be done selectively, and any ions that are not completely ejected can be removed by background subtraction. This is not true for electron impact excitation, since any ions trapped in the electron beam may undergo fragmentation, regardless of mass. Hence, electron impact excitation will require a more careful use of ion ejection for parent ion selection. For this reason, the use of a "notch ejection" (*10*), or preferably a tailored excitation (*11*), is to be preferred over successive swept-frequency ejection for the EIEIO experiment.

The fact that ions must be trapped in the electron beam in order to perform the excitation means that the method will be restricted to positive ions. We believe that this limitation will not prevent the technique from being applied to solving a broad range of chemical problems. It should be possible to substitute a positive ion beam (e.g., a Cs^+ beam) for the electron beam to produce fragmentation of negative ions.

Electron impact excitation offers some characteristics which might make it a desirable alternative to collision-induced dissociation in some cases. For example, with the dual-cell geometry, ions may be transferred to the analyzer cell for CID experiments (*7*). This isolates the ions from reactive neutrals, prevents daughter ions from undergoing ion–molecule reactions, and permits higher resolution to be obtained. In order to perform a CID experiment, ions must be excited to a larger orbital radius. Once their orbital radius has been increased, they cannot be transferred through the conductance limit. This limitation does not apply in an EIEIO experiment, since the ions remain on-axis throughout the experiment until detected. This is an important capability for making full use of the dual-cell geometry for ion–molecule reaction experiments with different reactant of collision gases present in the source and analyzer cells, permitting multiple MS/MS experiments.

For CID experiments in the dual-cell instrument, it is necessary to move ions into the analyzer cell (isolated from any reactive neutral species) and then introduce a collision gas through a pulsed valve (*12*). This requires several hundred milliseconds for accelerated ions to collide with the collision gas and for the gas to be pumped out of the analyzer region to obtain high resolution. Since no collision gas is required for the electron impact experiment, less time is required for the experiment. This may make EIEIO a more desirable choice for experiments where time is a factor, such as GC–MS.

Registry No. Acetophenone, 98-86-2; isophorone, 78-59-1; cumene, 98-82-8.

LITERATURE CITED

(1) Cody, R. B.; Freiser, B. S. *Anal. Chem.* **1979**, *51*, 547-551.
(2) Fedor, D. M.; Cody, R. B.; Burinsky, D. J.; Freiser, B. S.; Cooks, R. G. *Int. J. Mass Spectrom. Ion Phys.* **1981**, *39*, 55-64.
(3) McLuckey, S. A.; Sallans, L.; Cody, R. B.; Burnier, R. C.; Verma, S.; Freiser, B. S.; Cooks, R. G. *Int. J. Mass Spectrom Ion Phys.* **1982**, *44*, 215-229.
(4) Freiser, B. S. *Int. J. Mass Spectrom Ion Phys.* **1980**, *33*, 263-267.
(5) Comisarow, M. B.; Grassi, V.; Parisod, G. *Chem. Phys. Lett.* **1978**, *57*, 413-416.
(6) U.S. Patent Application Serial No. 610502.
(7) Ghaderi, S.; Littlejohn, D. P. *Collected Abstracts of the 33rd Annual Conference on Mass Spectrometry and Allied Topics*; ASMS: San Diego, CA, 1985; 727-728.
(8) Cody, R. B.; Kinsinger, J. A.; Ghaderi, S.; Amster, I. J.; McLafferty, F. W.; Brown, C. E. *Anal. Chim. Acta* **1986**, *178*, 43-66.
(9) Ghaderi, S.; Kulkarni, P. S.; Ledford, E. B., Jr.; Wilkins, C. L.; Gross, M. L. *Anal. Chem.* **1981**, *53*, 428-437.
(10) Noest, A. J.; Kort, C. W. F. *Comput. Chem.* **1983**, *7*, 81-86.
(11) Marshall, A. G.; Wang, T.-C. L.; Ricca, T. L. *Collected Abstracts of the 33rd Annual Conference on Mass Spectrometry and Allied Topics*; ASMS: San Diego, CA, 1985; 717-718.
(12) Carlin, T. J.; Freiser, B. S. *Anal. Chem.* **1983**, *55*, 571-574.

