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PURIFICATION OF DIETHYLENETRIAMINE-N,N,N′,N″-TETRAACETIC ACID-N″-PROPIONIC ACID USING ANION-EXCHANGE DISPLACEMENT CHROMATOGRAPHY

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SUMMARY

The synthesis, purification and characterization of dietylenetriamine-N,N,N′,N″-tetraacetic acid-N″-propionic acid are reported. The purification is achieved using anion-exchange displacement chromatography.

INTRODUCTION

A new chelating agent, diethylenetriamine-N,N,N′,N″-tetraacetic acid-N″-propionic acid (DTTAP), has been successfully prepared in our laboratory. This new chelating agent shows promise as a ligand that can assist in the successful separation of trivalent actinides from the trivalent lanthanides[1]. A successful Ln–An separation is necessary for the effective disposal of high-level radioactive wastes that contain the transuranic actinides.

The synthesis of DTTAP, has been carried out in two steps. In each step, good separation of the reaction products was achieved using ion displacement chromatography. In the first experiment, cation-exchange displacement chromatography was used to isolate pure diethylenetrianic-N-propionic acid. Anion-exchange displacement chromatography was used to isolate pure DTTAP in the second separation.

Few examples of anion-exchange displacement chromatography have been reported in the literature. In 1952, Partridge and Brimley[2] reported the use of this technique for the separation of several mixtures of amino acids. This work illustrates the tremendous value of this technique for resolving mixtures of aminocarboxylic acids possessing very similar pK_a values. Peterson and Torres[3] and Peterson[4] have reported the use of carboxymethyldextrans as spacers and displacers in anion-exchange displacement chromatography. In these reports, dextran was carboxymethylated to varying degrees with chloroacetate. The derivatives containing the largest number of carboxylate groups (*ca.* 400) were used as displacers, and the intermediately

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substituted derivatives were used as spacers in an anion-exchange separation of various proteins. Gueron *et al.*[5] reported the separation of mixtures of acrylic and methacrylic acids using anion-exchange displacement chromatography. In 1968, Coleman and Gilbert[6] showed that chromium(III) thiocyanate complexes can also be separated using this technique.

After reviewing the work of Peterson[3] and Partridge and Brimley[2], it is not surprising that anion-exchange displacement chromatography is the most successful method of separation for the purification of this new polyaminopolycarboxylate.

EXPERIMENTAL

Materials

All chemicals were of reagent-grade. Deionized water in this laboratory was prepared by passing condensed steam through a mixed bed of cation- and anion-exchange resins.

^{13}C NMR

All ^{13}C NMR spectra were recorded on a Nicolet 300 MHz Fourier transform NMR spectrometer. The chemical shifts are reported relative to a dioxane standard (66.5 ppm).

Elemental analyses

All elemental analyses were performed by Desert Analytics, Tucson, AZ, U.S.A. Analyses were performed using a Perkin-Elmer 240C elemental analyzer.

Mass spectrometry

The mass spectral analysis was performed using a Kratos-MS-50 mass spectrometer.

Procedure

Step 1. One mole of 3-chloropropionic acid (108.5 g) was dissolved in a minimum of deionized water and neutralized slowly with one mole of sodium hydroxide in an ice-water bath. This solution was reacted with 3 moles of diethylenetriamine at 50°C, with magnetic stirring. The pH was kept between 8 and 10 with periodic additions of sodium hydroxide. After 24 h, the reaction solution was adjusted to pH 2 with concentrated sulfuric acid. The desired intermediate diethylenetriamine-N-propionic acid was isolated by cation-exchange displacement chromatography.

Step 2. A concentrated aqueous solution containing 0.232 moles of the mono-propionic acid intermediate was prepared and neutralized to pH 8 with sodium hydroxide. This solution was reacted with an aqueous solution containing 1.02 moles of chloroacetate at 50°C with magnetic stirring. The pH was kept between 8 and 10 by periodic additions of sodium hydroxide. After $2\frac{1}{2}$ h, the pH was adjusted to pH 2 with concentrated hydrochloric acid. The desired product, DTTAP, was then isolated by anion-exchange displacement chromatography.

