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Analysis of Organic N-Chloramines in Chlorinated Drinking Water

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ANALYSIS OF ORGANIC N-CHLORAMINES IN CHLORINATED DRINKING WATER

by

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ABSTRACT

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IN CHLORINATED DRINKING WATER

John Po Wen Yang
Old Dominion University, 1982
Director: Dr. Frank E. Scully, Jr.

This thesis describes the development of a sensitive method for the separation and quantitation of organic N-chloramines in chlorinated tap water. 5-Dimethylamino-naphthalene-1-sulfinic acid has been synthesized and characterized. It yields highly fluorescent dansyl derivatives on reaction with N-chloramines. Conditions of the derivatization were optimized for the detection of N-chloropiperidine at concentrations in aqueous solution of 10^{-7} M. The technique gives a quantity of derivative which is proportional to the concentration of chloramine present over the range examined (10^{-7} M to 10^{-4} M). High pressure liquid chromatographic separation of dansyl derivatives of amines and amino acids has been optimized using a fluorescence detector. This technique has been applied to the analysis of tap water. Liquid chromatograms of derivatized tap water reveal the presence of many organic N-chloramines.

DEDICATION

I wish to dedicate this work to my parents, Mrs. K. C. Yang and Mr. K. S. Yang, for their love.

ACKNOWLEDGEMENTS

I wish to thank my thesis advisor, Dr. Frank E. Scully, Jr., for the opportunity to work with him on this project, as well as his advice and guidance. Thanks are also extended to the members of my research committee, Dr. B. T. Upchurch, Dr. C. E. Bell, Jr., and Dr. J. H. Yuan for their advice and encouragement.

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John Po Wen Yang

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INTRODUCTION

Since the introduction of chlorine as a disinfectant in drinking water, the incidence of waterborne diseases has been dramatically reduced. However, recent studies have suggested that there are by-products of water chlorination which are mutagenic (1), and individual compounds identified in chlorinated water have been found to be carcinogenic (2-3).

For almost seventy years chlorination as a means of disinfection has been viewed as a public health breakthrough. Epidemic outbreaks of waterborne diseases such as typhoid and cholera were prominent in the late 1800's. Then in the early 1900's the state of New Jersey was the first in the nation to utilize chlorine as a means of disinfection (4). Shortly after this, the U. S. Public Health Service was established to ensure the safety of drinking water. After World War II when there was a boom in population, industry, technology and agriculture, there was also a dramatic increase in the amount of fertilizer, pesticides, and waste materials discharged into water supplies. The health implications of these practices were not realized until the 1960's when these chemicals were found to accumulate in water and aquatic life. In 1962 the Public Health Service Standards, as decreed by Congress, required that drinking water be obtained from a "protected source" (5). A "protected source" was defined to include

one or more such processes as dilution, storage, sedimentation, aeration, exposure to sunlight, infiltration through soil, percolation through underlying material, storage below the ground water table, and chlorination. Of these, chlorination was the most widespread. In the early 1970's 66 organic substances were identified in the New Orleans water supply. Several suspected carcinogens which are by-products of water chlorination were among these. People are now realizing that water purification by the use of chlorine, which was seen as a means of protection from viral and bacterial hazards, may actually introduce a new health hazard. Several toxicological and epidemiological studies support this view.

Loper, et al., analyzed the organic concentrates from water of five U. S. cities for mutagenic activity by Ames' assay and by in vitro cell transformation assay (1). They showed that mutagenic activity was detected from water samples in each city, with the high molecular weight non-volatiles exhibiting the most activity. Cell transformation tests revealed characteristics typical of transformed cells including disoriented growth, decreased adhesiveness, increased saturation density, and increased plating efficiency in soft agar.

A study of gastrointestinal (GI) and urinary tract (UT) cancer deaths over a three year period has been studied by Alavanja, et al. (6). He reported on GI and UT cancer mortality between January 1, 1968 and December 31, 1970 in

five New York State counties. One of the variables studied included residence in an area served by chlorinated or non-chlorinated water. Results of the study showed that GI and UT cancer mortality was greater in most areas utilizing chlorinated water.

In 1978 the E.P.A. released a list of 600 organic compounds identified in U.S. drinking waters through 1976 (3). Among those compounds identified, there is a conspicuous absence of aliphatic amines. Organic amines are important components of biological systems. They enter the biosphere by enzymatic breakdown of amino acids, alkaloid discharge from plants, and organic waste disposal from industrial processes. In addition, free aliphatic amines have been identified in foods including cheese (7), fish (8-9), beer (10), tea (11), and milk (12). Among the amines most commonly identified are methylamine, dimethylamine, and ethylamine, as well as three-, four-, and five-carbon aliphatic amines including the heterocyclic amines, piperidine, and pyrrolidine. Piperidine and propylamine have been found in drinking water. Piperidine, pyrrolidine, and dimethylamine have been identified in human blood and urine (13-14). In fact, Blau has reported that the mean basal excretion in the urine of normal human males over 24 hours is 17 mg of dimethylamine, 15 mg of pyrrolidine, and 5 mg of piperidine. There have been only a few relatively recent studies on the identification of amino compounds in natural waters or wastewaters. Gardner

and Lee (15) identified a number of amino acids in fresh water, and Glaze (16) identified 18 amino acids in high concentrations in raw sewage. Despite these data, the chemistry of amines in chlorinated water indicates that they are found as their N-chloro derivatives rather than as amines.

Margerum, et al., have shown that the equilibrium constant for the formation of N-chloromethylamine from methylamine and hypochlorite is $10^{14}\ M^{-1}$ (17). Higuchi, et al., have shown that the equilibrium constant for the formation of organic chloramine from the amine and hypochlorite is approximately 10^{12} (equation 1) and that the hydrolysis of the chloramines at pH 7 to amine and hypochlorous acid is negligible (18).

$$R_2NH + HOCl \longrightarrow R_2NHCl + H_2O \qquad (1)$$

Morris, et al., (19) have shown that the reactions of ammonia, methylamine, and dimethylamine with aqueous chlorine at 25°C and about pH 6 have second order rate constants of 3.4×10^8, 3.0×10^{10}, and 3.2×10^{10} l/mole min. respectively (equations 2, 3, and 4).

$$NH_3 + HOCl \longrightarrow NH_2Cl + H_2O \qquad (2)$$

$$CH_3NH_2 + HOCl \longrightarrow CH_3NHCl + H_2O \qquad (3)$$

$$(CH_3)_2NH + HOCl \longrightarrow (CH_3)_2NCl + H_2O \qquad (4)$$

Therefore, a discussion of nitrogenous organic compounds in chlorinated water must be directed to organic N-chloramines and N-chloramino acids rather than to free amino compounds.

The fact that organic N-chloramines have not been identified in chlorinated water raises two questions: Are the organic N-chloramines which are formed short-lived and, if they are long-lived, how can they be identified?

Recently Scully and Bempong reported a study of the aqueous decomposition of N-chloropiperidine (NCP) and N-chlorodiethylamine (NCDEA) (20). The following main points emerged. NCP and NCDEA are considerably longer-lived compounds (7 days and 2.2 days, respectively, at 25^{o} at pH 7.0) than the N-chloramino acid, N-chloroalanine (55 min., calculated from the data of Stanbro and Smith) (21). The half lives of all three compounds do not change from pH 3.5 to pH 9, but increase dramatically below pH 3.0. Although more information is needed about the effects of light and trace metals on the stability of chloramines in the environment, it appears quite likely that some organic N-chloramines are long-lived species.

Because of their thermal lability, organic N-chloramines are not likely to withstand the harsh conditions of concentration, gas chromatographic separation, and mass spectrometric identification. Therefore, if direct isolation is precluded, derivatization is required.

Glaze showed that "superchlorination" (2000 mg/L of chlorine) of raw sewage greatly diminishes the concentration of amino acids (16). However, it is not clear in his report whether decomposition of the corresponding N-chloramino acids is responsible for the loss or whether

he analyzed for amino acids in the presence of their N-chloro derivatives. In either case his method, which involved dechlorination of the water with sodium sulfite, binding of the amines to an ion-exchange column, elution of the amines, concentration, derivatization, and GC/MS identification, was long and tedious. Furthermore, each step opened the possibility for extraneous contamination. In addition, his chromatogram of amino products, isolated from chlorinated sludge and treated as described above, reveals many more supposed amino contaminants than he was able to identify. To facilitate further studies of chlorinated amino compounds in disinfected water, a simple analytical technique that is specific for N-chloramines is needed.

Recently Scully and Bowdring reported a reaction of organic N-chloramines with sodium salts of arenesulfinic acids (equation 5) (22). The reaction gives good to high yields of stable arenesulfonamides at or below room temperature as shown in Table I.

$$R_2N\text{-}Cl + ArSO_2^- \longrightarrow ArSO_2NR_2 + Cl^- \qquad (5)$$

Many chloramines cannot be distilled and isolated pure the way the three listed in Table I can. However, they can be generated in aqueous solution by reaction of the amine with sodium hypochlorite. This can be done either in the presence the sodium arenesulfinate salt or the chloramine can be generated first and the sulfinate salt added subsequently. In this way inorganic chloramine (89%) and

TABLE I: DERIVATIZATIONS OF ORGANIC N-CHLORAMINES

Chloramine	Derivative	Percent Yield
N-chlorodimethylamine	p-Toluenesulfonamide	100
N-chlorodiethylamine	p-Toluenesulfonamide	63
N-chloropiperidine	Benzenesulfonamide	78

N-chloro derivatives of pyrrolidine (100%), sec-butylamine (95%), aniline (85%), ethylenediamine (81%), as well as others could be derivatized in good yield. This method is also applicable to the N-chloro derivatives of the amino acids L-leucine (78%) and glycine (70%). A total of 14 compounds of widely varying structure and reactivity were derivatized in this manner to illustrate the broad scope of the reaction.

The mechanism of the reaction of chloramines with sulfinate salts involves nucleophilic attack of the sulfinate anion on the chloramine chlorine to form a sulfonyl chloride (equation 6). The second step if the reaction (equation 7) is the well-characterized Hinsberg reaction of an amine with an arenesulfonyl chloride to form the arenesulfonamide.

$$ArSO_2^- + R_2NCl \xrightarrow{H_2O} ArSO_2Cl + R_2NH \quad (6)$$

$$ArSO_2Cl + R_2NH \longrightarrow ArSO_2NR_2 + HCl \quad (7)$$

Consequently, when sodium benzenesulfinate is added to dilute solutions of chloramines, the yield of arenesulfonamide derivative decreases dramatically as hydrolysis of the arenesulfonyl chloride (equation 8) competes with sulfonamide formation.

$$ArSO_2Cl + H_2O \longrightarrow ArSO_3H + HCl \quad (8)$$

Scully and Liu (23) have found that when dilute aqueous solution of N-chloropiperidine are derivatized with sodium

p-toluenesulfinate (saturated solution) only an 8% yield of sulfonamide could be obtained on derivatization of a 10^{-4} M N-chloropiperidine and no sulfonamide was detectable when a 10^{-5} M solution of N-chloropiperidine was derivatized.

It is the objective of this project (1) to adapt the reaction of N-chloramines with sulfinates to dilute aqueous solution, (2) to synthesize a sulfinate which will react with N-chloramines to form a highly fluorescent derivative, (3) to develop a method of separating the derivatives by high pressure liquid chromatography (HPLC), and (4) to apply this technique to the analysis of chlorinated drinking water.

The approach will involve the use of a polar, aprotic cosolvent with the derivatization of aqueous solutions of chloramines in hopes of inhibiting competing solvolysis of the amine and sulfonyl chloride intermediates.

To enhance the sensitivity and selectivity of detection of the sulfonamide products, a sulfinic acid will be synthesized which gives highly fluorescent sulfonamide products. Dansyl chloride (5-dimethylamino-1-naphthalene-sulfonyl chloride) is a well-characterized fluorescent derivatizing reagent for amines (24), amino acids (25-26), and proteins (27). It forms stable, highly fluorescent sulfonamides which are detectable at nanomolar concentrations and can be separated by TLC or HPLC.

RESULTS

Derivatization of N-chloramines

Recently Scully and Bowdring reported that N-chloramines react with arenesulfinate salts in concentrated solution to yield arenesulfonamides in high yield. Although these derivatizations were performed on amine or chloramine solutions of approximately 0.2 M concentration, with slight modification the method is applicable to much more dilute solutions (28). In addition, there is no interference from hypochlorite which is usually present in real chlorinated water systems. Sulfinate salts have been shown to be oxidized in basic solution to sulfonate salts (29).

To enhance the sensitivity and selectivity of detection of the sulfonamide products, a sulfinic acid was synthesized which yields fluorescent sulfonamides on reaction with chloramines. Dansyl chloride (5-dimethylamino-1-naphthalenesulfonyl chloride) reacts with sodium sulfite in aqueous solution at 70-80°C over five hours to give a 54% yield of 5-dimethylamino-1-naphthalenesulfinic acid monohydrate ($DANSO_2H$). The compound is an amino acid and hence exists in a zwitterionic form at its isoelectric point (approximately pH 4). The sodium salt has also been prepared by dissolution of the acid in dilute ethanolic sodium hydroxide and precipitation by concentration of the solution *in vacuo*.

In dilute base aqueous solutions of the sodium salt of $DANSO_2H$ react with chloramines to give the same dansyl

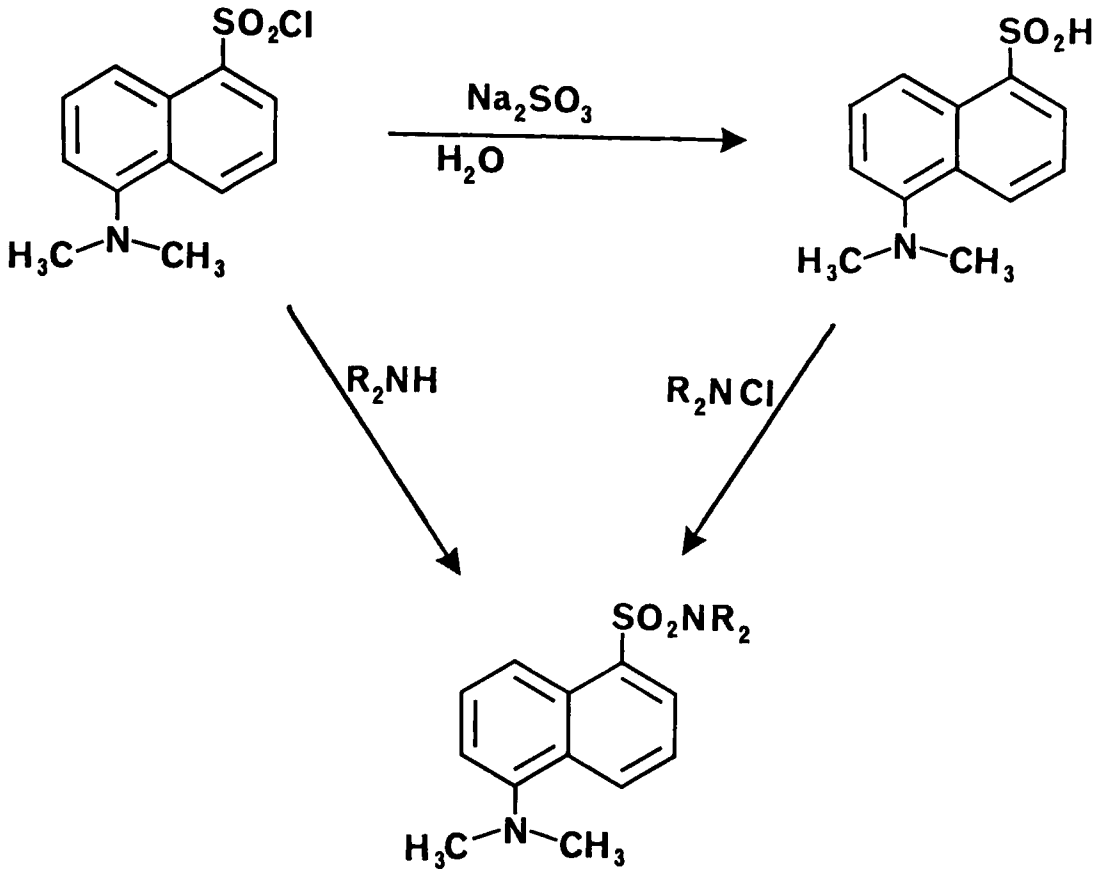
derivatives (Scheme I) formed by the reaction of dansyl chloride with the unchlorinated amine.