Robert B. Cody*

Nicolet Analytical Instruments
5225-1 Verona Road
Madison, Wisconsin 53711

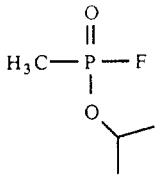
Ben S. Freiser

Department of Chemistry
Purdue University
West Lafayette, Indiana 47917

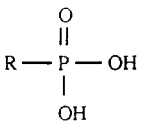
RECEIVED for review February 13, 1986. Resubmitted November 7, 1986. Accepted November 7, 1986.

Fluorescence Detection of Alkylphosphonic Acids Using *p*-(9-Anthroyloxy)phenacyl Bromide

Sir: Organophosphonates have found wide use as herbicides, insecticides, and antibiotics (*1*). Given their biocidal potency, sensitive means for monitoring trace amounts of organophosphonates in the environment are needed. Laser-based detection methods have provided excellent sensitivities in the determination of a variety of compounds (*2–7*). This report elaborates an analysis technique for methylphosphonic acid (**1a**), a residue resulting from complete hydrolysis of sarin.

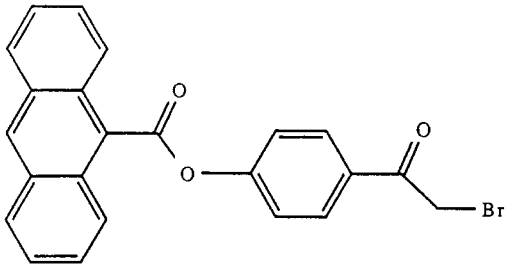


sarin



1a R = CH_3
2a R = CH_3CH_2
3a R = $(CH_3)_2CH$
4a R = $CH_3(CH_2)_5$

The analysis methodology is applicable to other alkylphosphonic acids such as **2a**, **3a**, and **4a**. Laser-induced fluorescence is used in tandem with microcolumn high-pressure liquid chromatography. Central to the methodology is derivatization with a fluorescent labeling agent, *p*-(9-anthroyloxy)phenacyl bromide (**5**). Laser-induced fluorescence analysis allows detection of these derivatives in the femtomole range.



5

To date, organophosphonates have been detected via a variety of techniques. Conversion of organophosphonates to

inorganic phosphate (*8*) facilitates quantitation by colorimetric assay. Similar colorimetric analysis is used for inorganic phosphate derived from organophosphonates separated by ion exchange chromatography (*9, 10*). Volatile organophosphorus compounds have been preferentially ionized and analyzed by using molecular secondary ion mass spectrometry (*11*). Determination of organophosphonates has been achieved by using a dual-flame photometric phosphorus-sensitive detector subsequent to ion-pair reverse phase, high-pressure liquid chromatographic separation (*12*). Sensitivities in the nanogram range are obtained by derivatization of the monoesters of alkylphosphonic acids with *p*-bromophenacyl bromide and determination of the adducts formed with absorbance detection (*13*). Derivatization of the phosphonic acid moiety is also useful in gas chromatographic analysis of organophosphonates. Derivatizing reagents include 3-benzyl-1-*p*-tolyltriazene (*14*), diazoalkanes (*15–17*), *O*-methyl-*N,N*-dicyclohexylisourea (*18*), BCl_3/2-chloroethanol (*19*), *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (*18*), and trifluoroethanol/trifluoroacetic anhydride (*20*). Gas chromatographic analysis can facilitate detection in the 10–100 ppb range (*20*).