Isolation of intermediate

Pure diethylenetriamine-N-propionic acid was isolated from the other products

of Step 1, by cation-exchange displacement chromatography. The mixture was first placed onto a 48 in. × 2 in. diameter column containing approximately 4.5 moles of Dowex 50-X8 cation-exchange resin capacity, 40–50 mesh, in the H^+ form. The mixture was then rinsed with deionized water to remove any highly acidic species. The mixture was then displaced with 0.1 *M* aqueous ammonia solution along the 2-in. diameter column and then, along two 48 in. × 1 in. diameter columns, each containing approximately 1.1 moles of Dowex 50W-X8 cation-exchange resin capacity in the H^+ form. The flow-rate of the displacing ammonia solution was a slow 2.5 ml/min. Ammonia was a convenient displacing molecule, as it did not displace unreacted diethylenetriamine. After several days, the lightly colored band reached the bottom of the third column.

Thirty-four fractions were collected over a period of three days. Each sample contained 200–250 ml of solution. Samples 1–30 (pH 9.32–9.41) were combined after they were each found to contain the pure end-substituted monopropionate by ^{13}C NMR spectroscopy. Samples 31–34 were found to contain the middle-substituted monopropionate by ^{13}C NMR spectroscopy.

Isolation of final product

The reaction mixture from Step 2 was first displaced through a series of two cation-exchange columns in an effort to separate the polyaminopolycarboxylate products from the other substances (*i.e.* Na^+, $Cl–CH_2COO^-$). The columns used were 48 in. × 2 in. Dowex 50-X8 (H^+ form) and 48 in. × 1 in. Dowex 50W-X8 (H^+ form). The displacer was 0.15 *M* ammonia at a flow-rate of 3 ml/min.

All fractions from the above displacement within pH 2–6 were combined and the products were further separated by anion-exchange displacement chromatography.

The mixture was displaced through a series of six 48 in. × 1 in. diameter columns, each containing approximately 1.1 moles of Dowex-2 anion-exchange resin capacity in the OH^- form. The columns were regenerated with a filtered solution containing 1 *M* barium hydroxide and 1 *M* potassium hydroxide. The carbonate-free solution was protected with an Ascarite trap. The presence of carbonate must be avoided, otherwise carbon dioxide bubbles will form on the column when the pH becomes acidic. The light colored band was displaced with 0.10 *M* hydrochloric acid solution at a flow-rate of 2 ml/min. Sixteen fractions were collected having pH 1–3. Samples 8–12 were found, using ^{13}C NMR spectroscopy, to contain the major reaction product in high purity.

RESULTS AND DISCUSSION

^{13}C NMR

The proton-decoupled ^{13}C NMR spectrum of diethylenetriamine-N-propionic acid in shown in Fig. 1. The chemical shifts of the seven observed peaks are listed in Table I.

The end-substituted product is the only product of Step 1 that would give a proton-decoupled ^{13}C NMR spectrum containing seven lines. Peaks 1 and 7 from Table 1 have been assigned to the carbons labeled 1 and 7 in the formula. The five remaining peaks occur in the region expected for the five remaining carbon atoms, each adjacent to an amine and to a methylene group[7].

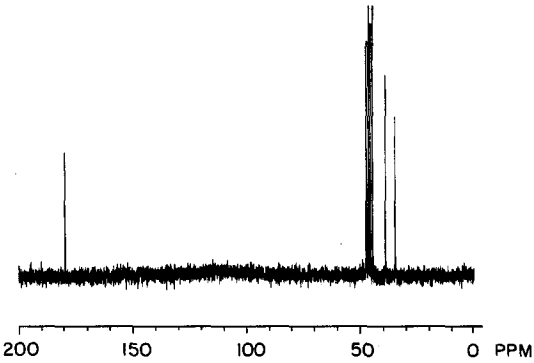


Fig. 1. Proton-decoupled ^{13}C NMR spectrum of diethylenetriamine-N-propionic acid.

The proton-decoupled ^{13}C NMR spectrum of diethylenetriamine-N'-propionic acid is shown in Fig. 2. The chemical shifts of the five observed lines are listed in Table II.

Five lines are expected in the ^{13}C NMR spectrum of this compound. All lines of this spectrum have been assigned based on their relative intensities and the expected relative chemical shifts of the carbon atoms in this compound[7]. The assignments are indicated by the numbering of the carbon atoms in the formula, which corresponds to the numbered peaks in Table II. The spectrum of this middle-substituted product contains lines of minor intensity corresponding to the end-substituted propionate, indicating an incomplete separation of the two isomers.