Several important steps in the method of handling dilute solutions of chloramines are worthy of specific note for the successful utilization of this technique. These include using a co-solvent, controlling the pH and temperature of the system, and allowing sufficient time for completion of the reaction.

For instance, unless a volume of tetrahydrofuran (or similar polar aprotic solvent) is used, which is equivalent to the volume of solution to be derivatized, no sulfonamide product is formed. In the early stages of this project acetonitrile was used as the co-solvent. However, it contains trace quantities of amines which react in chlorinated water, such as tap water, to form chloramines which are subsequently derivatized. This is not a problem when solutions of stable chloramines are derivatized in the absence of sodium hypochlorite nor when the concentrations of chloramines in the presence of dilute sodium hypochlorite are high i.e., greater than 10^{-4} M. However, the concentrations of the amine contaminants in UV-grade acetonitrile are far greater than the chloramines in tap water and consequently they interfere with the analysis. UV-grade tetrahydrofuran does not contain any interfering substances.

Another important aspect of the method is the proper adjustment of pH during the derivatization. When three

SCHEME I

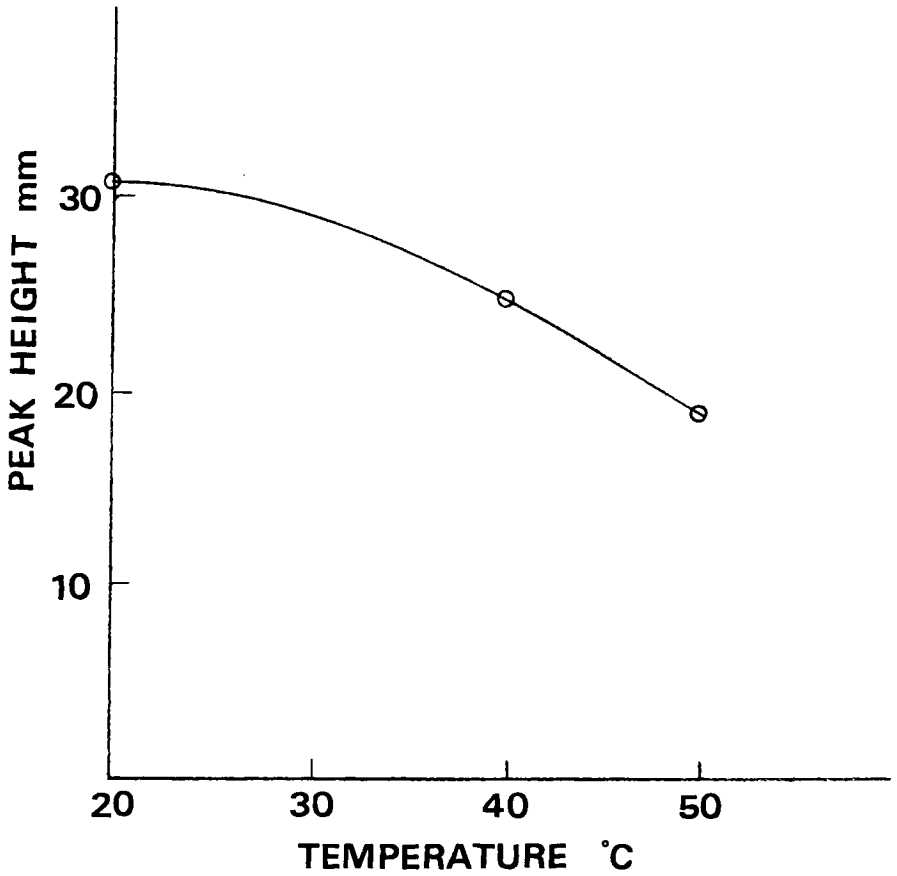


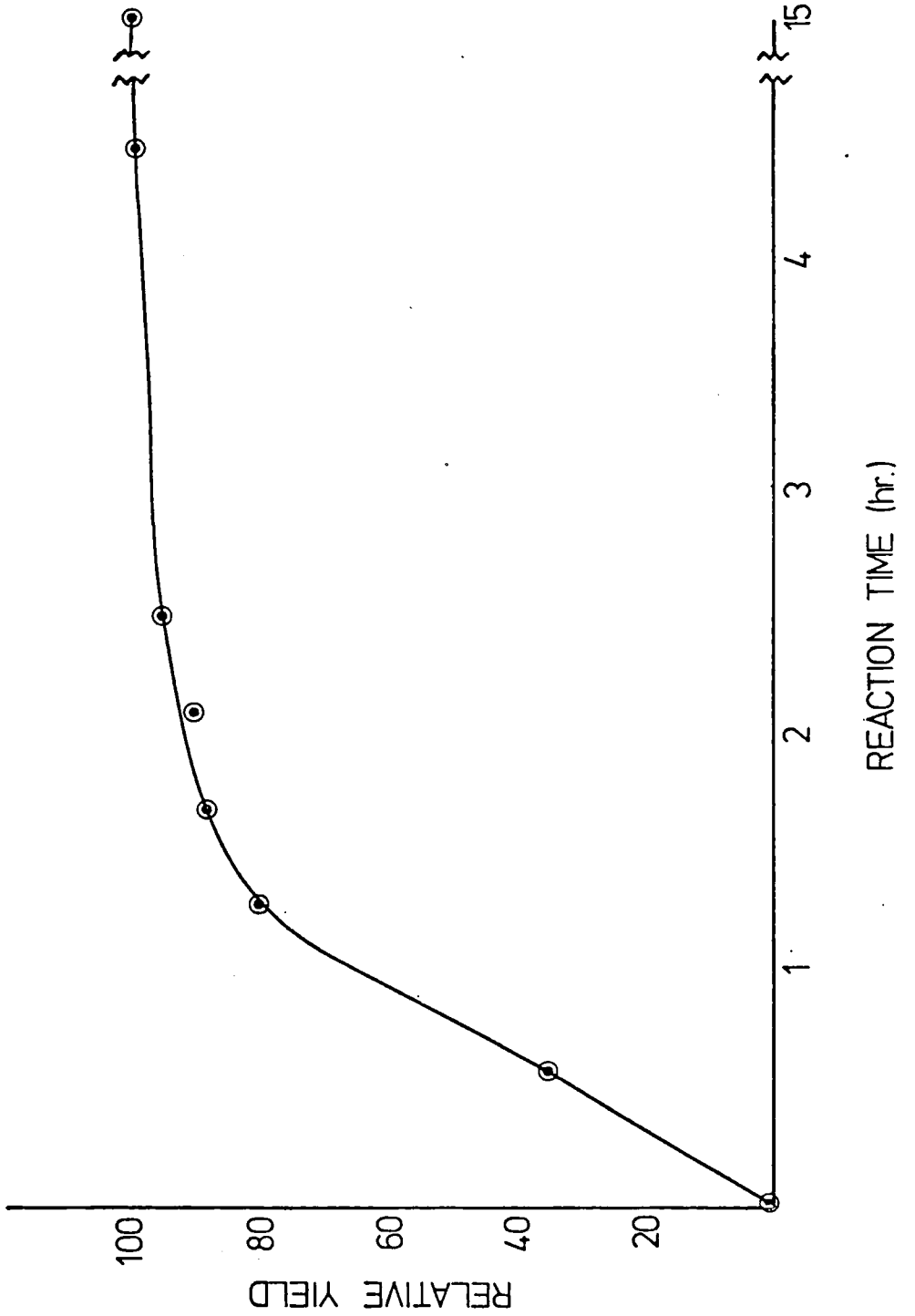
separate solutions of N-chloropiperidine in a phosphate buffer are adjusted to different pH's (pH 5, 7, and 9) and then derivatized, only the pH solution gives a product. Typically, a sodium bicarbonate solution buffered to about pH 10 is used for all derivatizations.

A third aspect of the reaction which has been examined is the effect of reaction temperature on the yield of chloramine derivative. Three 1 mL aliquots of a 10^{-5} M solution of isobutylamine in 10^{-4} M NaOCl have been derivatized in the standard manner for 30 minutes each at different temperature: room temperature, 40, and 50°C. HPLC analysis of each solution has shown that the yield of dansyl isobutylamine decreases with increasing temperature as illustrated in Figure 1.

The effect derivatization time has on the yield of dansyl piperidine has also been examined. Seven aliquots (1 mL) of a 10^{-5} M solution were derivatized in the standard manner. At various time intervals the reactions were quenched by adding acid and analyzed for the amount of dansyl piperidine formed. As Figure 2 shows, the reaction is greater than 95% complete in 2.5 hours and is essentially complete overnight. Typically, derivatizations of dilute solutions of chloramines have been carried out overnight in this project with good reproducibility.

These yields, however, are only relative yields. Since competing reactions may occur, an absolute yield of dansyl piperidine from derivatization of a 1.0×10^{-6} M

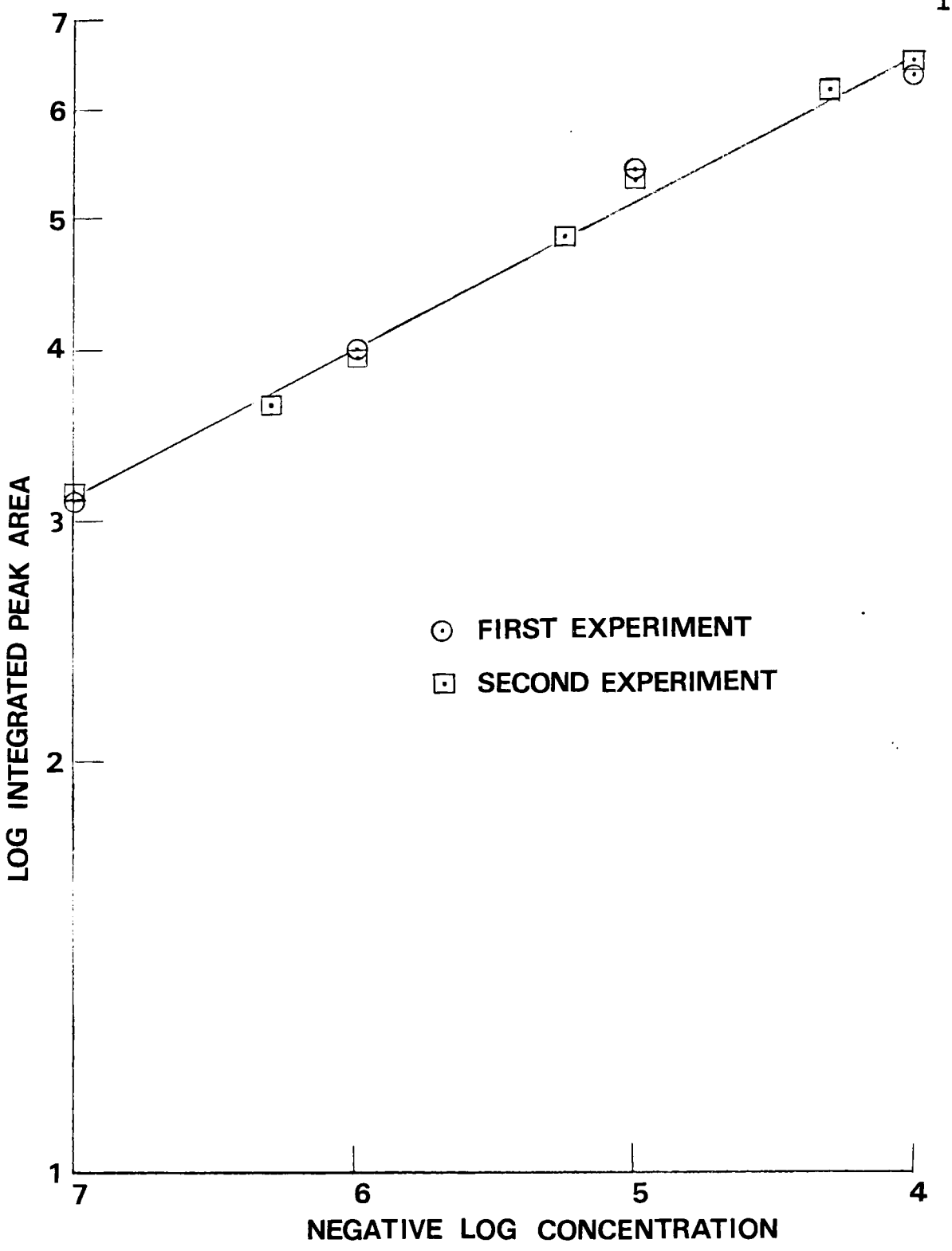

PEAK HEIGHT mm
30
20
10
20
30
40
50
TEMPERATURE °C


RELATIVE YIELD
100
80
60
40
20
1
2
3
4
15
REACTION TIME (hr.)

solution was determined. After standardization of the fluorescence detector of the HPLC was carried out, it was determined that approximately 90% of the N-chloropiperidine is converted to its dansyl derivative by this method.

That the derivatization reaction is quantitative is further supported by the fact that the amount of fluoresent dansyl derivative formed on derivatization of dilute aqueous solutions of N-chloropiperidine is directly proportional to the concentration of N-chloropiperidine in the solution over at least three orders of magnitude (Figure 3). The concentration is measured by integration of the area of the chromatographic peak.

Dansyl sulfinic acid does not itself react with amines to form sulfonamides. However, when solutions of chloramines containing unchlorinated amines are derivatized, the sulfonamide products of both the chloramine and the amine are found. The product ratios reflect the relative reactivities of the amine or the parent amine of the chloramine toward dansyl chloride. Thus, when a dilute solution equimolar in both the chloramine, N-chloropiperidine, and the unchlorinated amine, diethylamine, is derivatized in 50% aqueous acetonitrile, only dansyl piperidine is detected. However, when solutions which are equimolar in N-chloropiperidine and unchlorinated methylamine are derivatized, both dansyl piperidine and dansyl methylamine were found in a 1.6 ratio. When N-chloromethylamine is derivatized in the presence of


7
6
5
4
3
2
1
LOG INTEGRATED PEAK AREA
FIRST EXPERIMENT
SECOND EXPERIMENT
7
6
5
4
NEGATIVE LOG CONCENTRATION

equimolar piperidine both sulfonamides are again formed, but in a 8.2:1 ratio of dansyl piperidine to methylamine derivative (See Table II). Competition studies between N-chloropiperidine and pyrrolidine as well as between N-chloropyrrolidine and piperidine revealed sulfonamide products of each compound. The results are summarized in Table III.

Chromatography and Detection

Fluorescence is one of the most highly sensitive methods of detection in liquid chromatography. Solutions (1 mL) of N-chloropiperidine (10^{-6} M) can be dansylated by the techniques described here and analyzed by HPLC quite simply without need of further concentration. Solutions of N-chloropiperidine (10^{-7} M and lower) as small as 10 mL require only slight concentration.

Initially, derivatized solutions of chloramines with concentrations of 10^{-7} M and lower were extracted with chloroform which was then dried and concentrated *in vacuo*. The pH of the extracted solution was not altered from that used during derivatization (pH 10). These conditions were satisfactory for isolation of derivatized chloramines, but not N-chloramino acids. Roughly five times more dansyl leucine or dansyl glycine is isolated when the derivatized solution is adjusted to pH 4 (the isoelectric point of dansyl amino acids) before extraction. In addition, methylene chloride appears to be a slightly better (about 20%) extraction solvent than chloroform.

