for all other applications.

Warning. Although we have not experienced any problems concerning the stability of these reagents, it is possible that an explosion may occur when using these reagents and proper eye and body protection should be worn at all times!

Labelled picryl ethers. Allyl picryl ether, pentyl picryl ether, octyl picryl ether and 1-hydroxy-2-picryl cyclohexane are the derivatives formed by reaction of silver picrate with allyl halides, pentyl halides, octyl halides, and cyclohexene oxide, respectively. These were prepared by heating, in acetonitrile, equal molar amounts of silver picrate and the iodinated halide, or the cyclohexene oxide. Since the halogenated compounds all give the same derivative, the iodinated compounds were used because they react faster than their brominated or chlorinated analogs. Allyl iodide and cyclohexene oxide were reacted at 70°C for 30 min; pentyl and octyl iodide were reacted at 70°C for 3 h. The post reaction mixtures were filtered and subjected to semi-preparative HPLC using a μBondapak CN column, 30 cm × 7.8 mm I.D. The mobile phase was water–acetonitrile (65:35), for the allyl and cyclohexene oxide derivatives, and water–acetonitrile (55:45) for the pentyl and octyl derivatives. The flow-rate in all cases was 4.5 ml/min. The picryl ethers were collected and rotoevaporated to dryness leaving the pure product. Fig. 1 shows the underivatized substrates and their corresponding labelled picryl ethers.

The physical and spectral characteristics of the labelled derivatives are summa-

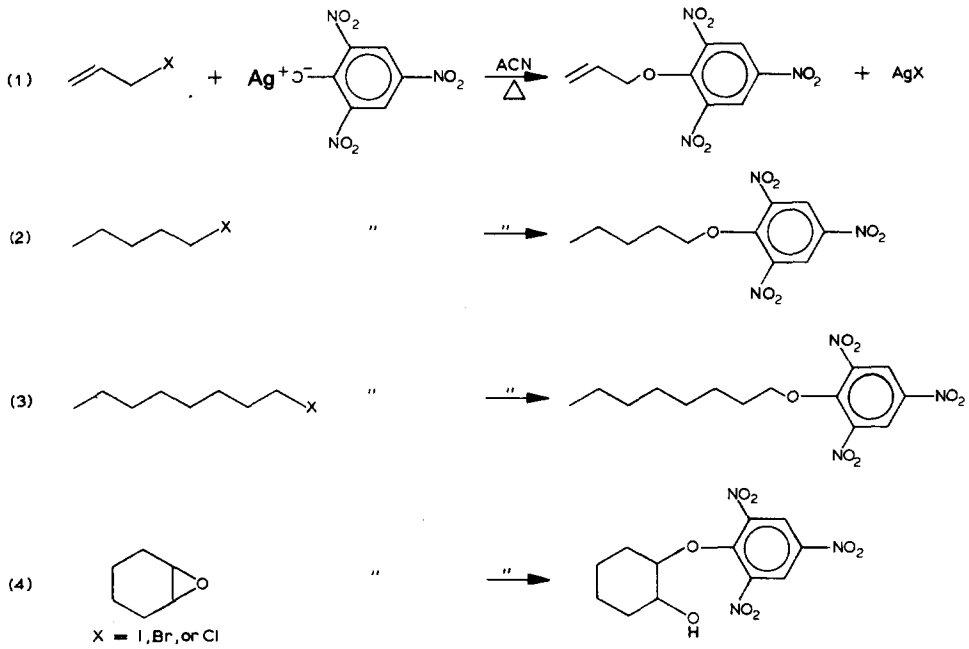

Fig. 1. Derivatization reactions of silver picrate with allyl halides (1), pentyl halides (2), octyl halides (3) and cyclohexene oxide (4) showing the substrates and products derived through the labelling reactions. The labelled products are allyl picryl ether, pentyl picryl ether, octyl picryl ether, and 1-hydroxy-2-picryl-cyclohexane, respectively.

rized in Tables I and II. For all of the compounds, except 1-hydroxy-2-picryl-cyclohexane, nuclear magnetic resonance spectroscopy (NMR) was the most useful technique to confirm the chemical identity of the derivatives. Poor solubility of the cyclohexene oxide derivative in a variety of NMR solvents prevented us from obtaining a useful spectrum, but in this case, the infrared spectrum (IR) was particularly