The proton-decoupled ^{13}C NMR spectrum of the final product, DTTAP, is shown in Fig. 3. The chemical shifts of the thirteen observed lines are listed in Table III. The peaks observed at 169.5 ppm and 56.21 ppm have been assigned to the carbons labeled 3 and 5, respectively. These two peaks are of double intensity compared to the others and are undoubtedly a result of the two equivalent acetate groups. The peak at 169.22 ppm is assigned to the carboxylic acid of the propionate group, as this carbon is expected to resonate upfield relative to the other carboxylic acid carbons. The peaks at

TABLE I

CHEMICAL SHIFTS OBSERVED IN THE ^{13}C NMR SPECTRUM OF DIETHYLENETRIAMINE-N-PROPIONIC ACID

$NH_2CH_2CH_2NHCH_2CH_2NHCH_2\overset{7}{C}H_2\overset{1}{C}OOH$

Peak	*Chemical shift (ppm)*
1	179.4
2	47.2
3	46.2
4	45.5
5	44.5
6	38.7
7	34.5

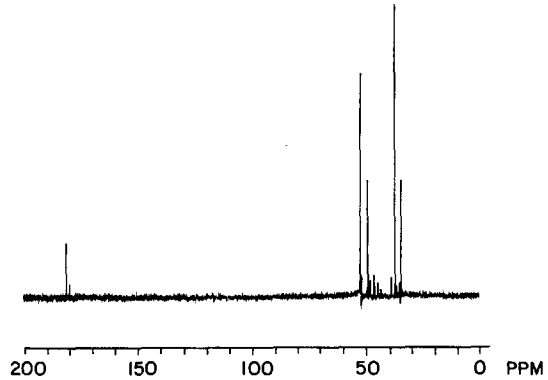


Fig. 2. Proton-decoupled ^{13}C NMR spectrum of diethylenetriamine-N'-propionic acid.

173.8 ppm and 173.7 ppm have been assigned to the carboxylic acid carbons labeled 1 or 2 in Figure 4. Distinguishing which of these nearly coincident peaks belongs to which of these carbonyl carbons is not possible without further experimentation. The peak at 28.2 ppm has been assigned to the carbon atom labeled "13" on the structure in Table III. The remaining seven peaks are currently unassigned. The chemical shifts of these peaks are, however, in the range one would expect for the remaining seven carbon atoms of DTTAP.

Elemental analyses

Diethylenetriamine-N-propionic acid was isolated as the trihydrochloride. Found: %C = 29.79, %H = 7.28, %N = 14.77, %Cl = 36.38; calculated for $C_7H_{20}N_3O_2Cl_3$: %C = 29.53, %H = 7.03, %N = 14.76, %Cl = 37.43; yield = 50% (pure compound).

DTTAP was isolated as the monohydrate from ethanol solution, mp = 159–162°C. Found: %C = 42.09, %H = 6.13, %N = 9.44; calculated for $C_{15}H_{25}N_3O_{10} \cdot H_2O$: %C = 42.35, %H = 6.35, %N = 9.88; yield = 2% (pure compound) based on original chloropropionic acid.

TABLE II

CHEMICAL SHIFTS OBSERVED IN THE ^{13}C NMR SPECTRUM OF DIETHYLENETRIAMINE-N'-PROPIONIC ACID

$^{1\ 5\ 3\ \ 4\ 2}$
$HOOCCH_2CH_2N(CH_2CH_2NH_2)_2$

Peak	*Chemical shift (ppm)*
1	181.0
2	51.8
3	48.8
4	36.9
5	34.1

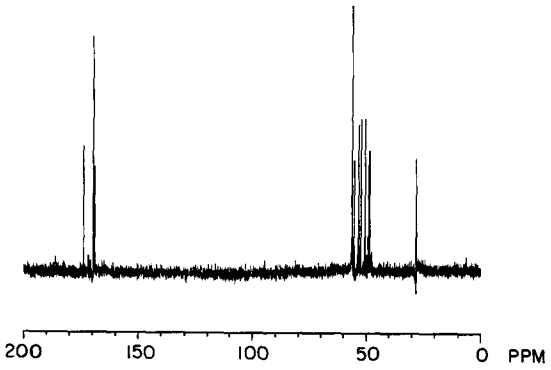


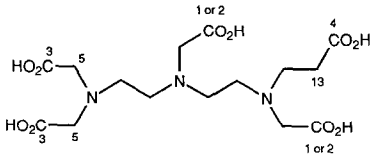
Fig. 3. ^{13}C NMR spectrum of DTTAP.

Titration data

Two equal-volume aliquots of aqueous DTTAP were titrated with standard base. The bare acid was titrated in the first experiment. The acid was titrated in the presence of one equivalent of Sm(III) in the second experiment. The ratio of equivalence point volumes for the two experiments is 3.0:5.0, indicating that five protons are released upon complexation[8].