EXPERIMENTAL SECTION

Apparatus. The HPLC system consisted of an HPLC Micropump (Brownlee Labs., Santa Clara, CA), a Model 7410 Rheodyne injector fitted with a 1-μL loop and a 250 x 1 mm i.d. Adsorbosphere C_{18} column (Alltech Associates, Waukegan, IL). The 325-nm output of a Model 4050B Liconix He–Cd laser (Sunnyvale, CA) is utilized as the excitation source. The laser radiation (ca. 10 mW) was focused into a 75-μm fused silica capillary connected to the end of the microcolumn. Fluorescence collected at right angles to the excitation beam was isolated with a saturated solution of sodium nitrite (to remove Rayleigh and Raman scatter) and a broad-band interference filter (no. 57530, Oriel Corp., Stratford, CT) and focused on a Centronic Model Q 4249B photomultiplier tube (Bailey Instruments Co., Saddle Brook, NJ). The PMT signal was conditioned with a current-to-voltage converter and a low-pass filter before being output to a strip-chart recorder.

^1H NMR spectra were recorded on a Varian XL-400 spectrometer and chemical shifts reported in parts per million relative to tetramethylsilane ($(CH_3)_4Si$, δ 0.0) with $CDCl_3$ as solvent. ^{13}C NMR spectra were also recorded on a Varian XL-400 spectrometer. Chemical shifts were reported in parts per million relative to tetramethylsilane ($(CH_3)_4Si$, δ 0.0).

Chemicals. Phosphonic acids were purchased from Alfa Products (Danvers, MA) and water was removed as its azeotrope with toluene immediately before use. Tetra-*n*-butylammonium hydroxide was purchased from Sigma Chemical Co. (St. Louis, MO). *N,N*-Diisopropylethylamine, 98%, was purchased from Aldrich and distilled from calcium hydride under nitrogen. *N,N*-Dimethylformamide was distilled from activated Linde 4A molecular sieves under nitrogen. Copper(II) bromide from Baker was ground in a mortar and pestle to a powder. All other chemicals were purchased from Aldrich (Milwaukee, WI) and used without further purification.

Synthesis of *p*-(9-Anthroyloxy)phenacyl Bromide (5). Anthracenecarboxylic acid (10 g, 44.1 mmol) and *p*-hydroxyacetophenone (5.57 g, 40.5 mmol) were mixed with 500 mL of toluene in a two-necked, 1-L, round-bottom flask fitted with a reflux condenser and a dropping funnel. Trifluoroacetic anhydride (18.5 g, 88.2 mmol) was added dropwise with stirring. Upon dissolution of the reactants, the solution was heated to 60 °C for 2 h. After cooling, the toluene was removed under reduced pressure and the resulting yellow solid stirred with cold ethanol for 10 min. Filtration on a Büchner funnel yielded *p*-(9-anthroyloxy)acetophenone which can be brominated directly or recrystallized from $CHCl_3$/ethanol. For bromination (*21*) copper(II) bromide (6.63 g, 29.4 mmol) was placed in a two-necked, 1-L, round-bottom flask fitted with a reflux condenser and 200 mL of ethyl acetate added and the solution brought to reflux. *p*-(9-Anthroyloxy)acetophenone (5.0 g, 14.7 mmol) was dissolved in 200 mL of hot chloroform and added to the flask. The reaction was refluxed for 2 h with vigorous stirring. Upon completion of the reaction (indicated by a color change from green to yellow and conversion of the black $CuBr_2$ to white CuBr) the solution was cooled and filtered through Florisil. The Florisil was washed with chloroform and the combined filtrates were evaporated under reduced pressure. *p*-(9-Anthroyloxy)phenacyl bromide was purified by flash chromatography (*22*) using 3:2 dichloromethane/hexane (v/v) as the mobile phase. The overall yield for synthesis of derivatization agent **5** was 77%.

