EDGEWOOD
RESEARCH, DEVELOPMENT & ENGINEERING CENTER
U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-480

NUCLEAR MAGNETIC RESONANCE (NMR) PROCEDURE FOR THE CHARACTERIZATION OF HD HYDROLYSATES

Linda L. Szafraniec
William T. Beaudry

RESEARCH AND TECHNOLOGY DIRECTORATE

March 1998

Aberdeen Proving Ground, MD 21010-5423

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1998 March	Final; 95 Sep - 97 Oct

4. TITLE AND SUBTITLE
Nuclear Magnetic Resonance (NMR) Procedure for the Characterization of HD Hydrolysates

5. FUNDING NUMBERS
Sales Order No. 7J1P4A

6. AUTHOR(S)
Szafraniec, Linda L., and Beaudry, William T.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
DIR, ERDEC, ATTN: SCBRD-RTC, APG, MD 21010-5423

8. PERFORMING ORGANIZATION REPORT NUMBER
ERDEC-TR-480

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
PMCD, ATTN: SFAE-CD-A, APG, MD 21010-5401

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

A useful and facile nuclear magnetic resonance (NMR) method for characterizing hydrolysates of HD (2,2'-dichlorodiethylsulfide) has been developed. The method uses both ^{1}H and ^{13}C NMR to identify the major components (>0.1 mole%) in the hydrolysate and to determine the mole ratios of these compounds in solution. This NMR method is especially useful since no sample preparation is required, and all soluble compounds, including the polar and ionic hydrolysis products of HD, are easily observed.

This report outlines a step-by-step procedure for characterizing HD hydrolysates using NMR spectroscopy and describes, in detail, the analysis of a typical hydrolysate sample. Interpreted ^{1}H and ^{13}C spectra and reproducibility data are included as well as a list of representative NMR parameters for compounds typically present in the hydrolysates.

14. SUBJECT TERMS
HD, Thiodiglycol, Hydrolysis, Sulfonium Ion, NMR, Neutralization, Hydrolysate

15. NUMBER OF PAGES
37

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

Blank

PREFACE

The work described in this report was authorized under Sales Order No. 7J1P4A, Alternate Technologies Program. This work was started in September 1995 and completed in October 1997.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Blank

CONTENTS

FIGURES

TABLES

NUCLEAR MAGNETIC RESONANCE (NMR) PROCEDURE FOR THE CHARACTERIZATION OF HD HYDROLYSATES

1. INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful techniques for elucidating the molecular structure of organic compounds. The sample (usually a liquid or a solid dissolved in an appropriate solvent) is placed within a strong, homogeneous magnetic field. Nuclei possessing magnetic moments within the sample align themselves with or against the direction of the static field. The sample is then irradiated with radio frequency (RF) electromagnetic radiation, causing these nuclei to resonate and flip orientation with respect to the magnetic field. This ultimately results in an NMR spectrum - a plot of resonance frequency *vs* peak intensity. The RF frequency used to irradiate the sample at a particular magnetic field strength dictates which nuclei will be observed in the NMR spectrum. Thus, for a magnetic field of 9.4 T, protons will be observed at 400 MHZ, ^{19}F nuclei at 376 MHZ, ^{31}P nuclei at 162 MHZ, and ^{13}C nuclei at 100 MHZ.

The three NMR parameters of interest are: (1) the chemical shift or δ (delta) value, which gives information about the immediate environment of the nuclei of interest; (2) the coupling constant or J value, which gives information about the number and types of neighboring magnetic nuclei; and (3) the integrated area of the resonance, which is directly proportional to the number of nuclei experiencing a particular magnetic environment. With this information, supplemented by complementary mass spectrometry (MS) and infrared (IR) data, NMR can be used to detect and confirm the presence of chemical warfare (CW) related compounds, their precursors and degradation products. Furthermore, these NMR parameters can be used to elucidate the structures of unknown organic components that may also be of interest.