TABLE I

NMR DATA OF LABELLED PICRYL ETHERS AND THEIR IODINATED STARTING SUBSTRATES

δ (ppm)			
Picric acid[a]	*Allyl picryl ether*[a]	*Pentyl picryl ether*[b]	*Octyl picryl ether*[b]
9.1 (2,s)[c]	4.9 (2,d)[e]	4.3 (2,t)[h]	4.2 (2,t)[h]
9.3 (1,s)[d]	5.4 (2,t)[f]	0.6-2.2 (9.6[i],m)[j]	0.8-2.2 (15,m)[k]
	6.0 (1,m)[g]	8.7 (2,s)[c]	8.7 (2,s)[c]
	9.1 (2,s)[c]		
	Allyl iodide[a]	*1-iodopentane*[b]	*1-iodooctane*[b]
	3.9 (2,d)[e]	3.2 (2,t)[h]	3.3 (2,t)[h]
	5.0 (2,t)[f]	0.6-2.2 (9,m)[j]	0.8-2.2 (15,m)[k]
	5.9 (1,m)[g]		

[a] Solvent: [$^2H_6$]acetone; [b] solvent: C^2HCl_3; [c] aromatic; [d] phenolic; [e] aliphatic; [f] terminal olefinic; [g] olefinic; [h] C_1; [i] the integration ratio should be 9 instead of 9.6. This is due to a solvent impurity; [j] C_2–C_5; [k] C_2–C_8.

TABLE II

PHYSICAL AND SPECTRAL CHARACTERISTICS OF LABELLED PICRYL ETHERS

	Allyl picryl ether	*1-Hydroxy-2-picryl-cyclohexane*	*Pentyl picryl ether*	*Octyl picryl ether*
Melting point	79.5–80.0°C	84.5–85.0°C	Liquid at 25°C	Liquid at 25°C
Elemental analysis*	C: 39.22 (40.16) H: 2.83 (2.62) N: 15.83 (15.61) O: 41.78 (41.61)	C: 43.01 (43.04) H: 4.09 (4.00) N: 12.31 (12.84) O: 38.19 (39.11)	C: 43.93 (44.15) H: 4.39 (4.38) N: 14.27 (14.04) O:*37.41 (37.43)	C: 49.46 (49.27) H: 5.27 (5.61) N: 12.45 (12.31) O: 32.17 (32.81)
HPLC retention time**	4 min 34 sec	4 min 25 sec	4 min 51 sec	5 min 10 sec
Mass spectrum***	*m/z* 243 (M−26) *m/z* 213 (M−56) *m/z* 196 (M−73)	*m/z* 327 (M)	NT§	NT§
Infrared spectrum (cm^{-1})§§	3100 s 1350 m 2970 w 1250 m 2880 w 1200 w 1835 w 1090 m 1610 s 980 w 1545 s 950 s 1460 m 1420 m	3530 s 1450 w 3100 w 1350 s 2930 m 1265 w 2860 w 1080 w 2340 w 980 m 1600 s 920 w 1545 w 720 w 1530 s	3100 w 1265 s 2960 w 1090 w 2925 w 960 w 2860 w 920 w 2340 w 720 w 1605 m 1545 s 1350 s	3100 w 1265 w 2960 w 1090 w 2925 w 960 w 2860 w 920 w 2340 w 720 w 1605 m 1545 s 1350 s

* Elemental analysis was performed at Galbraith Laboratories, Knoxville, TN, U.S.A. The numbers in parentheses represent the theoretical value. * = Obtained by difference.

** Alltech 600 CN column, 25 × 0.46 cm; mobile phase, water–acetonitrile; (50:50); flow-rate, 1 ml/min.

*** Direct probe; electron impact (70 eV); source = 250°C, $7 \cdot 10^{-10}$ Torr.

§ Not taken. See text for details.

§§ KBr pellet; s = strong; m = moderate; w = weak.

useful. Epoxides show infrared absorbances near 1250 cm^{-1} and between 950 and 810 cm^{-1}. These bands are also present in the IR of picric acid, therefore it is not possible to show the absence of these bands in the ether derivative. What the IR does show is an alcoholic function in the ether which does not appear in the IR spectrum of cyclohexene oxide, which indicates that the ring has opened. The IR of cyclohexene oxide was taken neat between salt plates.

The mass spectrum of allyl picryl ether did not give a molecular ion, although the fragments are consistent with the expected product. The mass spectrum of 1-hydroxy-2-picryl-cyclohexane gave a molecular ion and a variety of other peaks that appeared in the mass spectrum of picric acid. The mass spectrum of both pentyl picryl ether and octyl picryl ether were of no use, because the solvent used to transfer the sample to the probe (acetone), probably polymerized the sample and many high-molecular-weight ions were found. Because NMR was very useful, no attempt to re-run the mass spectrum of these samples was made. The infrared spectra of all of the derivatives were consistent with a picric acid derivative, there are many similar absorbances in all of the spectra.