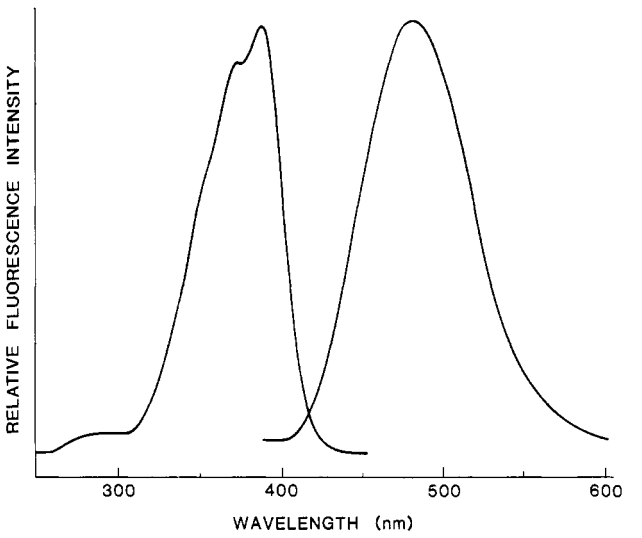


Figure 1. Excitation and emission spectra of adduct of methylphosphonic acid derivatized with *p*-(9-anthroyloxy)phenacyl bromide. Solvent that was used for dissolving the adduct is acetonitrile.

Derivatization Method A. The alkylphosphonic acid (10 μmol) was dissolved in distilled, deionized water and the pH adjusted to 7.1 with tetra-*n*-butylammonium hydroxide. After removal of water under reduced pressure, the residue was further dried by removing the remaining water as its azeotrope with toluene. **5** (0.21 g, 50 μmol) dissolved in 2 mL of dimethylformamide was added to the phosphonate residue. The solution was stirred at 40 °C for 2 h and then injected onto the column after suitable dilution with acetonitrile.

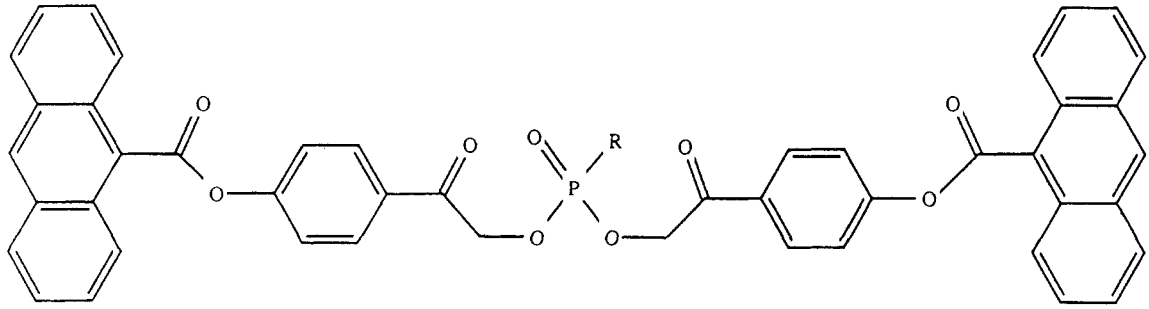
Derivatization Method B. Alkylphosphonic acid (10 μmol) dissolved in dimethylformamide was mixed with **5** (0.021 g, 50 μmol) and diisopropylethylamine (0.004 g, 30 μmol). The total volume was adjusted to 2 mL. The solution was heated with stirring at 80 °C for 30 min and then injected onto the column after suitable dilution with acetonitrile.