TABLE II. DERIVATIZATION OF AMINES WITH $DANSO_2H$ IN THE PRESENCE OF CHLORAMINES[a]

Chloramine	Amine	Dansylated Amines	Product Ratio[b,c]
N-chloropiperidine	methylamine	piperidine/methylamine	1.6
N-chloromethylamine	piperidine	piperidine/methylamine	8.2
N-chloropiperidine	pyrrolidine	piperidine/pyrrolidine	1.1
N-chloropyrrolidine	piperidine	piperidine/pyrrolidine	2.0

a. Chloramine and amine concentrations were equivalent in all cases (5×10^{-6} M). Derivatization time = 8 hours.

b. Ratio was determined by integration of fluorescence peak areas of liquid chromatogram on a μBondapak Phenyl column.

c. Corrected for relative response of fluorescence detector.

TABLE III. RELATIVE REACTIVITIES OF AMINES TO DANSYL CHLORIDE[a]

Amine #1	Amine #2	Product Ratio[b,c] Dansyl Amine #1/Dansyl Amine #2
piperidine	pyrrolidine	1.1
piperidine	methylamine	1.6

a. Reaction of equimolar amine mixtures (5.0×10^{-6} M) with 0.1 M dansyl chloride in 50% aqueous acetonitrile. Reaction was analyzed 2.0 minutes after mixing by HPLC.

b. Each entry is an average of two determinations. Ratio was determined by integration of fluorescence peak areas of liquid chromatogram on a μBondapak phenyl column.

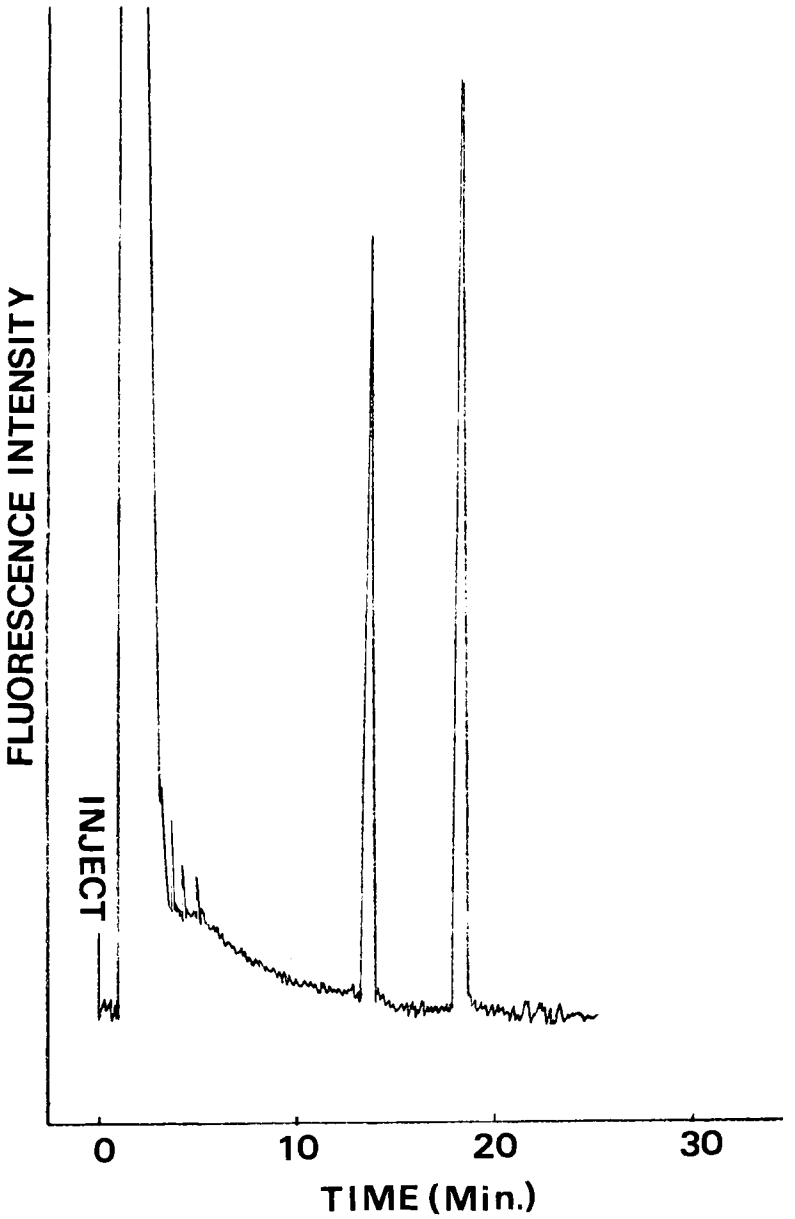
c. Corrected for relative response of fluorescence detector.

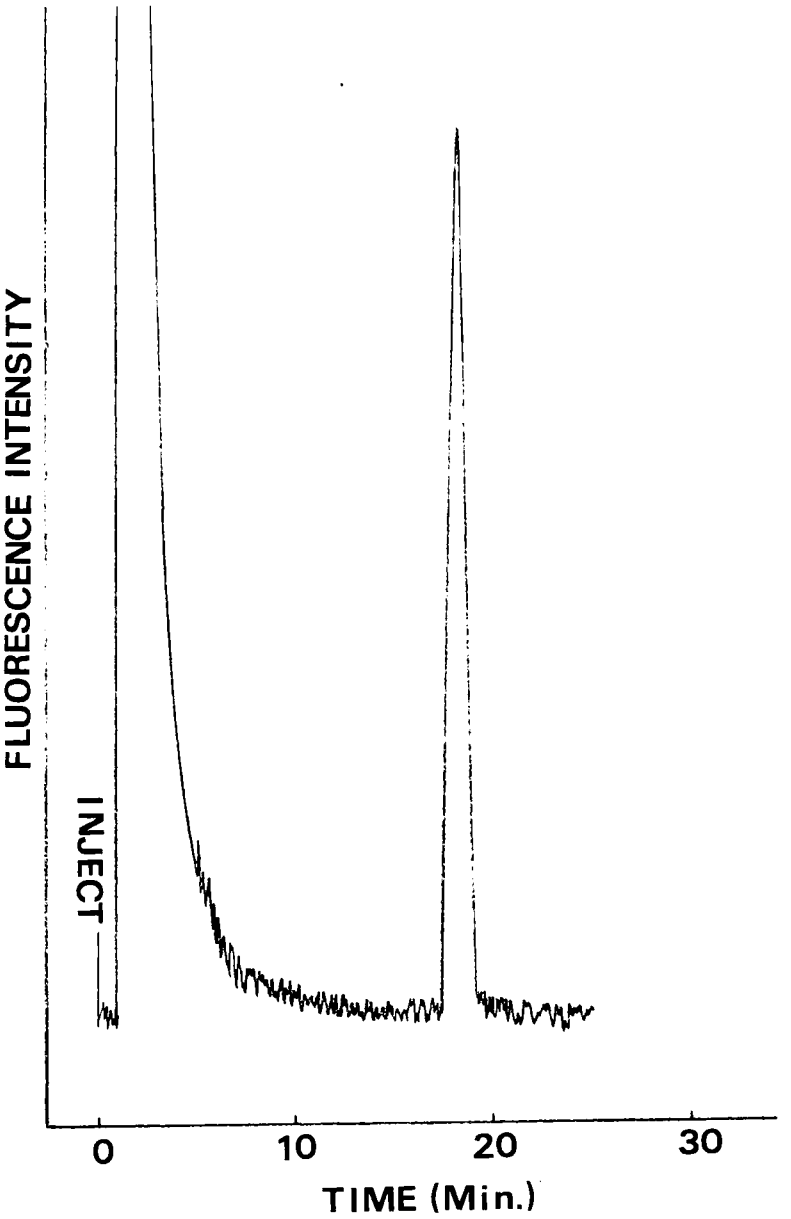
The separation of dansyl derivatives of amino acids by HPLC has been described (25-26). We have found a reversed-phase column to be most suitable for the separation of dansylated amines and Figures 4 and 5 show typical chromatograms of dansyl derivatives formed by derivatization of dilute solutions of N-chloramines. Both the HPLC conditions of Bongiovanni (32) as well as those described here have been used to characterize chlorinated drinking water.

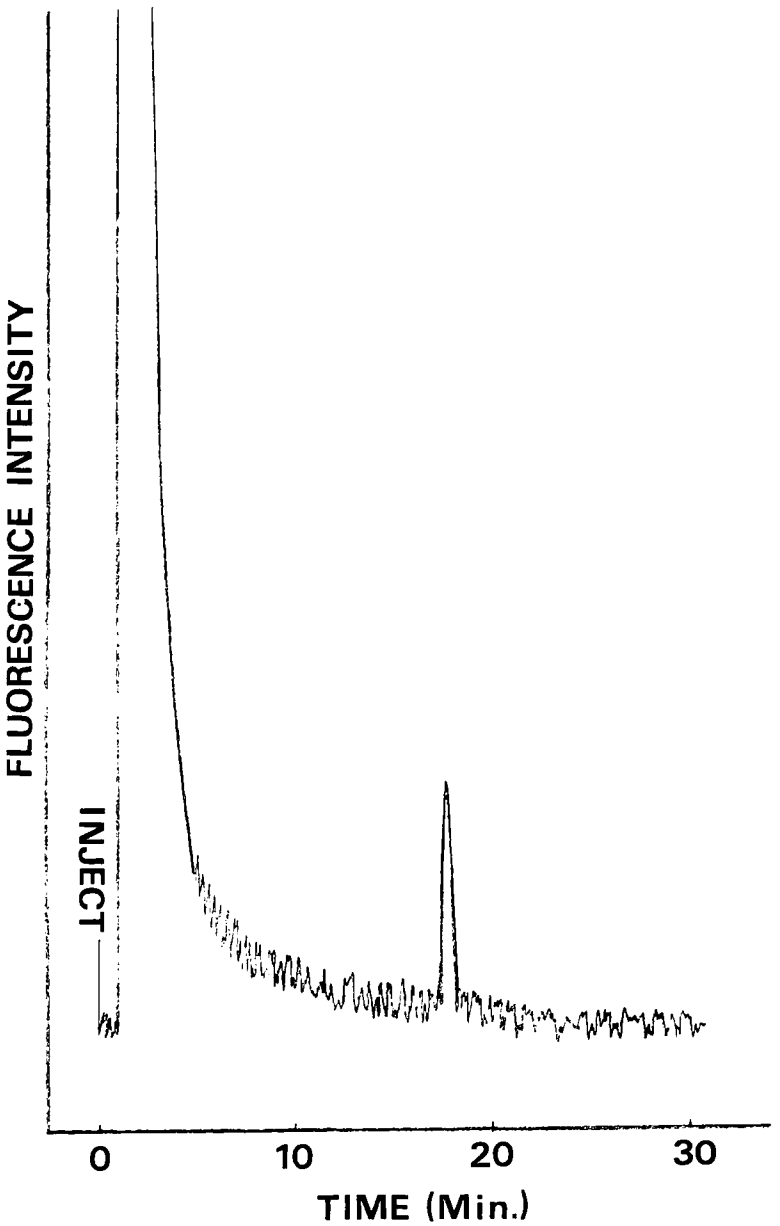
The lower limit of detectability of N-chloropiperidine by this derivatization method and the chromatographic analysis just described is approximately 1 ng (Figure 6). This allows for a signal to noise ratio of 2:1. Since concentrations of individual N-chloramines in chlorinated aqueous solutions are expected to be about 10^{-7} M, a protocol was developed for handling concentrations at this level. Figure 5 is the chromatogram of concentrate obtained from a 10^{-7} M solution of N-chloropiperidine by the method described above. It demonstrates the ease of measuring concentrations even in the 10^{-8} M range.

Analysis of Chlorinated Drinking Water

To demonstrate the applicability of this method to real-life situations, drinking water from the Norfolk Municipal Water System was obtained from the tap and treated as described above for the derivatization of chloramines in the 10^{-7} M range. Initially, a blank consisting of distilled deionized water, which had been


FLUORESCENCE INTENSITY
INJECT
0
10
20
30
TIME (Min.)


FLUORESCENCE INTENSITY
INJECT
0
10
20
30
TIME (Min.)


FLUORESCENCE INTENSITY
INJECT
0
10
20
30
TIME (Min.)

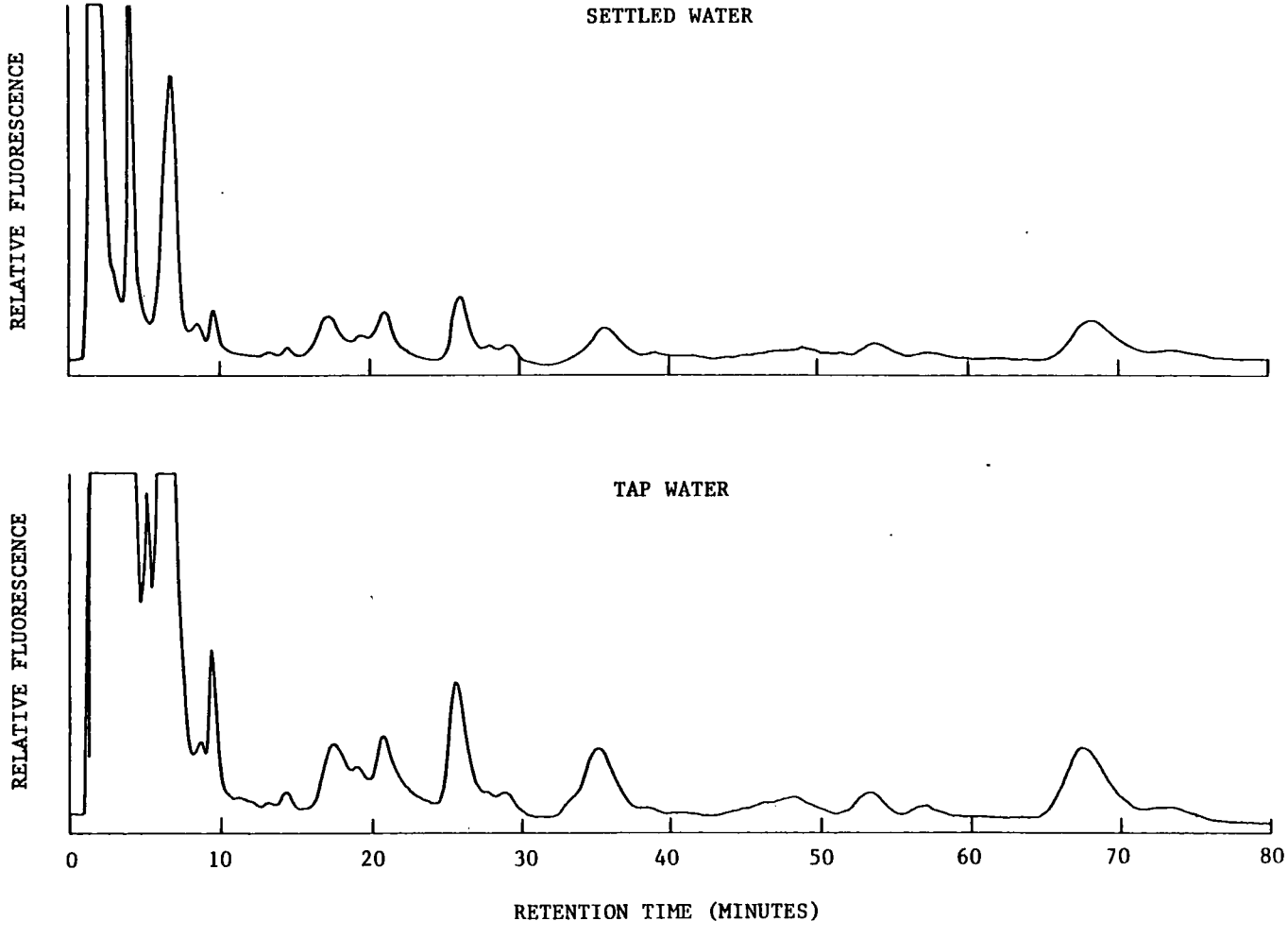
chlorinated to a free residual chlorine level of 2 ppm, was treated concurrently in the same manner. However, derivatization and analysis of the blank revealed significantly high background levels of chloramines. When chlorine-demand-free water, chlorinated to 2 ppm free chlorine, was used instead, no background levels of chloramines were found. Figures 7 and 8 showed chromatograms of concentrates of CH_2Cl_2 extracts of derivatized samples of tap water and settled water using a μBondapak phenyl column with isocratic elution [40% CH_3CN/60% H_2O (1% HOAC)] at a flow rate of 2 mL/min. The very large peak which first elutes from the column in every one of these cases corresponds to dansyl sulfinic acid and dansyl sulfonic acid. In addition to this peak the chromatograms show many other peaks suspected of being organic N-chloramine derivatives. The two chromatograms are remarkably similar except within the first ten minutes. The chromatogram of the blank shows only a peak corresponding to dansyl sulfinic and sulfonic acids.