Preparation of the supported picrate reagents

Silica supported silver picrate, sodium picrate, and 18-crown-6-complexed potassium picrate. The silica gel used for all of these supported reagents was Waters normal-phase liquid chromatography packing material, 37–55 μm. The silica was refluxed in 2 *M* HCl for 2 h, washed with water and methanol, and dried in a drying pistol at 110°C (reflex solvent, toluene) over phosphorous pentoxide for 12 h. Enough silver picrate to prepare a 10% (w/w) loading was added (in acetonitrile) to the silica, and the solvent was removed by rotoevaporation. The resulting solid supported reagent was dried in a drying pistol at 61°C (reflux solvent, chloroform) for 12 h. The sodium picrate was prepared by mixing equal molar amounts of sodium hydroxide and picric acid in water and then removing the water. The crown-ether-complexed potassium picrate was prepared by first mixing equal molar amounts of potassium hydroxide and picric acid in water and then, after removing the water, the potassium picrate was mixed with an equal molar amount of 18-crown-6 in acetonitrile, stirred for 30 min, and the solvent was removed by rotoevaporation. These picrate salts were then adsorbed onto silica as described above. *Warning.* These reagents may be explosive and proper eye and body protection should be worn!

Reaction procedure

For reactions using a solid support, the solvent containing the substrate is added to the supported reagent contained in a reaction vial. After the substrate is added, there should be some dry reagent still present (all of the liquid should be absorbed by the reaction bed). The vials were heated by a water bath at a specific temperature for a specific time. The post reaction mixture was then washed through a filter into a volumetric flask with the mobile phase to be used for the HPLC analysis, and injected onto the HPLC for quantitation of the derivatization.

Determination of minimum detection limits

In this report, all minimum detection limits (MDLs) were normalized to a signal-to-noise ratio of 2.5 with the exception of MDLs reported of the picryl ethers using the reductive electrochemical detector. The MDLs using this system were limited primarily by the mobile phase composition required to adequately separate the ether from the oxygen peak present in the samples injected. All samples were deoxygenated by bubbling nitrogen (saturated with mobile phase) through the solutions, but even with pre-treatment, an oxygen peak often was present, especially at high sensitivity settings on the detector. The mobile phase was designed to elute the ether shortly after the oxygen peak. In all cases, the signal-to-noise ratio using the reductive system was higher than 2.5. The MDLs of the labelled picryl ethers are listed in Table III.

RESULTS AND DISCUSSION

Solvent optimization

One of the initial experiments was to determine which solvent would be best for this type of derivatization. Solvents tested included triethylamine, pyridine, acetonitrile, methanol, hexane and ethyl acetate. The first two solvents were immediately eliminated because a solution of allyl iodide in triethylamine formed a precipitate

TABLE III

DETECTION LIMITS (ppb) OF THE LABELLED PICRYL ETHERS FOR 200-μl INJECTIONS

Ether	*Reductive electrochemical detection**	*Oxidative electrochemical detection after photolysis***	*UV detection at 220 nm****
1-Hydroxy-2-picryl-cyclohexane	5.8	3.3	62
Allyl picryl ether	4.1	6.0	26
Pentyl picryl ether	3.3	3.5	44
Octyl picryl ether	16	6.1	62

* All MDLs were normalized to a 30 × 0.39 cm Waters μBondapak CN column. Flow-rate, 1.5 ml/min. The allyl and cyclohexyl ether MDLs were determined using a mobile phase of water containing 0.2 *M* sodium chloride–methanol (79:21); applied potential, −0.9 V *vs.* Ag/AgCl. The pentyl ether MDL was determined using a mobile phase of water containing 0.2 *M* sodium chloride–methanol (60:40); applied potential, −0.9 V *vs.* Ag/AgCl. The octyl ether MDL was determined using a mobile phase of water containing 0.2 *M* sodium chloride–methanol (50:50); applied potential, −0.5 V *vs.* Ag/AgCl. Working electrode, glassy carbon.

** All MDLs are normalized to a 30 × 0.39 cm Waters μBondapak CN column. Flow-rate, 1.4 ml/min; mobile phase, water containing 0.2 *M* sodium chloride–methanol (50:50); applied potential, 0.9 V *vs.* Ag/AgCl; working electrode, glassy carbon.

*** All MDLs were determined using a 30 × 0.39 cm Waters μBondapak CN column. UV detection at 220 nm. Flow-rate, 1.6 ml/min; mobile phase, water–acetonitrile (55:45).

before addition of the reagent, and pyridine had a very large peak that obscured the derivative peak when a UV detector was used. Of the remaining solvents, acetonitrile showed the best reactivity, followed by hexane, methanol and ethyl acetate. Here, and throughout this paper, the percent reaction will be referred to as the percentage of the original substrate converted to the labelled derivative, not the percentage of the original compound that is gone at the completion of the reaction time, because this would also include the competing (and undesirable) elimination reactions. One point to note from this solvent study is that the reaction using a supported reagent can be carried out in hexane. A solution reaction could not be carried out in this solvent because of the limited solubility of the reagents.