Product Characterization. *p-(9-Anthroyloxy)phenacyl Bromide*: ^1H NMR ($CDCl_3$) (ppm): δ 4.50 (s, 2 H), 7.56–7.64 (m, 6 H), 8.09 (s, 1 H), 8.11 (s, 1 H), 8.18 (s, 1 H), 8.20 (s, 1 H), 8.22 (s, 1 H), 8.25 (s, 1 H), 8.65 (s, 1 H). ^{13}C NMR ($CDCl_3$) (ppm): 30.5, 122.2, 124.5, 125.7, 127.6, 128.8, 128.9, 130.6, 130.9, 131.8, 155.1, 167.2, 190.1. FTIR (NaCl, film) (cm^{-1}): 3126–3050 (br), 1736 (m), 1674 (s), 1638 (s), 1589 (m), 1271 (w), 1202 (w), 1160 (m), 1114 (m), 957 (m), 856 (m).

Bis[p-(9-anthroyloxy)phenacyl] Methylphosphonate: ^1H NMR δ ($CDCl_3$) (ppm): 1.89 (d, J_{CP} = 18 Hz, 3 H), 5.43 (dd, J = 16 Hz, J = 12 Hz, 2 H), 5.56 (dd, J = 16 Hz, J = 11 Hz, 2 H), 7.50–7.62 (m, 12 H), 8.04 (s, 2 H), 8.06 (s, 2 H), 8.08 (s, 2 H), 8.12 (s, 2 H), 8.21 (s, 2 H), 8.23 (s, 2 H), 8.59 (s, 2 H). ^{13}C NMR ($CDCl_3$) (ppm): 12.3 (d, J_{CP} = 148 Hz), 67.5 (J_{COP} = 5.8 Hz), 122.2, 124.5, 125.6, 127.6, 128.7, 128.8, 129.7, 130.5, 130.8, 131.8, 155.0, 167.1, 191.9. FTIR (NaCl, neat) (cm^{-1}): 3140–3165 (br), 1743 (s), 1707 (s), 1595 (s), 1226 (w), 1239 (w), 1201 (m), 1185 (s), 1165 (s), 1135 (s), 1092 (m), 969 (s), 916 (w).

RESULTS AND DISCUSSION

Derivatization of alkylphosphonic acids such as methylphosphonic acid (**1a**) serves two purposes. First, the product of derivatization facilitates separation of the alkylphosphonic acid by C_{18} microcolumn high-pressure liquid chromatography. This is essential to observing the alkylphosphonic acid among a diverse array of other solutes in biological and envrionmental systems. Second, the derivatized alkylphosphonic acid is



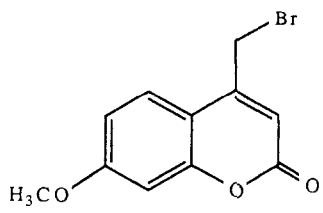
1b R = CH_3
2b R = CH_3CH_2
3b R = $(CH_3)_2CH$
4b R = $CH_3(CH_2)_5$

detectable by laser-induced fluorescence which provides sensitivity far exceeding that available for this class of compounds by previous HPLC methods.

A straightforward, inexpensive synthesis can be used to prepare derivatizing agent **5** from readily available starting materials (**5** is also available from Molecular Probes, Eugene, OR). Reaction of **5** and **1a** affords bis[*p*-(9-anthroyloxy)-phenacyl] methylphosphonate **1b**. Formation of the diesterified adducts of alkylphosphonic acids appears to be general in view of derivatization of **2a**, **3a**, and **4a** with **5** to form diesters **2b**, **3b**, and **4b**. The alkylphosphonate must be neutralized for derivatization to occur although excessive amounts of base lead to hydrolysis of derivatizing reagent **5**. Therefore, when the number of equivalents of alkylphosphonate are known, Method B is the most convenient protocol. Method A is designed for aqueous solutions of alkylphosphonic acids where the phosphonic acid concentrations are unknown. The tetra-*n*-butylammonium hydroxide functions as a titrant and, after removal of the water, as a phase transfer reagent which enhances the solubility of alkylphosphonates in dimethylformamide. Both methods afford approximately a 90% yield of derivatized methylphosphonic acid.