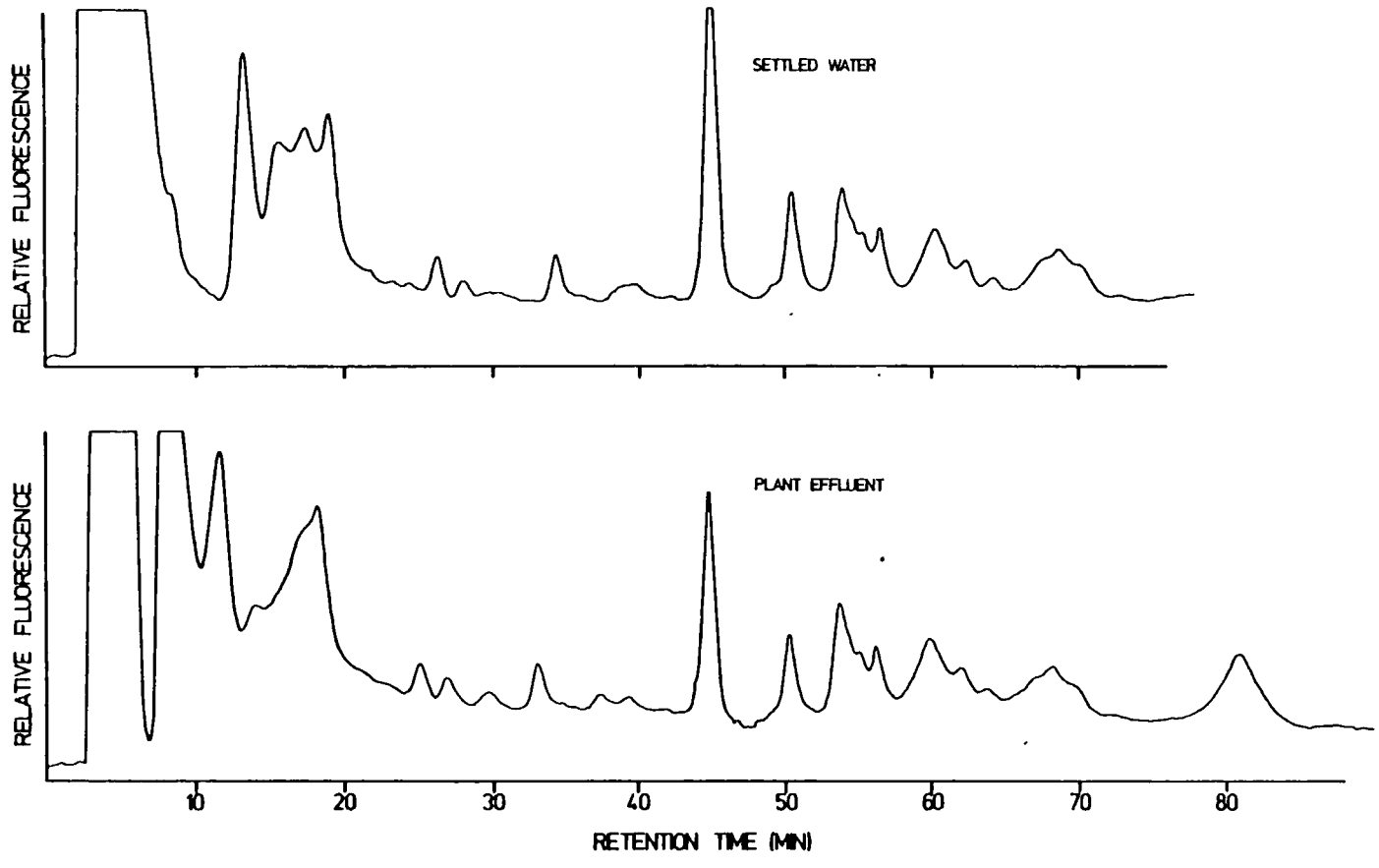
Standard dansylated amines, amino acids, and di-and tripeptides were chromatographed under these conditions (Table IV). In general, all the dansyl amino acids and peptides elute in less than 15 minutes. The less polar amines elute with longer retention times.

In order to determine whether dansyl amino acids were present in these derivatized solutions; the conditions of Bongiovanni and Dutton were used (32). Figures 9, 10, and

11 are chromatograms of derivatized drinking water samples from different points in the purification process using a uBondapak C_{18} column with isocratic elution [18% CH_3CN/ 82% H_2O (1% HOAC)] for 5 minutes followed by gradient elution (18% to 54% CH_3CN over 45 minutes curve 6) at a flow rate of 1.5 mL/min. Figure 9 is a chromatogram of derivatized settled water which contains 1.67 ppm total residual chlorine (1.52 ppm free residual chlorine). It does not differ significantly from plant effluent except in the first 20 minutes. However, tap water is considerably different and shows a number of distinctly different major peaks in the first 40 minutes of the chromatogram. The profile of the chromatograms of all three samples are remarkably similar beyond 40 minutes.

In an attempt to identify the peaks in these chromatograms, standard dansylated amines and amino acids were chromatographed (Table V).


SETTLED WATER
RELATIVE FLUORESCENCE
TAP WATER
RELATIVE FLUORESCENCE
0
10
20
30
40
50
60
70
80
RETENTION TIME (MINUTES)


SETTLED WATER
RELATIVE FLUORESCENCE
PLANT EFFLUENT
RELATIVE FLUORESCENCE
10
20
30
40
50
60
70
80
RETENTION TIME (MIN)

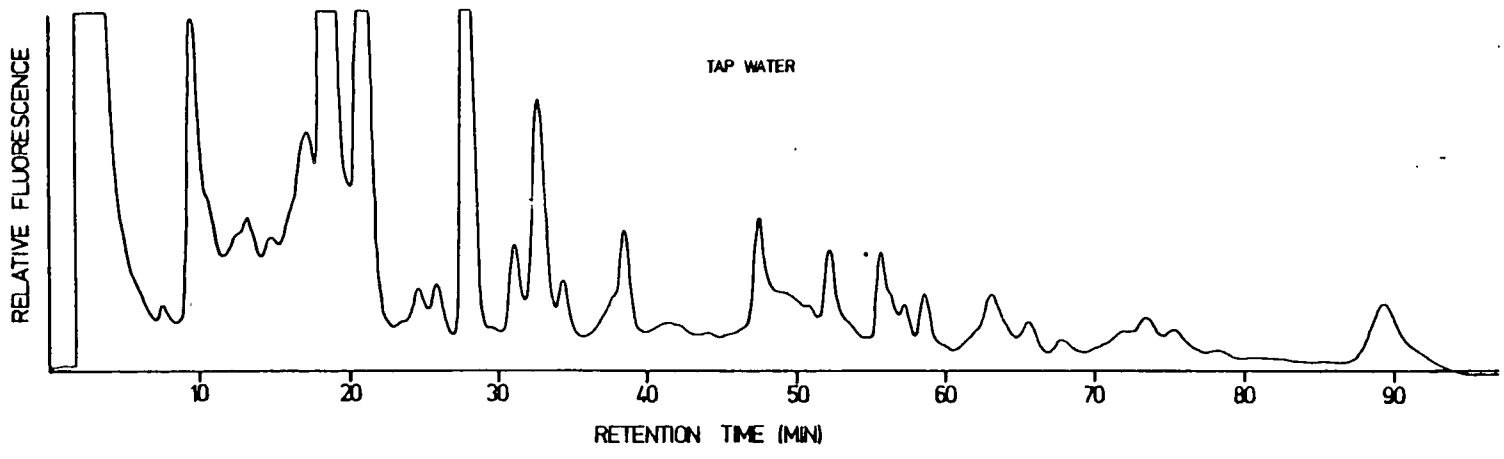

TAP WATER
RELATIVE FLUORESCENCE
10
20
30
40
50
60
70
80
90
RETENTION TIME (MIN)

TABLE IV. RETENTION TIMES OF DANSYL DERIVATIVES ON μBONDAPAK PHENYL COLUMN

Dansyl Amino Acids, Amines, and Di-and Tripeptides	Retention Time (min.)
Dansyl-Isoamylamine	23.7
Dansyl-2-Methylbutylamine	23.4
Dansyl-B-Phenylethylamine	22
Dansyl-Isobutylamine	16.5
Dansyl-L-Phenylalanyl-L-Leucine	13.4
Dansyl-Diethylamine	13.0
Dansyl-Pyrrolidine	12.8
Dansyl-DL-Leucyl-Glycly-DL-Phenylalanine	9.7
Dansyl-Propylamine	9.5
Dansyl-1,5-Diaminopentane	8.4
Dansyl-Phenylalanine	8.2
Dansyl-1,4-Diaminobutane	7.3
Dansyl-Ethylamine	7.0
Dansyl-Valine	6.2

TABLE IV (cont'd)

Dansyl Amino Acids, Amines and Di-and Tripeptides	Retention Time (min.)
Dansyl-Methylamine	5.8
Dansyl-L-Isoleucyl-Glycine	4.8
Dansyl-L-Alanyl-L-Valine	4.8
Dansyl-r-Amino-n-Butyric Acid	4.3
Dansyl-Alanine	4.2
Dansyl-Amide	4.0
Dansyl-Glycine	3.7
Dansyl-Glycyl-Glycine	2.8

TABLE V. RETENTION TIMES OF DANSYL DERIVATIVES ON μBONDAPAK C_{18} COLUMN

Dansyl Amino Acids and Amine	Retention Time (min.)
Dansyl-L-Isoleucine	33.4
Dansyl-L-phenylalanine	33.3
Dansyl-L-Methionine	27.8
Dansyl-L-Valine	27.3
Dansyl-L-Proline	25.8
Dansyl-L- -Alanine	14.2
Dansyl-L-Threonine	13.2
Dansyl-L-Glycine	10.6
Dansyl-Amide	9.9
Dansyl-L-Lysine	9.5
Dansyl-L-Serine	9.2
Dansyl-L-Glutamic Acid	8.0

DISCUSSION

The analytical procedure described here is the first method which utilizes the reactivity of chloramines for their identification and quantitation in dilute aqueous solution. The yield of dansyl derivatives of stable chloramines is high and proportional to the concentration of the N-chloramine in the solution being derivatized. Because the dansyl derivatives are fluorescent, the derivatization technique, in conjunction with high performance liquid chromatography and fluorescence detection, is sufficiently sensitive for the analysis of chloramines at environmentally significant concentrations.

There are no known interferences to the method, at least from the type of organic compounds which have been identified in chlorinated drinking water, specifically the halocarbons. Thus, chloroform, methyl iodide, and α-chloroacetone (all active substrates for nucleophilic displacement reactions) fail to yield a chromatographically distinguishable product under the conditions described here. Although, sulfinate salts have been shown to react with alkyl halides to form sulfones, the reaction requires high temperature, long reaction time, and organic solvents to produce even fair yields of sulfones (30). The conclusion, therefore, is that such a reaction is likely to be quite slow in an aqueous medium at room temperature and consequently will not give sulfone derivatives with $DANSO_2H$ under the conditions described here.

Scully and Liu (23) have noted that when derivatization is carried out in 100% aqueous solvent, i.e., no co-solvent used, no sulfonamide product is formed when the concentration of the chloramine is less than 10^{-4} M. This is easily explained by the mechanism elucidated by Scully and Bowdring (22) (see Scheme II).

Scheme II

$$DANSO_2^- + R_2NCl \xrightarrow{H_2O} DANSO_2Cl + R_2NH \longrightarrow DANSO_2NR_2$$

$$DANSO_2Cl \xrightarrow{H_2O} DANSO_3^-$$

The first step involves nucleophilic attack of the sulfinate anion on the chloramine chlorine to form a sulfonyl chloride and an amine. The second step of the reaction is the well-characterized Hinsberg reaction of an amine with an arenesulfonyl chloride to form an arenesulfonamide. However, the concentrations of both the sulfonyl chloride and amine intermediates depend on the concentration of the chloramine alone when a large excess of sulfinate is used. Therefore, at low concentrations of chloramines (10^{-4} M) the rate of hydrolysis of the sulfonyl chloride, a first order process (31) is sufficiently high to compete effectively with the second order process occurring in the Hinsberg reaction. The Hinsberg reaction is usually carried out under very basic conditions, but even when the pH is adjusted to 10.6 no product is formed. In water the amine and sulfonyl chloride must be rapidly solvated and, because of extensive protonation and hydrogen bonding in pure water,

the amine is less nucleophilic.

Addition of a dipolar, aprotic co-solvent enhances the reaction of the amine with the sulfonyl chloride either by suppressing hydrolysis of the sulfonyl chloride or by enhancing the nuclephilicity of the amine. The solvent deuterium isotope data of Swain (13) suggests that solvent reorganization in the transition state of hydrolysis of benzenesulfonyl chloride is minimal. Hence, initial-state interaction between the sulfonyl oxygens and the solvent is probably insignificant. It is therefore probably not much affected by a co-solvent. If anything, the presence of a co-solvent would enhance the nucleophilicity of the hydroxide ion which would hydrolyze the sulfonyl chloride. On the other hand, quantitative formation of dansyl piperidine from derivatization of dilute aqueous solutions of N-chloropiperidine suggests that the nucleophilicity of the amine intermediate is greatly enhanced by suppression of its solvation. Such a phenomenon has been noted in many nucleophilic reactions when a protic solvent has been replaced by a polar aprotic one (34).

While amines themselves do not react directly with $DANSO_2H$ to form sulfonamides, derivatives of both amines and chloramines are formed when solutions are derivatized which contain an amine of one structure and a chloramine of another. Thus, when a dilute aqueous solution of pyrrolidine and N-chloropiperidine, which is equimolar in each, is

derivatized with $DANSO_2H$ both dansyl piperidine and dansyl pyrrolidine are formed. There are two possible explainations for this. First, it is possible that the chlorine is exchanged between the two amino nitrogen substrates until an equilibrium mixture of the two chloramines is established. While there is evidence that N-chloramines can donate their chlorine atom to an amine of greater basicity, e.g., NH_2Cl with organic amines (35, 36) or N-chlorosuccinimide with organic amines (18), there are no reported evidence that this takes place among amines of similar basicities. There is more data available to support the second possibility, namely, that, when a chloramine reacts with $DANSO_2H$ to form the intermediate $DANSO_2Cl$, all unchlorinated amino compounds in solution compete to form a sulfonamide. Thus, when solutions equimolar in N-chloropiperidine and methylamine are derivatized with $DANSO_2H$ in the standard manner, a 1.6:1 ratio of dansyl piperidine to dansyl methylamine is formed. Similarly, when a solution of N-chloropiperidine and pyrrolidine of equal concentration is derivatized, a 1.1:1 ratio of dansyl piperidine to dansyl pyrrolidine is formed (see Table II). These products ratios are almost identical to the kinetic ratios (from initial rates) of products found when piperidine, pyrrolidine, and methylamine are allowed to compete for reaction with dansyl chloride. This suggests the mechanism shown in Scheme III where k_1 is much greater than either

k_2 or k_3 and the product ratio is $k_2 [R_2NH] / k_3 [\overset{*}{R}NH]$.