Temperature and time optimization

It was generally found that as both temperature and time increased, the percent reaction increased. An outer limit of 2 h and 90°C was set, and all of the compounds studied were reacted under these conditions using the silica supported silver picrate (prepared from silver acetate) as the SPR. The volume of sample derivatized was 100 μl. The data are summarized in Table IV. Each data point represents an average of three different reactions, each reaction mixture was injected three times, for a total of nine measurements per point. For all substrates listed in Table IV, except the tertiary halides, an external standard of the labelled derivative was used to determine the percent derivatization. For the tertiary halides, 1-hydroxy-2-picryl-cyclohexane was used as the external standard because (1) it has similar chromatographic properties as the derivative peak formed from the reaction of the tertiary halides and (2) the labelled derivatives only have the picrate chromophore in their structure and

TABLE IV

PERCENT REACTION FOR DIFFERENT SUBSTRATES

Reaction conditions: all substrate solutions were made equal molar to 1000 ppm allyl iodide. The reactions were carried out using a 10% (w/w) silica supported silver picrate loading as the reagent; reaction time, 120 min; temperature, 90°C.

Substrate	*Percent reaction (S.D.)*
Allyl iodide	16 (2.8)
Allyl bromide	51 (2.2)
Allyl chloride	2.6 (0.2)
1-Iodopentane	88 (1.6)
1-Bromopentane	27 (2.9)
1-Chloropentane	0.14 (0.014)
1-Iodooctane	71 (6.1)
1-Bromooctane	13 (0.55)
1-Chlorooctane	0.18 (0.013)
2-Iodo-2-methylpropane	2.3 *
2-Bromo-2-methylpropane	0.90 *
2-Chloro-2-methylpropane	0.30 *
Cyclohexene oxide	44 (1.1)
2-Cyclohexen-1-one	0 (0)

* A standard deviation (S.D.) is not reported because the reaction was only performed once.

therefore should have similar molar absorptivities. A standard deviation is not reported for the tertiary halide reactions because they were only performed once.

The data reported in Table IV are consistent with an S_N2 type mechanism. Considering the leaving groups, the expected reactivity of I > Br > Cl was found here with the exception of the allyl compounds. In this case, the lower reactivity of the iodinated compound we feel is due to the competing elimination reaction. The higher reactivity of the less substituted substrates is also consistent with an S_N2 mechanism.

Detectability as a result of derivatization

The reasons these derivatizations were performed was to increase the selectivity and sensitivity of these electrophiles. The previous papers discussing reactions of these electrophiles used in conjunction with HPLC only gave detection data concerning the labelled derivatives, not the starting compounds[5,6]. Most of the electrophiles derivatized here, however, show absorbance at 220 nm, and some can be detected using the electrochemical detectors. Table V lists the MDLs of the underivatized starting compounds. The detection limits using the UV detector were calculated from a knowledge of the molar peak height response ratios of the derivatized and underivatized compounds (Table VI), and the experimentally determined MDLs of the labelled derivatives (Table III). Table VII lists the MDLs of the starting compounds after derivatization with silica supported silver picrate for 120 min at 90°C.

The usefulness of this derivatization approach for each substrate can be seen by comparison of Tables V and VII. Cyclohexene oxide has the best improvement in detectability by being totally undetectable by all three of the detectors without derivatization, but after derivatization, ppb levels can be detected with all three detectors. For 1-bromopentane, although detectable underivatized by a UV detector at

TABLE V

DETECTION LIMITS (ppb) OF THE UNDERIVATIZED STARTING COMPOUNDS FOR 200-μl INJECTIONS

ND = not detected.

Starting compound	*Reductive electrochemical detection**	*Oxidative electrochemical detection after photolysis***	*UV detection at 220 nm****
Allyl iodide	2100	1.40	42.0
Allyl bromide	ND	ND	127
1-Iodopentane	ND	4.10	629
1-Bromopentane	ND	ND	373
1-Iodooctane	ND	10.3	1170
1-Bromooctane	ND	ND	652
Cyclohexene oxide	ND	ND	ND

* MDL determined using an applied potential of −0.9 V *vs.* Ag/AgCl. All other HPLC conditions are identical to those in the second foot note.

** MDLs experimentally determined using a post-column photolysis weave, a mobile phase of water containing 0.2 *M* sodium chloride–methanol (50:50), a μBondapak CN 30 × 0.39 cm column, a flow-rate of 1.4 ml/min, an applied potential of 0.9 V *vs.* Ag/AgCl, and a glassy carbon working electrode.