The excitation and emission spectra (uncorrected) of diester **1b** is shown in Figure 1. A mixture of methyl-, ethyl-, isopropyl- and *n*-hexylphosphonic acids was derivatized by method B at the 10 mM level and injected after suitable dilution. Figure 2 shows the separation of 10 pmol each of the derivatized mixture of alkylphosphonic acids. The sensitivity of the method was determined by derivatizing methylphosphonic acid, diluting by appropriate volumes, and determining the signal-to-noise ratio down to the detection limit ($S/N = 2$). Using 8 mW of 325-nm radiation of a He–Cd laser, the detection limit was found to be 20 fmol.

Functionalization of **1a** is unique to derivatizing agent **5**. Attempted derivatization with a range of other reagents, including 4-bromomethyl-7-methoxycoumarin (**6**), under a variety of conditions failed to provide any derivatized alkylphosphonic acids. This is particularly notable in view of 4-bromomethyl-7-methoxycoumarin's effective derivatization



6

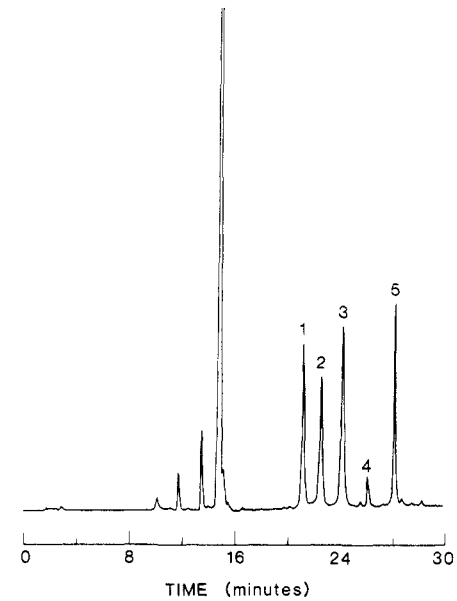


Figure 2. Separation of a mixture of (1) methyl-, (2) ethyl-, (3) isopropyl-, and (5) *n*-hexylphosphonic acid derivatives using a linear gradient of 60 to 100% acetonitrile in 30 min. The other solvent was water. Peak 4 is due to a reaction byproduct. The amount injected per phosphonic acid is 10 pmol. Flow rate is 50 μL/min.

of monoalkyl esters of methylphosphonic acid (*23*). The precedented ability of phenacyl halides to esterify mono- (*24*), di-, and tribasic carboxylic acids (*25*) implies that phenacyl halide **5** is more reactive than coumarin **6**. *p*-(9-Anthroyloxy)phenacyl bromide is thus a useful hybrid. As a phenacyl halide, **5** facilitates derivatization of alkylphosphonic acids, while its anthracyl moiety functions as the necessary chromophore for laser-induced fluorescence.

The results obtained show that alkylphosphonic acids can be readily derivatized by use of *p*-(9-anthroyloxy)phenacyl bromide. The reaction is rapid and the yield is quantitative, making this derivatizing agent an excellent choice for trace analysis. Disadvantages of the method are the low solubility of the resulting derivative in most HPLC compatible solvents, the need to remove water from the system during derivatization to minimize degradation of the reagent, and the fact that unreacted *p*-(9-anthroyloxy)phenacyl bromide is also fluorescent and shows up in the chromatogram requiring efficient separation of derivatives to avoid interference in quantitative analysis. The bulky derivatizing agent may tend to overwhelm the small structural variations of closely related alkylphosphonic acids making separation difficult. However, the use of a simple gradient can provide excellent separation between the derivatizing reagent and the alkylphosphonic acid

derivatives as shown in Figure 2. Derivatization with *p*-(9-anthroyloxy)phenacyl bromide combined with laser-induced fluorescence is an alternative to the aforementioned methods currently exploited in organophosphonate detection (*8–20*). The ease of derivatization and excellent sensitivity are particularly appealing as a substitute for the use of radiolabels (*26*) in organophosphonate analyses.