NMR is particularly suited to studying decontamination reactions of CW agents. Many of the solvent systems are aqueous, and the reactions can easily be monitored without extraction or sample manipulation. In addition, many of the products from these reactions are either acids, salts or pyrophosphate compounds that do not readily chromatograph. NMR allows us to follow not only the disappearance of the agent but, also, the appearance of these various products so that a complete reaction profile can be obtained. Furthermore, the NMR investigations can be carried out at agent concentrations closer to those expected for field conditions and demilitarization operations (0.01 M and greater) allowing us to observe interactions which may not exist at the dilute concentrations required by some other analytical methods.

Taking advantage of the unique capabilities of NMR, a method was developed to characterize samples of hydrolyzed sulfur mustard (HD, 2,2'-dichlorodiethylsulfide, CAS No. 505-60-2). The method, described in this report, utilizes ^{1}H and ^{13}C NMR to determine the mole ratios of the major products present in hydrolyzed HD, including any sulfonium ions that may have formed. (See Appendix A).

2. EXPERIMENTAL PROCEDURE

2.1 Application.

This method applies to the use of ^{1}H and ^{13}C NMR spectroscopy to evaluate the composition of HD hydrolysates.

2.2 Concentration Range.

The concentration range covered by this procedure ranges from the detection limit of the instrument to neat samples.

2.3 Detection Limits.

The detection limit for any compound depends on the field strength of the instrument used to evaluate the sample, the accumulation time for the Fourier transform (FT) experiment, the nucleus observed, and the diameter of the NMR tube used.

Examples:

^{1}H, 400 MHZ, 5-mm NMR tube, 16 hr accumulation time (over night): 1 μg/mL or greater.

^{13}C, 100 MHZ, 5-mm NMR tube, 16 hr accumulation time: 100 μg/mL or greater.

^{31}C, 150 MHZ, 5-mm NMR tube, 16 hr accumulation time: 50 μg/mL or greater.

2.4 Interferences.

Interferences include any compound or compounds that have the same resonance frequency(ies) as the compound(s) of interest (i.e., overlapping peaks). Also, the presence of paramagnetic ions (e.g., iron, nickel, copper, etc.) in solution may broaden the NMR resonances to the extent that NMR analysis may not be possible without special sample preparation (i.e., filtering the sample to remove suspended paramagnetic solids or the use of cationic exchange resins to remove soluble paramagnetic species).

2.5 Analysis Rate.

Depending on the detection limit desired, the analysis rate can range from 0.5 to >60 hrs/sample. At 400 MHZ, using a 5-mm NMR sample tube, a typical analysis with a detection limit of 0.5 mole% requires approximately 3-3.5 hrs/sample. This includes acquiring the ^{1}H and ^{13}C data, and plotting and evaluating the spectra.

2.6 Validation.

Compound identifications are validated by manual comparison of the spectra to those in a database library or to spectra of the authentic compounds. When required, the samples are spiked with the authentic compound for further validation of an assignment.

The NMR results provide a mole ratio (or a calculated weight ratio) of components identified in the sample. Absolute quantitation of any component (i.e., $\mu g/mL$), is accomplished using another analytical method which has undergone precision and accuracy testing for that analyte (e.g., gas chromatography (GC), liquid chromatography (LC), or inductively coupled plasma-atomic emission spectroscopy (ICP-AES)).

2.7 Instrumentation.

The sample described in this report was analyzed using a Varian Unity Plus 400 Fourier transform (FT) NMR spectrometer system operating at 400 MHZ for ^{1}H and 100 MHZ for ^{13}C observation. The spectrometer was equipped with a variable temperature (VT) controller, a high resolution liquid proton/broadband switchable VT probe, and utilized modulated hetero- and homonuclear decoupling schemes.

Any commercially available FTNMR spectrometer with a field strength greater than 4.7 T (200 MHZ) and capable of ^{1}H and ^{13}C observation can be used. The instrument should have hardware and software capabilities for solvent suppression during data accumulation.