*** These are calculated from the knowledge of the detection limits of their labelled derivatives, and the relative molar response factors (Table VI) of the starting compound and the labelled derivatives.

220 nm, a 60-fold improvement in detection limit was achieved through derivatization when the reductive electrochemical detector was used. The iodinated compounds are interesting in that even without derivatization, the photolysis liquid chromatography–electrochemical detector has ppb MDLs, and because of this, we

TABLE VI

HPLC-UV (220 nm) MOLAR ABSORBANCE RATIOS

This table indicates the relative response (peak height) of equal molar solutions of the starting substrates and their labelled derivative under the same HPLC conditions. μBondapak CN 30 × 0.39 cm column; mobile phase, water–acetonitrile (55:45); flow-rate, 2.0 ml/min; 25-μl injections.

Allyl picryl ether	1.000
Allyl iodide	0.612
Allyl bromide	0.204
Allyl chloride	0.108
Pentyl picryl ether	1.000
1-Iodopentane	0.070
1-Bromopentane	0.118
1-Chloropentane	0.001
Octyl picryl ether	1.000
1-Iodooctane	0.053
1-Bromooctane	0.095
1-Chlorooctane	≈0
1-Hydroxy-2-picryl-cyclohexane	1.000
Cyclohexene oxide	≈0

TABLE VII

DETECTION LIMITS (ppb) OF THE STARTING SUBSTRATES AFTER DERIVATIZATION FOR 200-μl INJECTIONS

MDLs after derivatization were calculated assuming (1) the same chromatographic conditions used to determine the MDLs of the labelled ethers listed in Table III; (2) reaction conditions of 90°C, 2 h; and (3) 200-μl injections.

Starting substrate	*Reductive electrochemical detection*	*Oxidative electrochemical detection after photolysis*	*UV detection at 220 nm*
Allyl iodide	16	23	100
Allyl bromide	3.6	5.3	23
1-Iodopentane	2.5	2.6	33
1-Bromopentane	6.2	6.5	82
1-Iodooctane	16	6.0	61
1-Bromooctane	69	26	269
Cyclohexene oxide	3.9	2.2	42

are further investigating the use of this detector for other iodinated compounds. If, however, one does not have this detector available, considerable improvements in MDLs are achieved through derivatization using UV or reductive electrochemical detectors. In all derivatization reactions, the HPLC analysis becomes more specific than without derivatization. Compounds can be identified not only by their retention time, but also by the formation of a new derivative peak from a specific organic reaction. Compounds, such as allyl iodide, that already show UV absorbance can be confirmed through specific derivatization reactions such as these.

To illustrate the increase in detectability that occurs following derivatization, a 200-μl solution of 1-iodopentane near its underivatized UV detection limit was derivatized under the conditions listed in Table IV. The post-reaction mixture was monitored using a UV detector. Fig. 2 shows the 1.2-ppm solution before derivatization; Fig. 3 shows the liquid chromatography–UV post-reaction solution. The detector setting in Fig. 3 is ten times less sensitive than in Fig. 2. After the derivatization, the excess picric acid is eluted first, followed by the labelled derivative. When working at low levels and high sensitivity settings, the large reagent front may show tailing in the area where the labelled derivative is eluted. This is undesirable and removal of the excess reagent is needed. This can be done by either gradient elution or through the use of pre-columns and switching valves. The switching valve arrangement used to obtain the chromatogram shown in Fig. 3 is illustrated in Fig. 4.

The operation of the switching valve system is as follows: in the initial position, the mobile phase is water which flows through a tee, a pre-column, and to waste. The pre-column consisted of μBondapak CN packing which was slurry packed into a 6 × 0.4 cm column. Under these conditions, an injection of the post-reaction mixture washed the silver picrate to waste, while the labelled ether was adsorbed onto the head of the pre-column. In this initial position, a plug after the detector prevented any flow onto the analytical column. After a suitable washing time with water (8 min at 2 ml/min), the solvent select valve on the pump was switched to solvent 2 [water–acetonitrile (55:45)], the detector plug was opened, and the switching valve