LITERATURE CITED

(1) The Role of Phosphonates in Living Systems; Hilderbrand, R. L., Ed., CRC Press: Boca Ratan: Fl, 1983.
(2) Sepaniak, M. J.; Yeung, E. S. *Anal. Chem.* **1981**, *54*, 1465A.
(3) McGuffin, V. L.; Zare, R. N. *Appl. Spectrosc.* **1985**, *39*, 847.
(4) Gluckman, J.; Shelly, D.; Novotny, M. J. *J. Chromatogr.* **1984**, *317*, 443.
(5) Morris, M. D.; Peck, K. *Anal. Chem.* **1986**, *58*, 811A.
(6) Nolan, T. G.; Hart, B. K.; Dovichi, N. J. *Anal. Chem.* **1985**, *57*, 2703.
(7) Smith, L. M.; Sanders, J. Z.; Kaiser, R. J.; Hughes, P.; Dodd, C.; Connell, C. R.; Heiner, C.; Kent, S. B. H.; Hood, L. E. *Nature (London)* **1986**, *321*, 674.
(8) Cook, A. M.; Daughton, C. G.; Alexander, M. *Anal. Chem.* **1978**, *50*, 1716.
(9) Jakob, F.; Park, K. C.; Ciric, J.; *Rieman*, W., III *Talanta* **1961**, *8*, 431.
(10) Waldoff, H.; Sladek, P. *Fresenius'* Z. *Anal. Chem.* **1985**, *320*, 163.
(11) Groenewold, G. S.; Todd, P. J. *Anal. Chem.* **1985**, 57, 886.
(12) Chester, T. L. *Anal. Chem.* **1980**, *52*, 1621.
(13) Bossle, P. C.; Martin, J. J.; Sarver, E. W.; Sommer, H. Z. *J. Chromatogr.* **1983**, *267*, 209.
(14) Daughton, C. G.; Cook, A. M.; Alexander, M. *Anal. Chem.* **1979**, *51*, 1949.
(15) Monsanto Chemical Co. *Pesticide Analytical Manual*; Food and Drug Administration: Washington, DC, 1977; Vol. 2.
(16) Rueppel, M. L.; Suba, L. A.; Marvel, J. T. *Biomed. Mass Spectrom.* **1976**, *3*, 28.
(17) Lores, E. M.; Bradway, D. E. *J. Agric. Food Chem.* **1977**, *25*, 75.
(18) Bardalaye, P. C.; Wheeler, W. B.; Moye, H. A. In *The Herbicide Glyphosate*; Grossbard, E., Atkinson, D., Eds.; Butterworths: New York, 1985; Chapter 17.
(19) Guinivan, R. A.; Thompson, N. P., Wheeler, W. B. *J. Agric. Food Chem.* **1982**, *30*, 977.
(20) Deyrup, C. L.; Chang, S.-M.; Weintraub, R. A.; Moye, H. A. *J. Agric. Food Chem.* **1985**, *33*, 944.
(21) King, L. C.; Ostrum, G. K. *J. Org. Chem.* **1964**, *29*, 3459.
(22) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(23) Gassmann, E.; Zare, R. N., Stanford University, 1985, unpublished results.
(24) Umeh, E. O. *J. Chromatogr.* **1971**, *56*, 29.
(25) Grushka, E.; Durst, H. D.; Kikta, E. J., Jr. *J. Chromatogr.* **1975**, *112*, 673.
(26) Hoskin, F. C. G. *Can. J. Biochem. Physiol.* **1956**, *34*, 75.

M. C. Roach
L. W. Ungar
R. N. Zare*
L. M. Reimer
D. L. Pompliano
J. W. Frost*

Department of Chemistry
Stanford University
Stanford, California 94305

RECEIVED for review September 15, 1986. Accepted November 24, 1986. Research was funded by the Department of the Army (Contract DAAG29-85-K-0242).