2.8 Hardware/Glassware.

The following hardware/glassware is required:

(1) Clean, dry commercially available Pyrex NMR tubes with pressure caps. Wilmad 5-mm o.d. tubes (Wilmad Glass, Buena, NJ) catalog nos. 507-PP, 526-PP, 527-PP or 528-PP may be used.

(2) Clean, dry, glass Pasteur pipettes.

(3) Glass wool, for filtering, if required.

(4) Parafilm (American National Can Co., Greenwich, CT) or equivalent.

2.9 Chemicals.

No chemicals and no reagent solutions are required for this analysis. A small amount (5-10 vol%) of any commercially available deuterium oxide (D_2O) may be added to the sample if a deuterium lock is required.

2.10 Standards.

No primary standards are required. The standards for calibrating the NMR spectrometer (i.e., pulse widths, field strengths, etc.) and for determining proper instrument performance are supplied by the manufacturer in sealed NMR tubes and are commercially available. For the data presented in this report, these include:

^1H Line Shape:	1% Chloroform, 99% Acetone-d_6
^1H Resolution:	1% o-Dichlorobenzene, 99% Acetone-d_6
^1H Sensitivity:	0.1% Ethyl Benzene in $CDCl_3$
^{13}C Sensitivity:	40% p-Dioxane, 60% Benzene-d_6

2.11 Procedure.

2.11.1 General Procedure.

HD hydrolysates are run neat (w/o solvent). An aliquot is placed into a clean, dry NMR tube of the appropriate size; no solids should be present in the sample. ^1H and ^{13}C NMR spectra are obtained using the standard software supplied by the manufacturer.

For each sample, the spectrometer frequency is tuned and the magnetic field homogeneity adjusted. Data for each nucleus of interest is then accumulated until the desired signal-to-noise (S/N) level has been achieved (2 min to >60 hrs). After data accumulation, the NMR spectra are stored on disk, and hard copies are plotted out. The spectra are manually interpreted: the compounds present are identified from their chemical shifts and coupling constants, and the integrals are measured. The results are reported as mole% when all components can be identified. When unknown components are present, an estimated mole% is reported by assigning the unknowns to likely compounds based on their NMR parameters and other available spectroscopic data.

2.11.2 Applicable Safety Regulations and Standard Operating Procedures (SOPs).

The following U.S. Army and Edgewood Research, Development and Engineering Center (ERDEC) regulations are applicable to the analysis of HD hydrolysates by NMR:

(1) AMC-R-385-100, Safety Manual (26 Sep 95).

(2) CRDEC-R-385-12, Hazard Communication Program (15 Jul 87).

(3) DA Pam 385-61, Safety. Toxic Chemical Agent Safety Standards (Mar 97).

(4) APG-R-385-4, Safety. The APG Safety and Occupational Health Program.

(5) SOP CR8-ISP006-97H, The NMR Experiment (12 Aug 96).

(6) SOP CR8-OSP004-96C, Handling and Sampling of Chemical Agents (5 Jun 97).

(7) SOP CR8-OSP005-96H, Filling of NMR Tubes (Sep 97).

(8) APGR-200-60, Hazardous Waste Management (15 Nov 95).

2.11.3 Instrument Calibration.

Calibration of the NMR spectrometer is performed under the following conditions:

(1) At least once per year.
(2) After loss of power.
(3) After emergency repair.
(4) After changing probes.
(5) If degradation in spectral quality is observed.

Measurement of the S/N ratio for ^{1}H and ^{13}C suffices to ensure instrument performance. The S/N specifications and test procedures for the instrument and probe used in the analysis are supplied by the instrument manufacturer.

For the work described in this report, the S/N specifications for each probe at each field strength are specified in Varian Publication No. 87-195329-00, "Unity INOVA Acceptance Tests Specifications" (e.g., 5-mm $^{1}H/^{19}F/^{15}N$-^{31}P switchable probe: ^{1}H = 100:1 and ^{13}C = 120:1), and the test procedures for obtaining the S/N ratio are described in Varian

Publication No. 87-195328-00, "Unity INOVA Acceptance Tests Procedures."