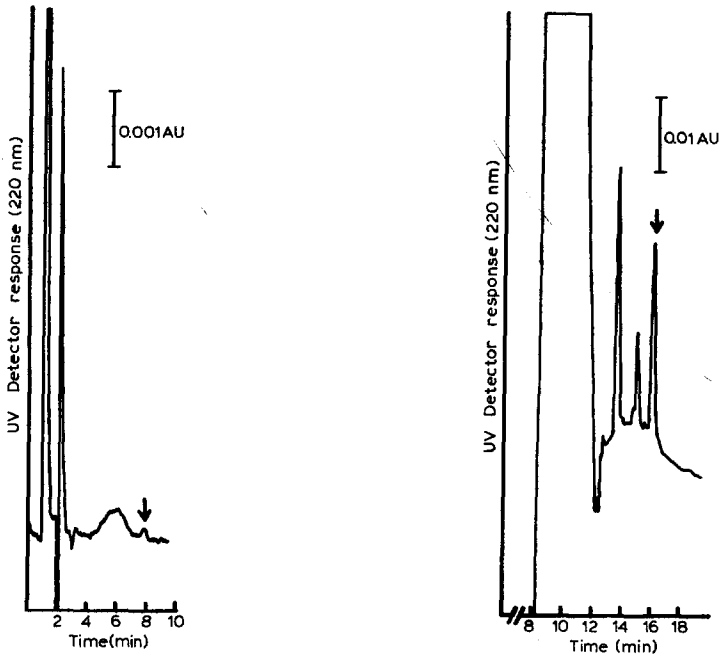

Fig. 2. Chromatogram of 1.2 ppm 1-iodopentane (arrow) without derivatization (injection volume, 200 μl). μBondapak CN column, 30 × 0.39 cm; mobile phase, water–acetonitrile (55:45); flow-rate, 1.6 ml/min.

Fig. 3. Chromatogram of 1.2 ppm 1-iodopentane after derivatization. The arrow shows the derivative (pentyl picryl ether). 1-Iodopentane solution (200 μl) was reacted at 90°C for 120 min with silica-supported silver picrate. The post-reaction mixture was analyzed using the instrumentation shown in Fig. 4.

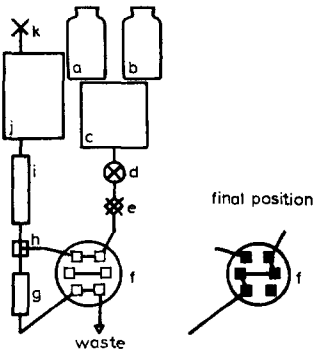

Fig. 4. Instrumental arrangement used to obtain the chromatogram shown in Fig. 3. a = Solvent 1 (water), b = solvent 2 [water–acetonitrile (55:45)], c = solvent delivery system, d = injection valve, e = pre-column filter, f = switching valve (shown in the initial and final position), g = μBondapak CN pre-column, h = SSI 01-01-65 tee, i = μBondapak CN analytical column, j = UV detector, k = plug. See text for details.

was changed to its final position. This backflushed the pre-column and washed the labelled derivative onto the analytical column.

Comparison of solution and solid phase reactions

All of the reactions described in this paper thus far were performed using picric acid salts on solid supports such as silica. All of these reactions can also be done in solution. We chose to study 1-iodopentane as a test substrate under solution and solid phase conditions. A 100-μl volume of a 1179-ppm 1-iodopentane solution in acetonitrile was injected into a reaction vial containing either silica supported silver picrate (10%, w/w), a C_2 bonded phase supported silver picrate (10%, w/w), or pure silver picrate. The reaction vial was heated in a water bath at 70°C for various times. The results are shown graphically in Fig. 5.

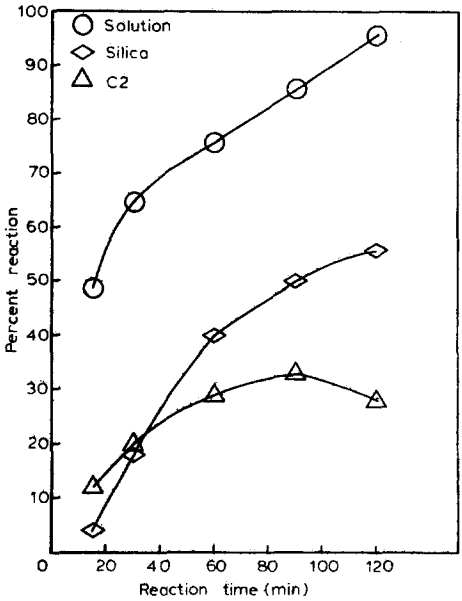

Fig. 5. Percent reaction of 1-iodopentane as a function of time and picrate reagent. See text for details.

The highest percent derivatization was achieved in solution followed by the silica supported reagent, and the C_2 bonded phase supported reagent. The lower reactivity of the silica supported reagent might have been due to the active silica surface, and that is why the C_2 bonded phase supported reagent was used. We have tried other supports such as neutral alumina under a variety of reaction conditions, but this was found to be even less active than the C_2 bonded phase supported reagent. The exact role the support plays in these derivatizations is unclear at this point in time, and is currently under further investigation in our laboratory. Using solution or supported reagents, significant improvements in UV detectability are achieved. The choice of reagent is up to the individual. Under most circumstances, the convenience and safety benefits of the supported reagent will outweigh the increase in percent reaction of the solution reagent. When lower levels of the substrates are encountered, the solution reaction may be most useful.