Optical Activity and Ultraviolet Absorbance Detection of Dansyl L-Amino Acids Separated by Gradient Liquid Chromatography

Sir: Dansyl [1-(dimethylamino)naphthalene-5-sulfonyl] chloride derivatization of amino acids was first used in peptide sequencing (*1*) and determination of proteins by fluorescence polarization (*2*). Recently dansylation has become a popular precolumn derivatization method for fluorescence or UV absorbance detection of amino acids. Reversed-phase or ion pair reversed-phase HPLC is employed in the separation of the product mixture. Much work has been done with dansyl derivatives including determination of reaction byproducts and optimum conditions for the reaction (*3,4*). De Jong (*5*) determined that quantitative data results if stringent reaction and chromatography conditions are used. Other workers (*6*) have found precolumn dansyl derivatization to be highly reliable and reproducible, producing variations of less than 5%. The separation of D and L isomers of dansyl amino acids has also been accomplished by using chiral β-cyclodextrin in the mobile phase (*7*) and by mixed-chelate complexation (*8*).

Many scientific investigations (e.g., geochronology, pharmaceuticals) have the need to determine enantiometric ratios of amino acids and other compounds. It has been reported that OA/UV or OA/RI (refractive index) are ideal methods for the determination of enantiomeric ratios without the need for chiral columns, chiral eluents, or diastereomer preparation (*9*). Unfortunately, only three amino acids are naturally UV absorbing (254 nm), and RI sensitivity for amino acids is low. Derivatization by several methods (*o*-phthalaldehyde, dansyl, phenylisothiocyanate, fluorescamine, 2,4-dinitrofluorobenzene, and phenylthiohydantoin) renders all amino acids UV absorbing and makes UV or fluorescence viable techniques for amino acid determinations. A previously neglected aspect of derivatization is the effect on optical activity. These highly polar groups influence the chiral center of amino acids drastically (electronic and steric effects). The shifting of the absorption band to the proximity of the wavelength used for OA measurements further enhances the importance of the substituent. We report here the determination of 17 dansyl amino acids in a mixture by UV absorbance and optical activity. This involves gradient elution. Previously, the optical activity detector (OAD) has been used only with isocratic HPLC. Unfortunately, the OAD is not perfectly selective and responds to large refractive index changes, very similar to observations in UV detectors. This is most evident at the void volume when eluent and sample solvent are not identical. The change in refractive index displaces and disturbs the collimation of the laser beam, which is interpreted by the detector as a change in rotation. Thus, gradient HPLC could not be coupled to the OAD without careful choice of eluents. By using a previously reported mixture of 0.13 M ammonium acetate and acetonitrile as eluent (*10*) in conjunction with a small change in solvent composition per unit of time, it was found that gradient separation of dansyl amino acids with the OAD was possible.

EXPERIMENTAL SECTION

Reagents. The dansyl L-amino acids were obtained from Sigma (St. Louis, MO). Dansyl-L-aspartic acid (Asp), Nα-dansyl-L-asparagine (Asn), dansylglycine (Gly), dansyl-L-proline (Pro), dansyl-L-phenylalanine (Phe), *N,N'*-didansyl-L-cystine (Cys), dansyl-L-glutamine (Gln), dansyl-L-cysteic acid (Cya), and Nϵ-dansyl-L-lysine (Lys) were obtained as the free acid; *N*-dansyl-L-serine (Ser), dansyl-L-alanine (Ala), dansyl-L-valine (Val), dansyl-L-methionine (Met), dansyl-L-isoleucine (Ile), and dansyl-L-α-amino-*n*-butyric acid (Abu) as the cyclohexylamine salt; *N*-dansyl-*trans*-4-hydroxy-L-proline (Hyp), dansyl-L-threonine (Thr), and Nα-dansyl-L-tryptophan (Trp) as the cyclohexyl-

0003-2700/87/0359-1059$01.50/0