When the chlorine atom is attached to the nitrogen of an amine other than piperidine, the data are not as clean. Thus, when solutions of equimolar piperidine and N-chloromethylamine or piperidine and N-chloropyrrolidine are derivatized, the product ratios reflect a greater amount of dansyl piperidine than predicted from the kinetic ratio of reactivities of the amines with dansyl chloride (see Table III). Interestingly, the amount of dansyl piperidine formed when N-chloromethylamine is the competing chloramine is about twice as much as when N-chloropiperidine and methylamine are derivatized. No satisfactory mechanistic explanation is apparent. One very basic difference in the experiments is that when N-chloropiperidine was used as the chloramine it was synthesized and purified by distillation before being used in the experiments. N-chloromethylamine and N-chloropyrrolidine cannot be purified in this manner, because they decompose. Consequently, they have been generated in situ from equimolar quantities of the amine and sodium hypochlorite. It is possible that more extensive oxidation of the amines could take place when the reagents are mixed. Typically, only 60% isolated yields of N-chloropiperidine can be isolated pure from the reaction of piperidine with sodium hypochlorite.

These results do not seriously affect the analysis of organic N-chloramines in chlorinated drinking water, since all primary and secondary amines are chlorinated and none

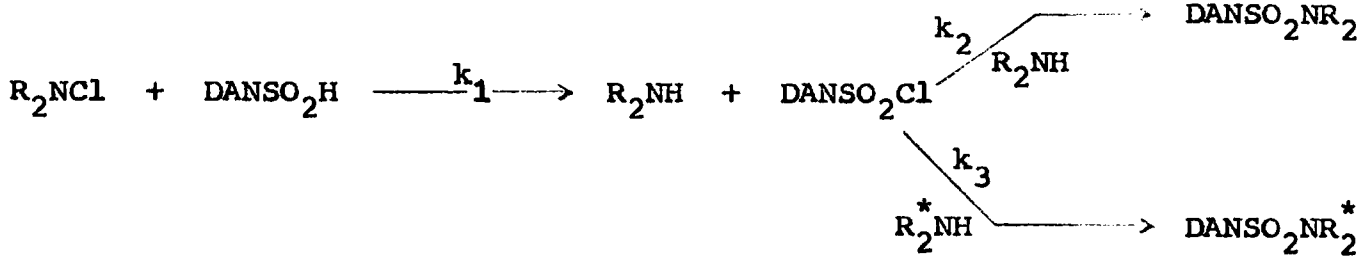
are unchlorinated. With one exception the sulfonamides formed are representative of the kinds of N-chloramines present as well as their concentration. Secondary N-chloramines may not be quantitatively derivatized since their parent amines appear to be less reactive toward dansyl chloride. Since N-chlorinated amino acids and proteins are suspected to be the major portion of amino nitrogen compounds in chlorinated drinking water and since these are primary amino compounds, derivatization of drinking water is expected to yield a mixture of sulfonamide derivatives which is roughly representative of the chloramines in solution.

However, in addition to dansyl amine derivatives, it may be possible that non-amino nucleophiles such as phenols and alcohols may also be derivatized, though in general alcohols are for less nucleophilic than amines.

With these limitations in mind derivatization of chlorinated drinking water was undertaken. In addition, the effect of each stage of the purification process on the concentration of chloramines was examined by comparing settled water, plant effluent, and tap water from the same source. The settled water and plant effluent were obtained from the Lambert's Point Water Treatment Plant of the City of Norfolk Utilities Department because it supplies Old Dominion University. The water supplied to this plant is obtained from Western Branch reservoir.

Chromatography of derivatized settled water and tap

SCHEME III


R2NCl + DANSO2H
k1
R2NH + DANSO2Cl
k2
R2NH
DANSO2NR2
k3
R2*NH
DANSO2NR2*

water on a μBondapak Phenyl column revealed numerous fluorescent compounds. That these were dansyl derivatives was confirmed by two experiments. The water samples were derivatized in the usual manner except that $DANSO_2H$ was omitted. After extraction and concentration chromatography revealed no fluorescent peaks. Furthermore, the same basic chromatogram was obtained using both 340 nm and 250 nm light as the excitation source in the fluorescence detector. At 340 nm dansyl amines have a more selective though weaker absorbance than at 250 nm.

A cursory comparison of the two chromatograms (Figures 7 and 8) shows the same basic profile for both water samples, especially in the compounds which elute from 15 to 80 minutes. A comparison of the retention times of dansyl derivatives of small chain aliphatic amines suspected of contaminating drinking water (see Table IV) showed poor correlation with peaks present in the derivatized drinking water. Dansyl piperidine was the only one examined which did correlate.

A more careful examination shows considerable differences in the first 15 minutes of the chromatograms of settled water and tap water. The peaks in the tap water chromatogram appear to be considerably larger in the first 15 minutes. An examination of Table IV reveals that the dansyl amino acids and small dansyl di - and tri-peptides all have retention times of less than 15 minutes on a μBondapak Phenyl column.

The conditions of Bongiovanni and Dutton (32) are more suitable for the analysis of dansyl amino acids. Therefore new samples of settled water, plant effluent, and tap water were obtained in mid-May, 1982, analyzed for free and combined residual chlorine levels, and derivatized with $DANSO_2H$. In the samples which gave the chromatograms shown in Figures 9 and 10 settled water and plant effluent had identical combined residual chlorine levels (chloramine levels) of 0.15 ppm while tap water had a level of 0.25 ppm. This higher chloramine level appears to correlate quite well with the larger quantities of suspected chloramines as determined by the relative heights of peaks shown in the chromatograms of the derivatized water samples. There are many similarities in the chromatograms of the derivatized settled water and plant effluent. However, the tap water which also has the higher combined residual chlorine level, gives a chromatogram with considerably higher peaks than the other two samples. The retention times of some of the peaks correspond to the dansyl derivatives of some of the non-polar amino acids but many do not correspond to any known compounds examined (see Table V).

One unexpected observation was the absence of dansyl amide, the derivative of inorganic chloramine, from the chromatograms. This may be an artifact of the analytical method and requires further investigation.

Although GC/MS or HPLC/MS analysis will be required

to identify the dansyl derivatives obtained from tap water, several implications can be drawn from the chromatograms shown above. For instance, the major portion of the dansyl derivative obtained from tap water, as determined by relative fluorescence peak heights, have a polarity more like dansyl amino acids and dansyl peptides than dansyl derivatives of aliphatic amines. However, the relative peak heights may be misleading since higher peaks may be due to a higher quantum of fluorescence for these derivatives than the less polar dansyl derivatives or else the parent amines may be more nucleophilic and give a higher reaction yield with dansyl chloride. On the other hand, there is a dramatic increase in the number of the more polar derivatives and in their quantity as the water proceeds through the purification system to the tap. The increase of these peaks corresponds to a 60% increase in the combined residual chlorine levels in the water. This is suggestive of the cleavage by hypochlorite of proteins which have only one amino terminus each and conversion of their amide nitrogens to primary amines and further to N-chloramino acids and terminal N-chlorinated peptides. Since the profile of the chromatogram is reproducible for different samples of tap water taken within the span of a few weeks it may be that certain small peptides of a particular amino acid sequence are unusually resistant to oxidation by hypochlorite.

EXPERIMENTAL

General:

High performance liquid chromatography was performed on a Waters Model 204 Liquid Chromatograph with a Model U6K Universal Injector. A Model 6000A Solvent Delivery System was used in conjunction with a Model M-45 pump and a Model 660 Solvent Programmer (Waters Associates Inc., Milford, Mass.). For chromatography involving all model studies and early tap water samples, an eluant of 40% acetonitrile/60% H_2O (1% acetic acid) at a flow rate of 2 mL/min. was used isocratically. A reversed-phase high performance liquid chromatographic column of stainless steel (3.9 mm I.D. x 300 mm) packed with μBondapak Phenyl (Waters Associates) was used in these studies. Later chromatograms of derivatized tap water were obtained on a μBondapak C_{18} column (3.9 mm I.D. x 300 mm) using isocratic elution [18% acetonitrile/82% H_2O (1% acetic acid)] for 5 minutes followed by gradient elution (18% to 54% acetonitrile over 45 minutes, curve 6) at a flow rate of 1.5 mL/minute. A Kratos Schoeffel Instruments Model FS 970 Spectrofluoromonitor was used at either 250 nm or 340 nm to detect all dansyl derivatives. A Shimadzu Recording Data Processor Model Chromatopac C-RIB was used to integrate the peak areas of liquid chromatograms using the following conditions: WIDTH 5, SLOPE 500, DRIFT 0, T-DBL 0, ATTEN 2, SPEED 10, METHOD A1, SPL WT 100, IS WT 1. Infrared spectra were recorded on a Perkin Elmer Model 137

Spectrophotometer. A JEOL Model FX 90Q Fourier Transform NMR Spectrometer was used to record the NMR spectra. Melting points were recorded on a Thomas Hoover melting point apparatus and are not corrected.

Materials

All materials used were reagent grade or better unless otherwise specified. Chemicals used as received from Mallinckrodt include dichloromethane, methylamine hydrochloride, chloroform, sulfuric acid, and ammonium hydroxide; from Aldrich include dansyl chloride, pyrrolidine, and piperidine; from Baker include sodium sulfite, acetonitrile, glacial acetic acid, potassium phosphate, and dimethylamine; from Fisher include chlorine, and sodium bicarbonate; from Sigma include all dansyl amino acids. Phenylarsine oxide (0.00564N) was used as received from Ricca Chemical Co., and tetrahydrofuran (UV grade) used as received from Burdick & Jackson and was stored under a blanket of nitrogen. Commercial grade sodium hypochlorite (Clorox) was used as received and was standardized by iodometric titration. Technical grade ethylamine, isopropylamine, N-propylamine, n-butylamine, and isobutylamine were used as received from Virginia Chemicals Co.

Methods:

Synthesis of 5-Dimethylamino-1-naphthalenesulfinic Acid Monohydrate ($DANSO_2H$, Dansyl Sulfinic Acid)

To vigorously stirred solution of sodium sulfite (10.7 g) in 50 mL of water warmed to 70° was added dansyl chloride (5g). The reaction mixture was kept at a temperature of 70-80° for five hours. Dansyl sulfinic acid was precipitated from the product mixture by acidifying the solution to pH 4 with sulfuric acid, following cooling and filtering. The precipitate was dissolved in a basic ethanol solution, filtered and acidified to yield a white precipitate. After filtration, washing with ethanol and drying in a vacuum desiccator, dansyl sulfinic acid was obtained about 54% yield as a pure white material which melted with decomposition at 237-239°.

IR: cm^{-1} 3700 (O-H stretch); 1620, 810, 775, 745 (aromatic H); 1470, 1380 ($-CH_3$); 1170 (S-O stretch); 1000, 920 (C-N stretch)

^{1}HNMR: ppm (DMSO - d_6); 8.3 - 8.0 (m, aromatic, 3H); 7.7 - 7.5 (m, aromatic, 3H); 3.6 (S, H_2O); 3.1 (S, $N(CH_3)_2$, 6H); 2.9 (S, SO_2H, 1H); 2.5 - 2.4 (m, DMSO)

^{13}CNMR: ppm (DMSO - d_6); 127.2, 124.1, 121.8, 116.9, 114.8 (aromatic C); 44.8 (methyl C); 41.8, 41.3, 40.4, 39.5, 38.6, 37.6 (DMSO)

Synthesis of 5-Dimethylamino-1-naphthalenesulfinic Acid, Sodium Salt

5-Dimethylamino-1-naphthalenesulfinic acid monohydrate (0.5, 1 g) was slurried in ethanol (15 mL). Sodium hydroxide (50% aqueous solution) was added dropwise until

all solid had dissolved. The resulting solution was concentrated to about 5 mL on a rotary evaporator during which a fine, white powder precipitated. The solid was filtered and washed with acetonitrile and dried in a vacuum desiccator. It had a m.p. $> 340^{\circ}C$.

IR: cm^{-1} 2900, 1570, 860, 810, 770, 720, 680 (aromatic H); 1480 - 1380 ($-CH_3$); 1280, 1040 - 1020, 960, 930 (C-N stretch); 1175, 1125 (S=O stretch)

$^{1}HNMR$: ppm (D_2O); 8.4 - 7.2 (m, aromatic, 6H); 4.8 (S, H_2O); 2.9 (s, $N(CH_3)_2$, 6H)

$^{13}CNMR$: ppm (D_2O); 130, 128.5, 128, 123, 121.5 (aromatic C); 47.5 (methyl C)

Preparation of Dansyl Sulfinic Acid Reagent Solution

Sodium bicarbonate (1.05 g, 12.5 mmoles) was dissolved in about 5 mL deionized water. Dansyl sulfinic acid (0.0633 g, 0.25 mmoles) was added and the resulting solution was diluted to 25 mL.

Synthesis of N-Chloropiperidine

Sodium hypochlorite (224 mL of a 5% aqueous solution) was saturated with sodium chloride in a 300 mL Erlenmeyer flask. The mixture was placed in an ice bath and allowed to cool to below $10^{\circ}C$. Next, piperidine (17 g, 200 mmoles) was added slowly to the mixture. After the piperidine was added and allowed to mix thoroughly, the mixture was placed in a 500 mL separatory funnel. The organic layer was withdrawn, put into a 25 mL round-bottomed flask, dried with

calcium chloride, and placed in the freezer. It was distilled at 45-48°C/20 mm before use.

Synthesis of Dansyl Piperidine from Piperidine

To a solution of dansyl chloride (2 g, 7.4 mmoles) and acetonitrile was added piperidine (0.63 g, 7.4 mmoles) and the temperature of the stirred reaction mixture was kept at room temperature for five hours, then cooled to below 0°C. The precipitated product was filtered and recrystallized from acetonitrile/water, mp 111.5 - 112°C (lit. mp 110°C).

IR: cm^{-1} 2900 - 2800, 1570, 826, 688 (aromatic H); 1430, 1380, 1330 ($-CH_3$); 1300, 1220, 1050, 930 (C-N stretch); 1150, 1125 (S=O stretch); 778 - 765 ($-CH_2-CH_2-CH_2-CH_2-$)

^{1}HNMR: ppm ($CDCl_3$); 8.6 - 8.1 (m, aromatic, 3H); 7.6 - 7.1 (m, aromatic, 3H); 3.2 (m, piperidine, 4H); 2.8 (S, N $(CH_3)_2$, 6H); 1.5 (m, piperidine, 6H)

^{13}CNMR: ppm ($CDCl_3$); 130.2, 127.7, 123.0, 119.9, 115.1 (aromatic C); 46.2 (methyl C); 45.2, 25.3, 23.6 (piperidinic C)

Synthesis of Dansyl Piperidine from N-Chloropiperidine

Dansyl sulfinic acid (1.8 g, 7.1 mmoles) was dissolved in 20 mL of 0.5 M sodium bicarbonate and the solution adjusted to pH 8 with sodium hydroxide. N-Chloropiperidine (0.85 g, 7.1 mmoles) in acetonitrile (20 mL) was added and the solution was stirred at room temperature for five hours.

The solvent was removed on a rotary evaporator. The crude product was washed with water and recrystallized slowly from acetonitrile. The purified compound had a melting point and mixed melting point identical to that of the dansyl piperidine prepared from piperidine and dansyl chloride.

IR: cm^{-1} 2950 - 2780, 1570, 825, 790, 690 (aromatic, H); 1430, 1380, 1340 ($-CH_3$); 1290, 1200, 1050, 925 (C-N stretch); 1140, 1130 (S=O stretch); 770 ($-CH_2-CH_2-CH_2-CH_2-$)

^{1}HNMR: ppm ($CDCl_3$); 8.6 - 8.1 (m, aromatic, 3H); 7.6 - 7.1 (m, aromatic, 3H); 3.2 (m, piperidinic, 4H); 2.8 (S, $N(CH_3)_2$, 6H); 1.5 (m, piperidinic, 6H)

^{13}CNMR: ppm ($CDCl_3$); 130.2, 127.7, 123.0, 119.9, 115.1 (aromatic C); 46.2 (methyl C); 45.2, 25.3, 23.6 (piperidinic C)

Effect of pH on the Derivatization of N-Chloropiperidine at a Concentration of 1.0×10^{-4} M in the Absence of a Co-solvent

A 100 mL stock solution of 10^{-2} M N-chloropiperidine was prepared in deionized water and a 50 mL stock solution of 10^{-2} M $DANSO_2H$ was prepared in 0.1 M KH_2PO_4. The pH's of three different 12 mL aliquots of the latter solution were adjusted to pH 5, 7, and 10. Then a 10 mL aliquot of each solution of different pH was pipetted into three

separate test tubes and 100 uL of the chloramine solution was added to each. The tubes were covered with Parafilm and allowed to react 30 minutes. Analysis of each tube by HPLC revealed that dansyl piperidine was formed only in the solution adjusted to pH 10.