Percent reaction as a function of substrate concentration

It is desirable that the percent reaction is independent of the concentration of the substrate. This was found to be true in previous on-line solid phase reductions of carbonyl compounds[37] and also was found in this study. Cyclohexene oxide was used as the test substrate and reacted at three different concentration levels as shown below:

Concentration	*Percent reaction (S.D.)*
584 ppm	6.5 (0.5)
58.4 ppm	6.3 (0.5)
5.84 ppm	6.6 (0.5)

The reagent was silica supported silver picrate (10%, w/w); cyclohexene oxide solutions were made in acetonitrile; time, 15 min; temperature, 50°C.

Effect of picrate's counter ion on reactivity

Several picrate salts were synthesized for this study including silver picrate, sodium picrate, and 18-crown-6-complexed potassium picrate. To determine the counterion effect, cyclohexene oxide and 1-iodopentane were chosen as test substrates. The results are tabulated in Table VIII. The dramatic effect of the silver ion is seen for 1-iodopentane. The higher reactivity of the crown-complexed picrate with the alkyl halide is consistent with naked anions being more reactive in these types of reactions. Cyclohexene oxide does not have a halide as a leaving group, and therefore its reactivity should be a reflection of the nucleophilicity of the entering group and how free the nucleophile is to attack. The large silver ion and the potassium–crown counter ions have approximately the same reactivity towards the epoxide; the smaller sodium ion is more tightly held to the attacking anionic nucleophile and is less reactive.

TABLE VIII

PERCENT REACTION *vs.* PICRATE'S COUNTER ION

Solutions equal molar to 1000 ppm allyl iodide were made for both substrates in acetonitrile. Each picrate salt–silica gel reagent was made to have the same number of moles picrate salt per gram silica gel as a 10% (w/w) loading of silver picrate on silica gel. Reaction conditions were 70°C for 30 min; 100 μl of substrate solution; quantitation by external standard (as before).

Counter ion	*Percent reaction (S.D.)*	
	Cyclohexene oxide	*1-Iodopentane*
Crown K^+	51 (0.96)	0.021 (0.0031)
Ag^+	47 (1.6)	18 (0.45)
Na^+	19 (0.31)	≈0 (0)

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REFERENCES

1 A. M. Cheh, J. Skochdopole, C. Heillig, P. M. Koskii and L. Cole, in R. L. Jolly, W. E. Brungs and R. B. Cumming (Editors), *Water Chlorination: Environmental Impact and Health Effects*, Ann Arbor Science, Ann Arbor, 1980, pp. 803–815.
2 L. Fishbein, in M. C. Bowman (Editor), *Handbook of Carcinogens and Hazardous Substances*, Marcel Dekker, New York, 1982, Ch. 10.
3 T. Okamoto and Y. Shirane, *Hiroshima-ken Kankyo Senta Kenkyu Hokoku*, 5 (1983) 39.
4 C.-Y. Wang and J. G. Tarter, *Anal. Chem.*, 55 (1983) 1175.
5 A. M. Cheh and R. E. Carlson, *Anal. Chem.*, 53 (1981) 1001.
6 K. W. Street and V. Hocson, *Anal. Lett.*, 16 (A17&18) (1983) 1403.
7 X.-D. Ding and I. S. Krull, *J. Agric. Food Chem.*, 32 (1984) 622.
8 C. M. Selavka, R. J. Nelson, I. S. Krull and K. Bratin, *J. Pharm. Biomed. Anal.*, (1985) in press.
9 I. S. Krull, X.-D. Ding, C. M. Selavka, K. Bratin and G. Forcier, *J. Forensic Sci.*, 29 (1982) 449.
10 I. S. Krull, X.-D. Ding, C. M. Selavka and R. J. Nelson, *LC, Liq. Chromatogr., HPLC Mag.*, 2 (1984) 214.
11 S. A. DiBiase and G. W. Gokel, *J. Org. Chem.*, 43 (1978) 447.
12 J. J. Christensen, D. J. Eatough and R. M. Izatt, *Chem. Rev.*, 74 (1974) 351.
13 L. F. Lindoy, *Chem. Soc. Rev.*, 4 (1975) 421.
14 E. S. Gould, *Mechanism and Structure in Organic Chemistry*, Holt-Dryden, New York, 1959, Ch. 4.
15 I. Roberts and L. P. Hammett, *J. Amer. Chem. Soc.*, 59 (1937) 1063.
16 O. T. Benfey, *J. Amer. Chem. Soc.*, 70 (1948) 2163.
17 H. Oelschlager and M. Muller, *Pharmazie*, 36 (1981) 807.
18 L. T. Wong, A. R. Beaubien and A. P. Pakuts, *J. Chromatogr.*, 231 (1982) 145.
19 H. E. Ganther and R. J. Kraus, *Anal. Biochem.*, 138 (1984) 396.
20 W. A. Jacobs and P. T. Kissinger, *J. Liq. Chromatogr.*, 5 (1982) 881.
21 H. Yoshida, T. Sumida, T. Masujima and H. Imai, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 509.
22 S. V. Vitt, M. M. Vorob'ev, E. A. Paskonova and M. B. Saporovskaya, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 158.
23 J. F. Lawrence and R. W. Frei, *Chemical Derivatization in Liquid Chromatography*, Elsevier, Amsterdam, 1976, pp. 111–209.
24 J. F. Lawrence, *Organic Trace Analysis*, Academic Press, New York, 1981.
25 W. P. Cochrane, L. A. Sternson and R. W. Frei, in R. W. Frei and J. F. Lawrence (Editors), *Chemical Derivatization in Analytical Chemistry, Volume 1; Chromatography*, Plenum, New York, 1981, Ch. 1, 3 and 4.
26 D. R. Knapp, *Handbook of Analytical Derivatization Reagents*, Wiley, New York, 1979.
27 K. Blau and G. King, *Handbook of Derivatives for Chromatography*, Heyden, London, 1977.
28 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, Ch. 17.