2.11.4 Operating Parameters.

The following instrument parameters were used to collect the ^{1}H NMR spectra described in this report:

Spectrometer Frequency	399.945	MHZ
Acquisition Time	1.500	sec
Number of Points	24000	
Sweep Width	8000	Hz
Filter Bandwidth	4400	Hz (with spectrum centered)
Pulse Width	14.1	μsec (77°)
Pulse Delay	0.500	sec
Number of Transients	48	(or greater)
Saturation Power	10	
Saturation Delay	2.500	sec
Fourier Number	65536	(or greater)
Gain	30	(maximum w/o overloading the analog to digital converter)

The pulse width is based on the Ernst pulse angle (Section 2.13, reference 3, p. 165) calculated by the manufacturer's software given a repetition rate of 4.5 sec, a 90° pulse width (PW90) of 16.5 μsec, and a T_1 value of 3 sec for the longest relaxation time measured in the sample (Appendix B).

The decoupler frequency, transmitter offset and saturation frequency were all set to the frequency of the water resonance. The data was collected in double precision mode, using the manufacturer's solvent suppression pulse sequence ("PRESAT") to reduce the large water resonance. With a pulse repetition rate of 4.5 sec (acquisition time + pulse delay + saturation delay), it took less than 4 min to acquire 48 transients.

The following instrument parameters were used to collect the ^{13}C NMR spectra:

Spectrometer Frequency	100.576	MHZ
Acquisition Time	1.600	sec
Number of Points	80000	
Sweep Width	25000	Hz
Filter Bandwidth	13800	Hz (with spectrum centered)
Pulse Width	12.2	μsec (72°)
Pulse Delay	2.500	sec
Number of Transients	>2000	(to detect 0.5% S^{+} ion)
Decoupler Mode	yyy	(decoupler on all the time)
Decoupler Mode Modulation	w	(WALTZ)
Fourier Number	65536	(or greater)

The pulse width is based on the Ernst pulse angle calculated by the manufacturer's software given a repetition rate of 4.1 sec, a PW90 of 15.2 μsec, and a T_1 value of 3.5 sec, slightly larger than the longest relaxation time measured in the sample (Appendix B). (NOTE: For best sensitivity, the PW90 should be determined directly on the HD hydrolysate sample, itself.) Because the detection of small components is of utmost importance, full proton decoupling was used in order to take advantage of the increased sensitivity provided by the nuclear Overhauser effect (NOE). Since all of the resonances of interest represent methylene carbons and since mole ratios by carbon will be determined, in most cases, by comparing the integrals of like carbons (i.e, $-SCH_2$ *vs* $-SCH_2$ and $-OCH_2$ *vs* $-OCH_2$), the greater sensitivity provided by the NOE outweighs any small errors in quantitation that may occur by slightly differing NOE's.

The data was collected in double precision mode. Since it required >2 hrs to obtain sufficient S/N to detect 0.5 mole% sulfonium ion, the ^{13}C data were usually collected in overnight, unattended runs, in order to free the instrument for other analyses during duty hours.

2.11.5 Step-By-Step Procedure.

(1) Using a clean, glass pipette, place an aliquot of the HD hydrolysate into a clean, dry NMR tube. At least 0.75 mL of hydrolysate will be required for a 5-mm tube and 1.5 mL for a 10-mm tube. If solids are present in the HD hydrolysate, filter the sample using glass wool so that no solids pass into the NMR tube. Do NOT add any reference compounds to the sample. However, D_2O (~10 vol%) may be added if a lock solvent is required.

(NOTE: If the HD hydrolysate has not been cleared for the presence of HD, this procedure should be carried out in accordance with the SOP's referenced in Section 2.11.2, above. If the sample has been cleared, Steps 1 and 2 should still be carried out in a fume hood, and the operators should wear laboratory smocks/aprons and surgeon's gloves, as a minimum).