TABLE III

^{13}C NMR CHEMICAL SHIFTS OF DTTAP



Peak	*Shift (ppm)*
1	173.78
2	173.71
3	169.54
4	169.22
5	56.21
6	55.31
7	53.43
8	52.31
9	52.27
10	50.54
11	48.88
12	48.50
13	28.17

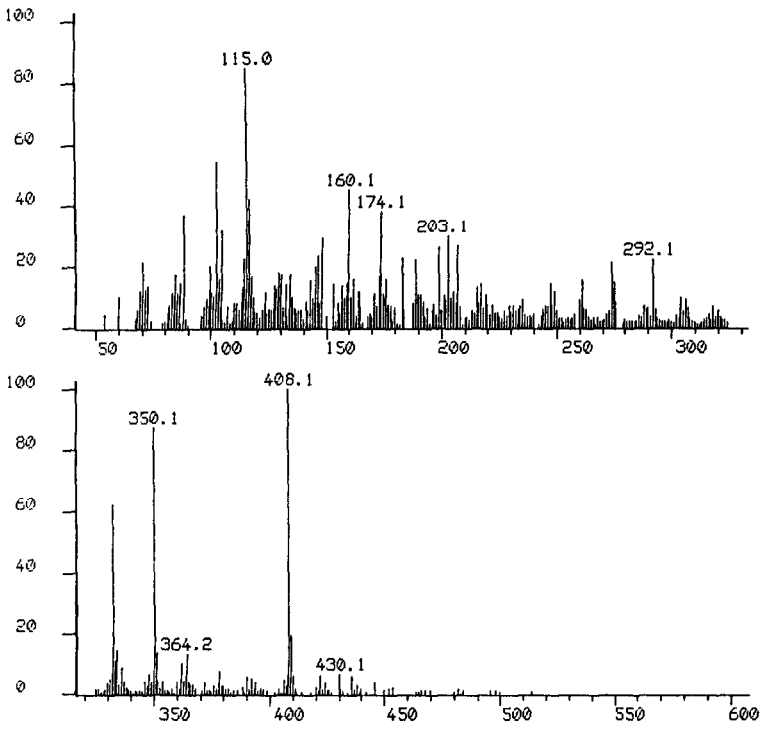


Fig. 4. Mass spectrum of DTTAP. Molecular formula DTTAP: $C_{15}H_{25}N_3O_{10}$; molecular weight: 407.1 a.m.u.

Mass spectrometry

The fast-atom-bombardment mass spectrum of DTTAP, in a glycerol–water matrix, was taken. The matrix subtracted mass spectrum can be seen in Fig. 4. The M+1 peak observed at 408.1 (*m/z*) provides further convincing evidence that the desired DTTAP has been formed. Other major peaks in the mass spectrum are due to fragments of the original DTTAP molecule. The peak at 115 (mass/charge) is probably due to a species formed by glycerol (mol. wt. = 92.1 a.m.u.) and sodium (mol. wt. = 22.99 a.m.u.)[9,10].

CONCLUSIONS

Diethylenetriamine-N-propionic acid and DTTAP have been prepared and purified for the first time. The 4:1 end to middle ratio of replaceable protons contributes to the observed dominance of the end-substituted product over the middle-substituted product in Step 1.

^{13}C NMR spectra for three compounds are reported for the first time. These spectra, along with the other evidence, allow for the unambiguous characterizaton of these compounds.

This work illustrates the value of ^{13}C NMR spectroscopy as a characterization tool for compounds of this type. The ^{1}H NMR spectra of these polyaminocarboxylates are often difficult to interpret. Unresolved multiplets are often observed, and the

isolation of the pure compounds in the absence of water or hydrochloric acid has not been achieved. Elemental analysis provides useful information, but is not an adequate characterization technique by itself. It is especially inadequate for characterizing products of a reaction where isomers are possible. The unambiguous characterization and evaluation of the purity of these compounds is accomplished when ^{13}C NMR spectroscopy is used.

Although cation-exchange displacement chromatography was successfully used to isolate the intermediate of the synthesis, this technique proved unsuccessful when used to isolate DTTAP. The impurities present in the second step are believed to be incompletely substituted tri- and tetra-carboxylic acids. DTTAP was isolated from these impurities using anion-exchang displacement chromatography.

The infrequent use of anion-exchange displacement chromatography is surprising. This work and the few examples that can be found in the literature illustrate that this technique deserves wider application for the preparative-scale separation of anionic species.

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