29 J. T. Stewart, *Trends Anal. Chem.*, 1 (1982) 170.
30 C. R. Clark and M. M. Wells, *J. Chromatogr. Sci.*, 16 (1978) 332.
31 J. F. Lawrence, *J. Chromatogr. Sci.*, 17 (1979) 147.
32 S. Ahuja, *J. Chromatogr. Sci.*, 17 (1979) 168.
33 J. C. Gfeller, J. M. Huen and J. P. Thevenin, *Chromatographia*, 12 (1979) 368.
34 G. Schwedt, *Ang. Chem. Int. Ed.*, 18 (1979) 180.
35 M. S. F. Ross, *J. Chromatogr.*, 141 (1977) 107.
36 I. S. Krull, K.-H. Xie, S. Colgan, U. Neue, T. Izod, R. King and B. Bidlingmeyer, *J. Liq. Chromatogr.*, 6 (1983) 605.
37 I. S. Krull, S. Colgan, K.-H. Xie, U. Neue, R. King and B. Bidlingmeyer, *J. Liq. Chromatogr.*, 6 (1983) 1015.
38 K.-H. Xie, S. Colgan and I. S. Krull, *J. Liq. Chromatogr.*, 6 (S-2) (1983) 125.
39 K-H. Xie, C. T. Santasania, I. S. Krull, U. Neue, B. Bidlingmeyer and A. Newhart, *J. Liq. Chromatogr.*, 6 (1983) 2109.
40 L. Nondek, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 55 (1983) 1466.
41 L. Nondek, *Anal. Chem.*, 56 (1984) 1194.
42 M. W. Bolme and S. H. Langer, *J. Phys. Chem.*, 87 (1983).
43 P. Vratny, J. Ouhrabkova and J. Copikova, *J. Chromatogr.*, 191 (1980) 313.
44 L. Nondek, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 282 (1983) 141.
45 J. F. Studebaker, *J. Chromatogr.*, 185 (1979) 497.
46 E. P. Lankmayer, B. Maichin, G. Knapp and F. Nachtmann, *J. Chromatogr.*, 224 (1981) 239.
47 K. Okamoto, T. Kinoshita, T. Tsukasa and T. Moriyama, *J. Chem. Soc. Perkin II*, (1978) 45.
48 R. A. W. Johnstone and M. E. Rose, *Tetrahedron*, 35 (1979) 2169.
49 B. A. Stoochnoff and N. L. Benoiton, *Tetrahedron Lett.*, 1 (1973) 21.
50 R. A. Rossi and A. B. Pierini, *J. Org. Chem.*, 45 (1980) 2915.
51 A. McKillop, J-C. Fiaud and R. P. Hug, *Tetrahedron*, 30 (1974) 1379.
52 G. Bram, T. Fillebeen-Khan and N. Geraghty, *Synth. Commun.*, 10 (1980) 279.
53 G. Gelbard and S. Colonna, *Synthesis*, (1977) 113.
54 T. Iversen and R. Johansson, *Synthesis*, (1979) 823.
55 M. Hassanein, A. Akelah and F. Abdel-Galil, *Polymer Preprints*, 26 (1985) 88.
56 J. P. de Kleijn, *Analyst (London)*, 107 (1982) 223.