Unreactivity of $DANSO_2H$ toward N-Chloropiperidine at a Concentration of 5.0×10^{-5} M in the Absence of a Cosolvent

A 100 mL stock solution of 10^{-4} M N-chloropiperidine was prepared in deionized water and a 10 mL stock solution of 10^{-2} M $DANSO_2H$ was prepared in 0.5 M $NaHCO_3$. The pH's of four different 1 mL aliquots of the letter solution were adjusted to pH 8, 9, 10, and 11. Then 1 mL of the chloramine solution was added to each and the pH of the mixtures determined (pH 8.10, 8.93, 9.90, and 10.55). The tubes were sealed with Parafilm and allowed to react 30 minutes. Analysis of each tube by HPLC revealed that no dansyl piperidine was formed in the solution.

Derivatization of Inorganic Chloramine in Aqueous Solution

Ammonium chloride (0.0054 g, 0.1 mmole) was diluted to 100 mL with chlorine-demand-free water in a volumetric flask. Solutions with concentrations of 1.0×10^{-7} M, 5×10^{-6} M, 1.0×10^{-6} M, 5×10^{-5} M, and 10^{-5} M were prepared by appropriate dilution of the stock solution with chlorine-demand-free water containing 2 ppm free chlorine. Solutions were derivatized in the standard manner described above for the appropriate concentration used. The

derivatized solutions were analyzed by HPLC. The chromatogram revealed that no dansyl derivative was present.

Effect of pH on the Derivatization of N-Chloropiperidine

A 100 mL stock solution of 1.0×10^{-5} M N-chloropiperidine was prepared in deionized water and a 50 mL stock solution of 10^{-2} M $DANSO_2H$ was prepared in 0.5 M $NAHCO_3$. The pH's of three different 10 mL aliquots the former solution were adjusted to pH 5, 7, and 9. At 1 mL aliquot of each solution of different pH was pipetted into three separate test tubes. Then 1 mL of acetonitrile and 100 uL of the dansyl sulfinic acid solution were added to each. The tubes were sealed with Parafilm and allowed to react 30 minutes. Analysis of each tube by HPLC revealed that dansyl piperidine was formed only in the solution adjusted to pH 9.

Effect of the Variation of Reaction Temperature on Derivatization

Isobutylamine (0.739 g, 0.01 mole) was diluted to one liter marked with deionized water in a volumetric flask. A solution of N-chloroisobutylamine with a concentration of 1.0×10^{-5} M was prepared by appropriate dilution of the stock solution with a 1.0×10^{-4} M NaOCl solution. Three 1 mL aliquots of a 1.0×10^{-5} M solution were derivatized in the normal manner for 30 minutes each at room temperature (approximately $20^{\circ}C$), $40^{\circ}C$ and $50^{\circ}C$. HPLC analysis of each solution showed that the yield of dansyl isobutylamine

decreases with increasing temperature. This experiment was repeated two additional times with similar results.

Standard Procedure for Derivatization of Dilute Solutions of Organic N-Chloramines with $DANSO_2H$

1. Aqueous solutions of chloramines between 10^{-6} M and 10^{-4} M.

The following reagents were mixed in a small test tube in the order and quantity given: $NaHCO_3$ (42 mg): 1 mL of chloramine solution, 1 mL acetonitrile or tetrahydrofuran, 100 uL of a 0.2 M solution of $DANSO_2H$ in 0.5 M $NaHCO_3$, and 2 drops of 1 M NaOH. The reagents were mixed thoroughly and the test tube was sealed and placed in the dark for at least 3 hours before HPLC analysis.

2. Aqueous solutions of chloramine of less than 10^{-6} M.

All quantities given above were increased by a factor of 10. After standing for at least 3 hours and typically overnight the reaction solution was extracted with chloroform (4 x 5 mL): the chloroform was dried over anhydrous Na_2SO_4, decanted, and concentrated under vacuum. The residue was dissolved up to a total volume of 0.5 mL and analyzed by HPLC.

Standard Procedure for Derivatization of Dilute Solutions of Amines with Dansyl Chloride

The following procedure was used to derivatize aqueous solutions of amines with concentrations greater than or equal

to 1.0×10^{-6} M. The following reagents were mixed in a small test tube: $NaHCO_3$ (42 mg), 1 mL of amine solution, 1 mL of a 0.2 M solution of dansyl chloride in acetonitrile or tetrahydrofuran, and 2 drops of 1 M NaOH. The reagents were mixed thoroughly, and the test tube was sealed and placed in the dark for at least 3 hours before HPLC analysis.

Quantitation of Dilute Solutions of N-Chloropiperidine

Linearity of Detector Response in the Range of 10^{-4} - 10^{-7}M

N-Chloropiperidine (0.12 g, 10 mmoles) was diluted to the mark with deionized water in a 100 mL volumetric flask. Solutions with concentrations of 1.0×10^{-7} M, 5.0×10^{-7} M, 1.0×10^{-6} M, 5.0×10^{-6} M, 1.0×10^{-5} M, 5.0×10^{-5} M, and 1.0×10^{-4} M were prepared by appropriate dilution of the stock solution. Solutions were derivatized in the standard manner described above for the appropriate concentration used. The derivatized solutions were analyzed by HPLC and the areas of the dansyl piperidine peaks integrated using a Shimadzu recording integrator.

The Percent Yield of Dansyl Piperidine Formed as a Function of Reaction Time

Separate aliquots of a 1×10^{-5} M solution of N-chloropiperidine were derivatized in the normal manner and allowed to react 30, 80, 100, 120, 150, 270 minutes or overnight. The solutions were analyzed by HPLC and the peak areas of the dansyl piperidine formed in each reaction time determined by integration.

Absolute Yield of Dansyl Piperidine from a 1.0×10^{-6} M Solution of N-Chloropiperidine

A solution of 1.0×10^{-6} M N-chloropiperidine was derivatized overnight with dansyl sulfinic acid in the normal manner. The derivatized solution was analyzed by HPLC and the peak area for dansyl piperidine integrated. An equivalent volume of a 1.0×10^{-6} M standard solution of dansyl piperidine (prepared from pure crystalline dansyl piperidine) was analyzed by HPLC and the peak area of the standard compared with the derivatized solution of the chloramine. A ratio of the peak areas revealed that 90% of the theoretical amount of dansyl piperidine was formed by derivatization of the chloramine.

Lower Limit of Detectability of N-Chloropiperidine

N-Chloropiperidine (0.12 g, 1.0 mmole) was diluted to the mark with deionized water in a 100 mL volumetric flask. Solutions with concentrations of 1.0×10^{-5} M, 1.0×10^{-6} M, 1.0×10^{-7} M, and 1.0×10^{-8} M were prepared by appropriate dilution of the stock solution. 1 mL of each solution was derivatized in the normal manner. Dansyl derivatives were analyzed by HPLC with the following detector conditions: range 0.01 uL, sensitivity 4.0, time constant 9.0 secs, and the excitation filter removed. A peak with a height of seventy percent of full scale was obtained by injecting 500 uL of a derivatized solution of 1.0×10^{-6} M, N-chloropiperidine. Injection of 500 uL of a derivatized solution of

1.0×10^{-7} M N-chloropiperidine gave a peak with a height of only seven percent of full scale and a signal to noise ratio of 5.

Unreactivity of $DANSO_2H$ toward Dilute Solutions of Chloroform, Methyl Iodide, α-Chloroacetone, N-Chloro-2'-Deoxyguanosine, and N-Chloro-2-Deoxycytidine

Solutions of chloroform (0.119 g, 0.001 mole), methyl iodide (0.142 g, 0.001 mole), and α-chloroacetone (0.0925 g, 0.001 mole) were prepared in 100 mL volumetric flasks. The compounds were solubilized in water by introducing them as solutions in methanol (1 mL). The concentration of each compound was 1×10^{-2} M. Solutions of 2'-deoxyguanosine (0.0026 g, 1×10^{-5} mole), and 2'-deoxycytidine (0.0022 g, 1×10^{-5} mole) were prepared in 1000 mL volumetric flasks using chlorine-demand-free water containing 2 ppm free chlorine. The concentration of each of these compounds were 1×10^{-5} M. One milliliter aliquots of each solution were derivatized in the normal manner and analyzed by HPLC. No evidence of any dansyl derivative was detected by HPLC analysis.

Derivatization of an Aqueous Solution of Diethylamine (unchlorinated) and N-Chloropiperidine

Diethylamine (0.73 g, 0.01 mole) and N-chloropiperidine (1.2 g, 0.01 mole) were diluted to the mark with deionized water in a 1000 mL volumetric flask. A solution with a concentration of 5.0×10^{-6} M in each compound was prepared

by appropriate dilution of the stock solution. A 1 mL aliquot of this solution was derivatized in the normal manner and analyzed by HPLC. The chromatogram revealed that the only dansyl derivative present was dansyl piperidine.

Derivatization of an Aqueous Solution of Diethylamine and Piperidine

Diethylamine (0.365 g, 0.005 mole) and piperidine (98%, 0.436 g, 0.005 mole) were diluted to the mark with deionized water in a 1000 mL volumetric flask. A solution with a concentration of 5.0×10^{-6} M in each compound was prepared by appropriate dilution of the stock solution. A 1 mL aliquot of this solution was derivatized with dansyl chloride in the normal manner for eight minutes and analyzed by HPLC. The chromatogram revealed that the only dansyl derivative present was dansyl piperidine.

Derivatization of an Aqueous Solution of N-Chlorodiethylamine and Piperidine

N-Chlorodiethylamine (98%, 0.549 g, 0.005 mole) and piperidine (98%, 0.436g, 0.005 mole) were diluted to the mark with deionized water in a 1000 mL volumetric flask. A solution with a concentration of 5.0×10^{-6} M in each compound was prepared by appropriate dilution of the stock solution. A 1 mL aliquot of this solution was derivatized in the normal manner for three hours and analyzed by HPLC. The chromatogram revealed that only dansyl piperidine was formed.

Derivatization of Methylamine, Pyrrolidine and Piperidine with $DANSO_2H$ in the Presence of Chloramines

Four solutions were prepared of binary mixtures of an amine with a chloramine (5.0×10^{-6} M in each): 1) N-chloropiperidine and methylamine, 2) N-chloromethylamine and piperidine, 3) N-chloropiperidine and pyrrolidine, and 4) N-chloropyrrolidine and piperidine. Each solution was derivatized in the normal manner with $DANSO_2H$ for 8 hours and analyzed by HPLC for dansyl amine derivatives. Peak areas from the chromatograms (fluorescence detector) were integrated and the ratio of products determined after correction for different response factors of the dansyl amines. Data is recorded in Table II.

Relative Reactivities Toward Dansyl Chloride of Methylamine and Pyrrolidine vs Piperidine

Two solutions were prepared each of which contained 5.0×10^{-6} M concentrations of two amines: 1) piperidine and pyrrolidine and 2) piperidine and methylamine. One milliliter of each solution was derivatized with dansyl chloride in the normal manner except that it was analyzed two minutes after mixing to determine the initial rates of reaction of each amine with the sulfonyl chloride. Peak areas from the chromatograms (fluorescence detector) were integrated and the ratio of products determined after correction for different response factors of the dansyl amines. Data is recorded in Table III.

Isolation of Dansyl Glycine and Dansyl Leucine at pH 10 and pH 4

Glycine (0.0075 g, 10^{-4} mole) and leucine (0.0131 g, 10^{-4} mole) were diluted to the mark with deionized water in a 1000 mL volumetric flask. A solution with a concentration of 1.0×10^{-6} M in each was prepared by appropriate dilution of the stock solution. A 10 mL aliquot of this solution was derivatized with dansyl chloride in the normal manner. The reaction solution was acidified with HCl to pH 4 before being extracted with CH_2Cl_2. A second 10 mL aliquot of the amino acid solution was also derivatized in the normal manner. When derivatization was complete the pH of the solution was adjusted to pH 10 with NaOH before being extracted with CH_2Cl_2. The CH_2Cl_2 extracts from each derivatization were dried over anhydrous Na_2SO_4, decanted, and concentration under vacuum. The residue was dissolved up to a total volume of 1 mL and analyzed by HPLC. The results showed the reaction solution was extracted with CH_2Cl_2 at pH 4 had five times higher peaks that pH 10.

Derivatization of Tap Water

1. Blank water (chlorine-demand-free water)

Deionized water was first distilled from acidic potassium permanganate. The distilled water was chlorinated to 2 ppm total chlorine and allowed to stand overnight before being irradiated with UV light (pen light, Ultra Violet

products model 11 lamp) until all residual chlorine had been discharged. This chlorine-demand-free water was rechlorinated to a free residual of 2 ppm and used as a blank to test the purity of the reagents.

2. Derivatization Procedure

The following reagents were mixed in a round bottomed flask in the order and quantity given: $NaHCO_3$ (0.42 g), 10 mL of tap water (or blank water), 10 mL of tetrahydrofuran, 1 mL of a 0.2 M solution of $DANSO_2H$ in 0.5 M $NaHCO_3$, and 2 drops of 50% NaOH solution. The reagents were mixed thoroughly and the round bottomed flask was sealed and placed in the dark overnight. The reaction solution (pH 8) was extracted with dichloromethane, or was acidified with HCl to pH 4 before being extracted with dichloromethane (4 x 5 mL); the dichloromethane was dried over anhydrous Na_2SO_4, decanted, and concentrated on a rotary evaporator. The residue was dissolved to a volume of 0.5 mL. The solution was transferred to a calibrated vial and concentrated under a stream of dry nitrogen gas to a final volume of 0.1 mL before being analyzed by HPLC.

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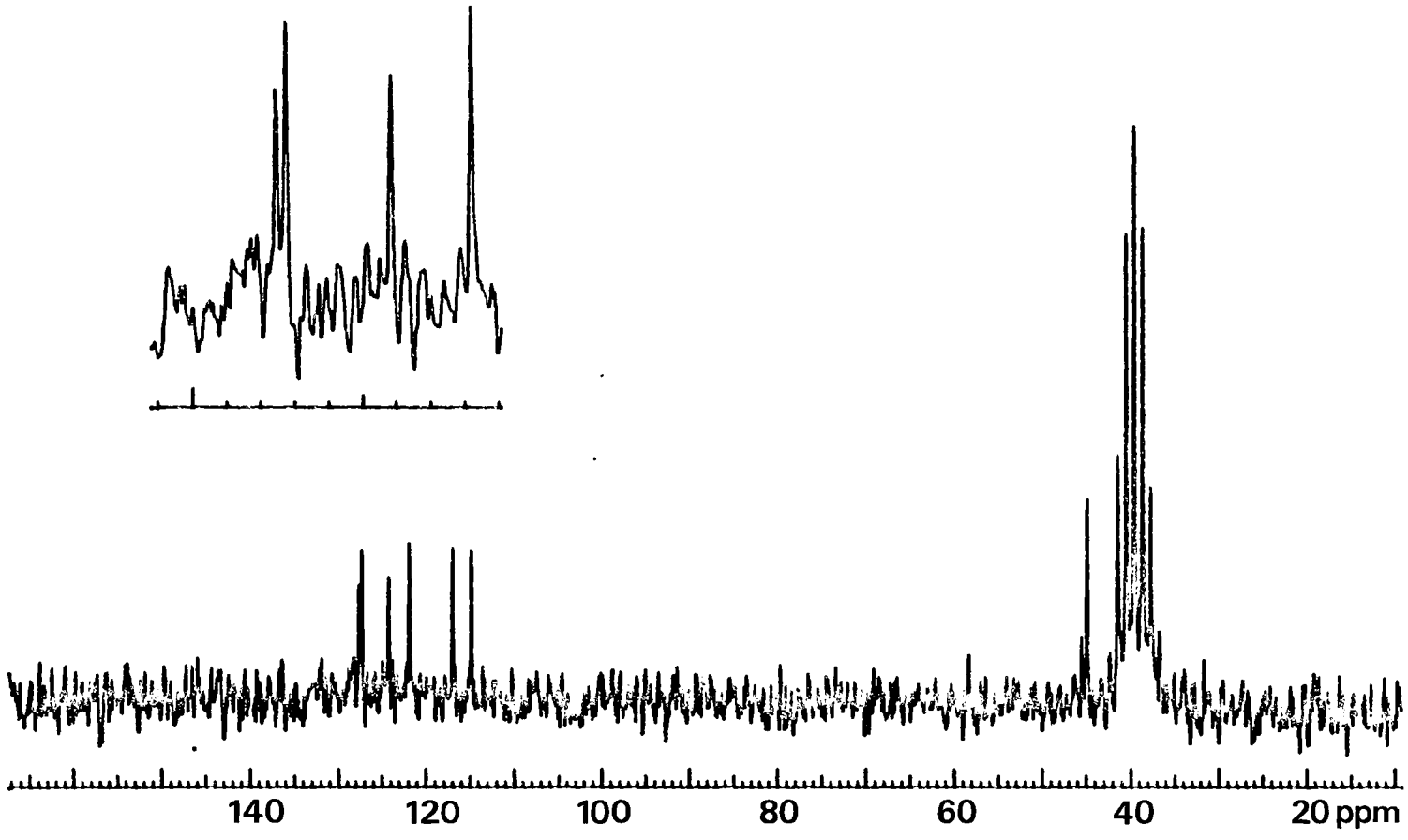
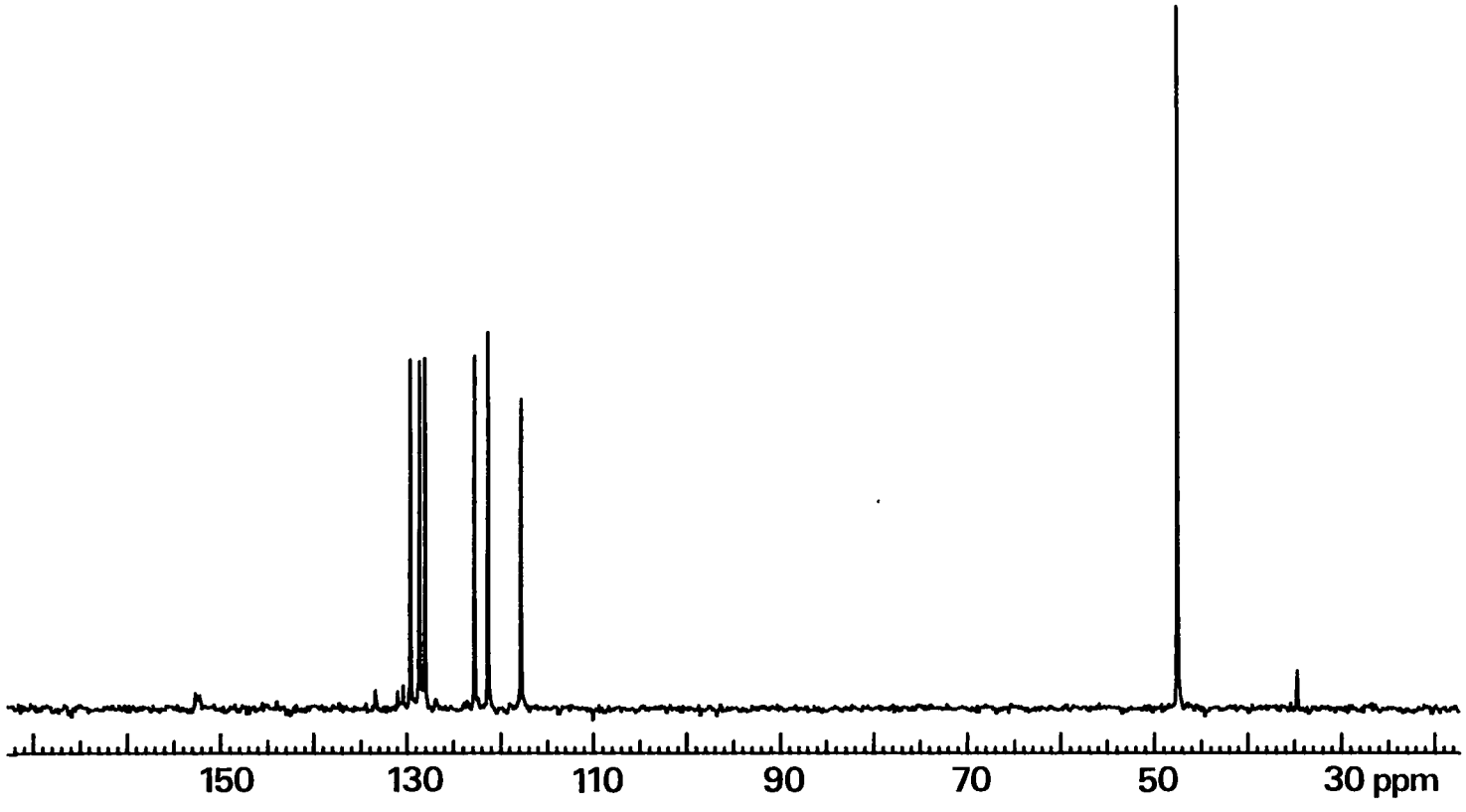
APPENDIX A. ^{13}C NMR SPECTRA



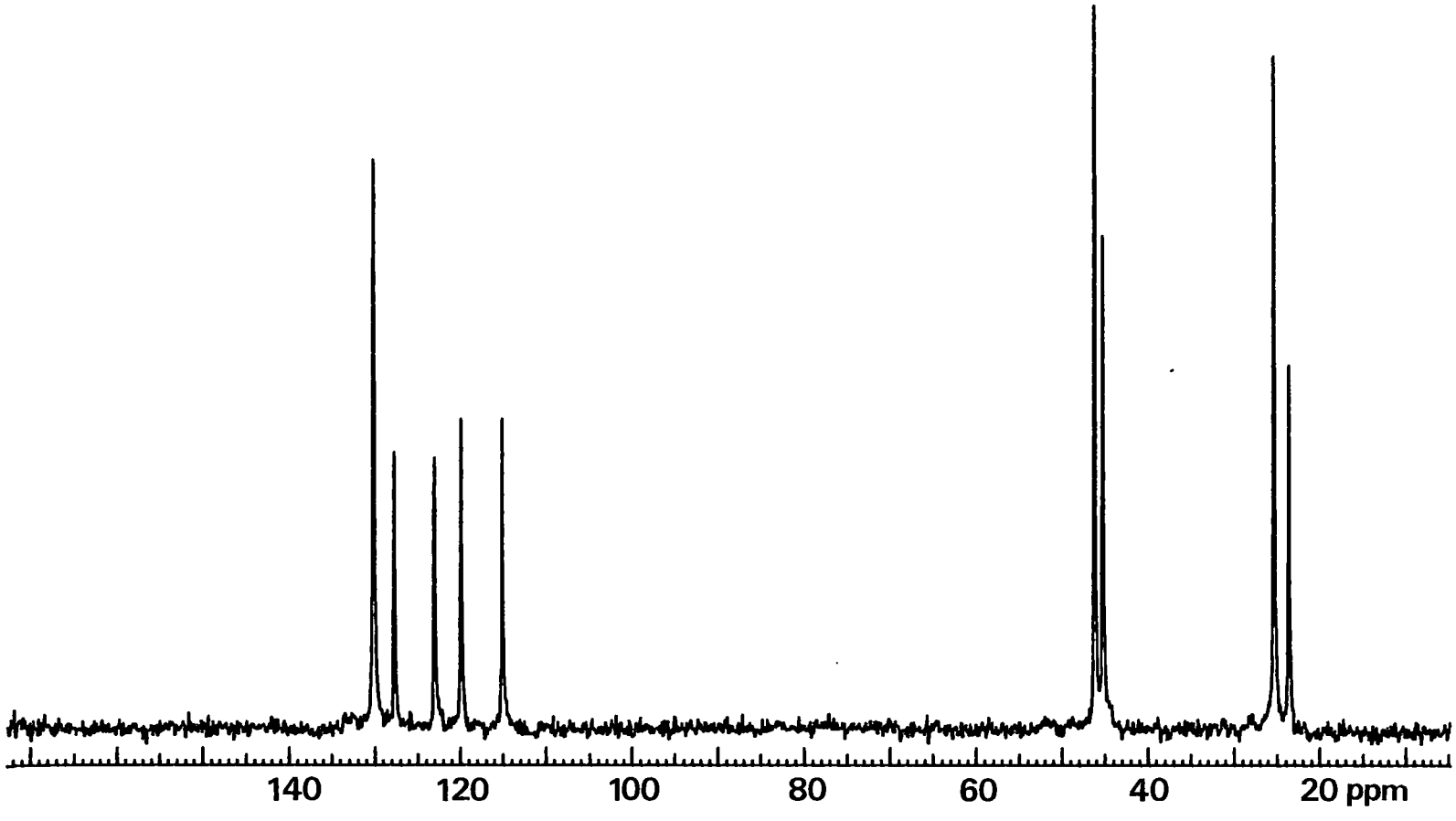
^{13}C NMR SPECTRUM OF DANSYL SULFINIC ACID

SOLVENT: DMSO - d_6



^{13}C NMR SPECTRUM OF 5-DIMETHYLAMINO-1-NAPHTHALENE SULFINIC ACID, SODIUM SALT

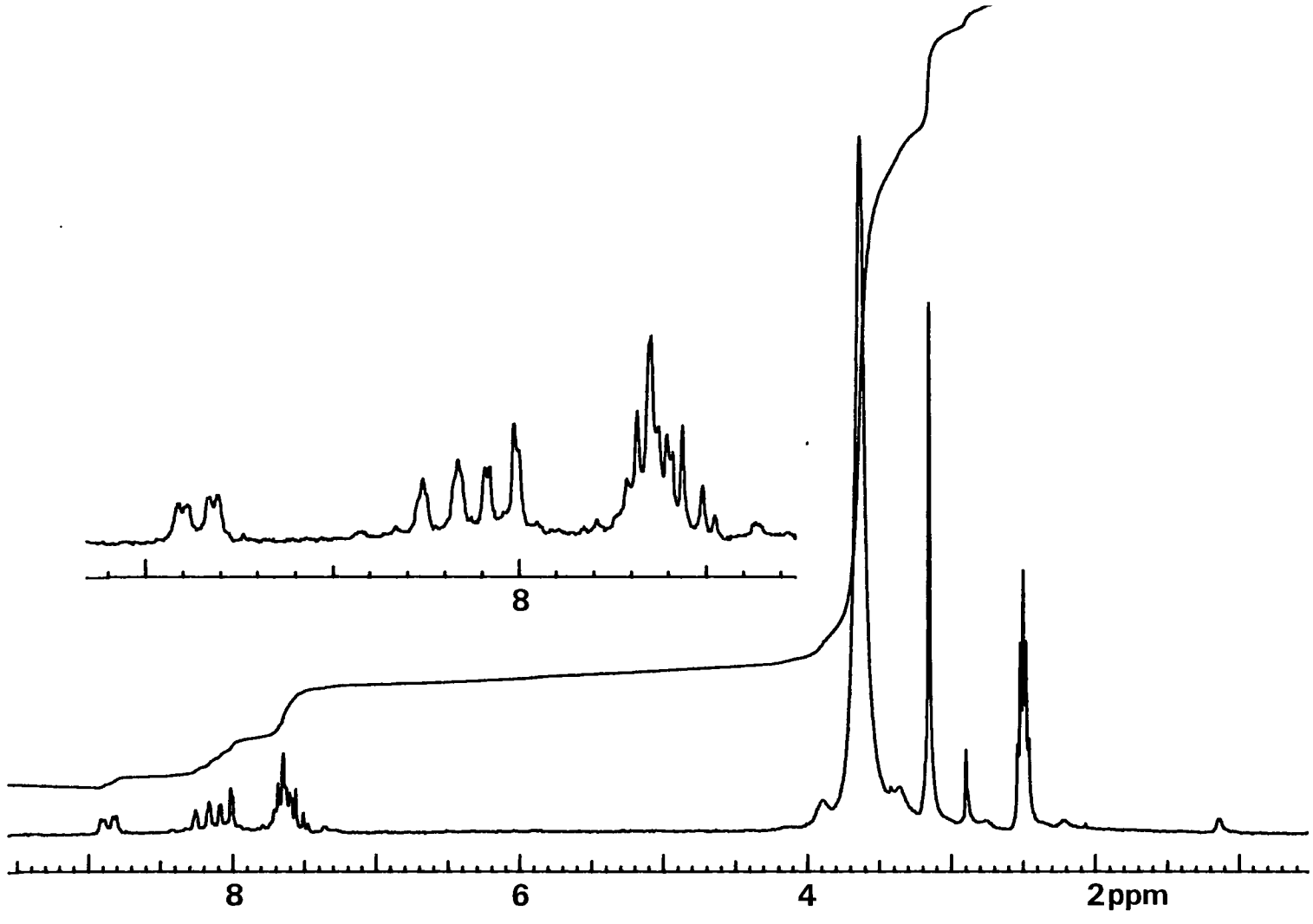
SOLVENT: D_2O



^{13}C NMR SPECTRUM OF DANSYL PIPERIDINE

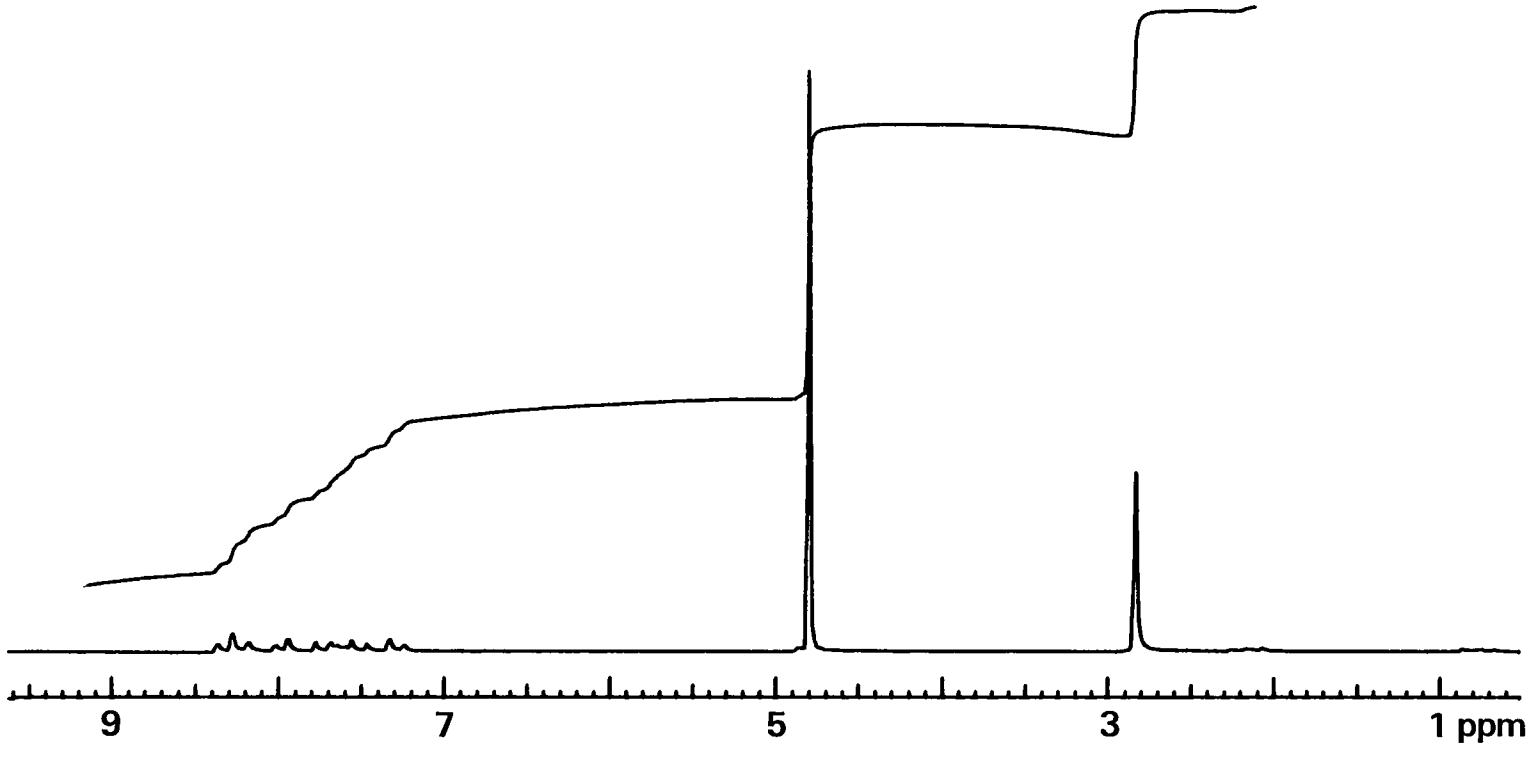
SOLVENT: $CDCl_3$

APPENDIX B. ^{1}H NMR SPECTRA



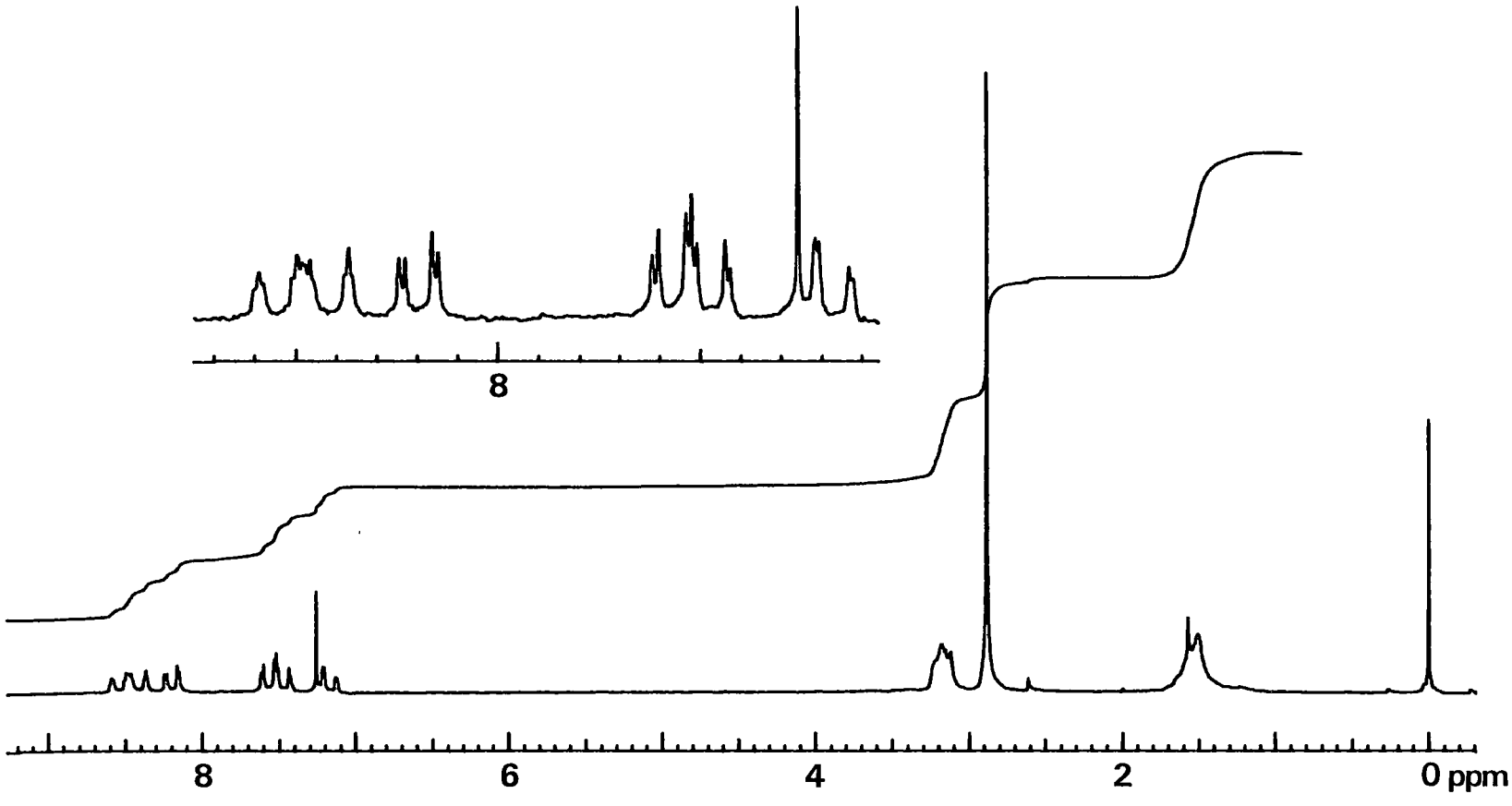
^{1}H NMR SPECTRUM OF DANSYL SULFINIC ACID

SOLVENT: DMSO - d_6



^{1}H NMR SPECTRUM OF 5-DIMETHYLAMINO-1-NAPHTHALENESULFINIC ACID, SODIUM SALT

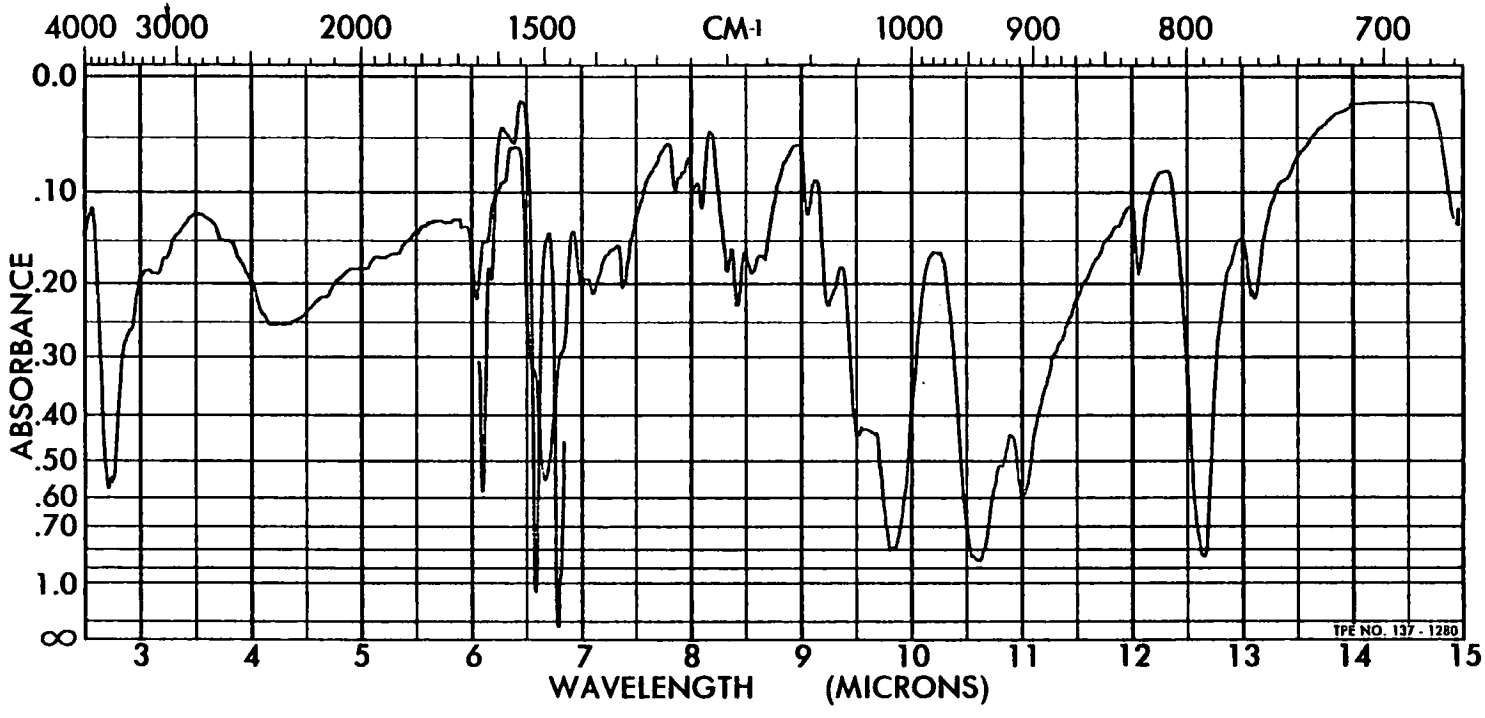
SOLVENT: D_2O



^{1}H NMR SPECTRUM OF DANSYL PIPERIDINE

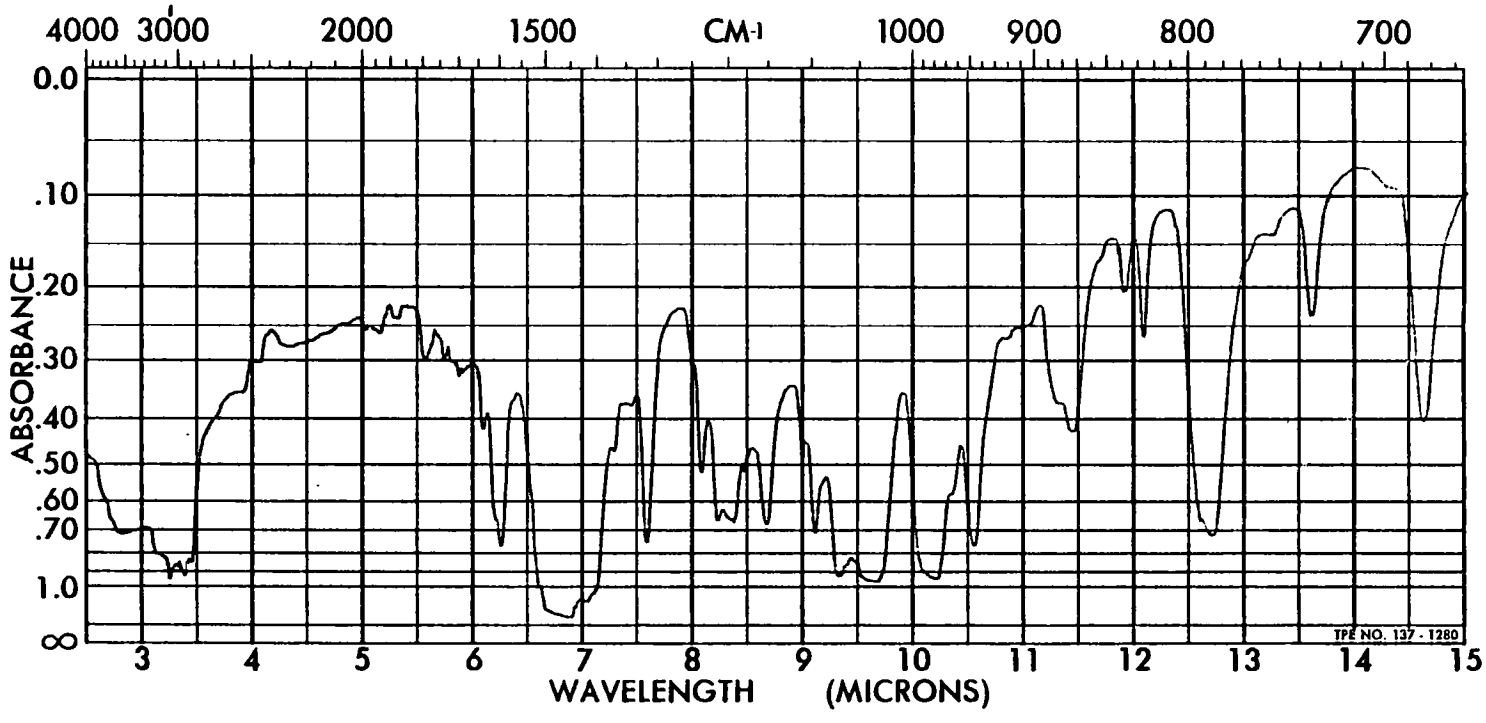
SOLVENT: $CDCl_3$

APPENDIX C. IR SPECTRA



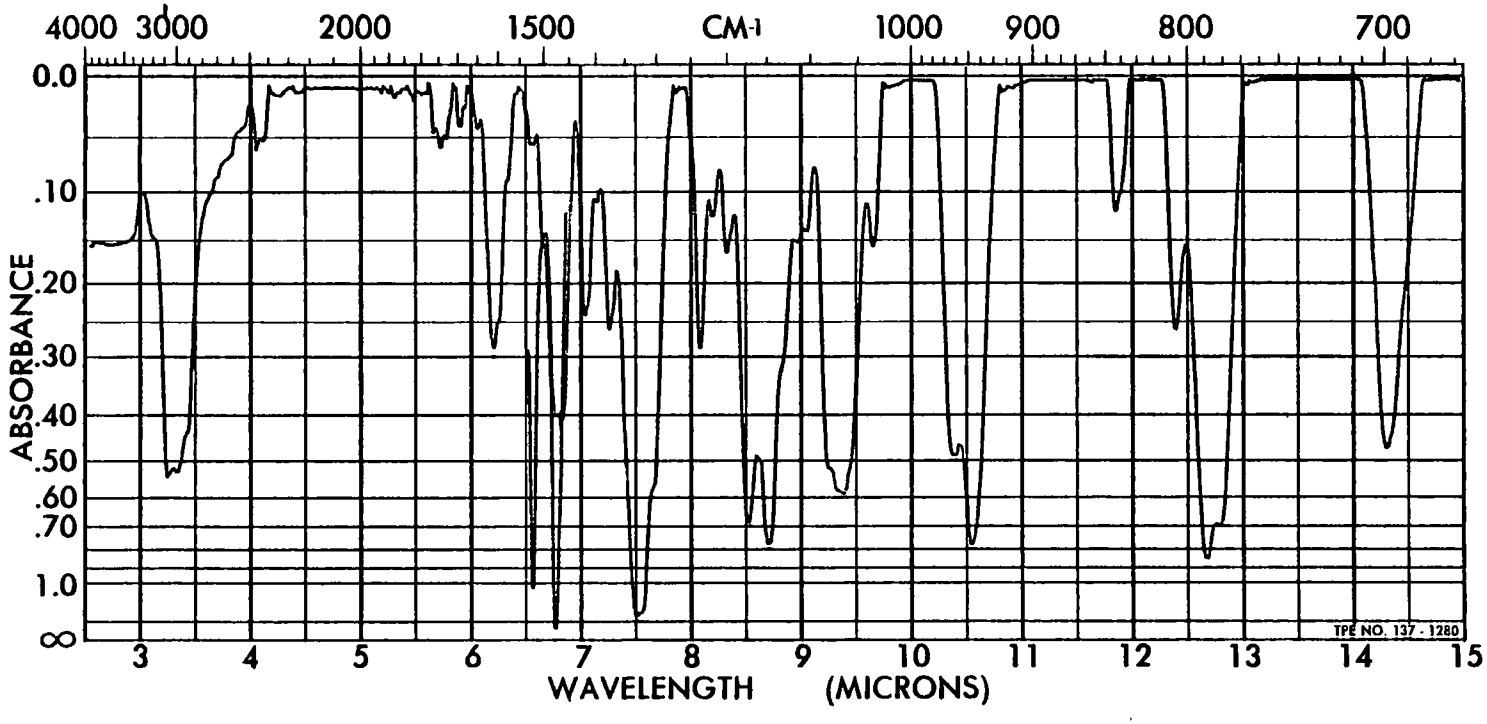
IR SPECTRUM OF 5-DIMETHYLAMINO-1-NAPHTHALENE SULFINIC ACID MONOHYDRATE

PHASE: KBr Pellet



IR SPECTRUM OF 5-DIMETHYLAMINO-1-NAPHTHALENESULFINIC ACID, SODIUM SALT

PHASE: KBr Pellet



IR SPECTRUM OF DANSYL PIPERIDINE

PHASE: KBr Pellet