(2) Cap the NMR tube with a pressure cap, wrap the top of the tube with Parafilm, and label the sample.

(3) Ensure that the spectrometer system is in good working order (see Section 2.11.3, above).

(4) Place the NMR tube into the spinner turbine to the correct depth, and place the sample into the spectrometer.

(5) If required, allow several minutes for the temperature of the sample to stabilize. Data may be accumulated

at probe temperature (ambient) or at a regulated temperature close to ambient (e.g., 25 °C).

(6) Set the spectrometer to acquire a ^{1}H spectrum using the manufacturer's standard software. Use a solvent suppression pulse sequence to minimize the large water resonance and acquisition parameters equivalent to those given in Section 2.11.4, above.

For very stable spectrometer systems that allow real-time homogeneity tuning directly on the spectrum or FID, the data may be accumulated with the lock turned off. For spectrometer systems that drift and/or cannot be tuned without a lock solvent, lock on the D_2O added in Step 1, above.

(7) Tune the probe frequency to the sample for maximum S/N at the ^{1}H frequency.

(8) Maximize the magnetic field homogeneity.

(9) Insert the sample number and other pertinent information into the text file of the ^{1}H experiment.

(10) Start data accumulation. Reference the spectrum by setting the $-SCH_2$ triplet of thiodiglycol (TDG) to $\delta 2.75$.

(11) When data accumulation is complete, file the data in the appropriate file for permanent storage.

(12) Plot a hard copy of the spectrum and the integral; list all acquisition parameters and the sample description on the first sheet, as a minimum. When plotting the data, increase the vertical scale to observe small resonances, and use smaller widths of plot to discern closely spaced peaks. Increase the integral amplitude 10, 100 or 1000 times, as required, to obtain a good representation of the area under the smaller peaks.

(13) Set the spectrometer to acquire a ^{13}C spectrum using the manufacturer's standard software. Use acquisition parameters equivalent to those given in Section 2.11.4, above.

(14) Tune the probe frequency to the sample for maximum S/N at the ^{13}C frequency.

(15) Maximize the magnetic field homogeneity, if there has been a change since the ^{1}H data was acquired.

(16) Insert the sample number and other pertinent information into the text file of the ^{13}C experiment.

(17) Start data accumulation. Reference the spectrum by setting the $-OCH_2$ resonance of TDG to $\delta 63.5$.

(18) Repeat Step 11.

(19) Repeat Step 12.

2.11.6 Control Charts.

No control charts are required for this procedure.

2.11.7 Corrective Measures.

If the spectrometer does not meet the manufacturer's specifications with regard to pulse widths, decoupler power, resolution, stability, lineshape and/or sensitivity (S/N), immediately call for emergency repair service.

2.12 Treatment of Data.

The following steps should be followed to interpret the NMR spectra obtained in accordance with the procedures outlined above:

(1) Assign the resonances in the ^{1}H spectrum to the various components of the HD hydrolysate. (See Appendix A for some compounds typically present in the hydrolysates.) Manually measure the integrals to determine the area of each resonance. Measure the integrals with a ruler calibrated in millimeters (mm), and record each integral to the nearest 0.1 mm directly on the spectrum. Divide this integral by the number of protons represented by the resonance to obtain the mm/proton for each component. Average the numbers for components represented by more than one resonance.

(2) Assign the peaks in the ^{13}C spectrum. For those components NOT detected in the ^{1}H spectrum, manually measure the integrals to determine the area under each peak of interest. Measure each integral to the nearest 0.1 mm, and record them directly on the spectrum. Divide each integral by the number of carbons represented by the peak to obtain the mm/carbon for each component.

(3) Choose a carbon resonance that represents a component for which the ^{1}H integral was measured in Step 1, above, and determine the mm/carbon for this resonance. If possible, this resonance should represent the same moiety (i.e., CH_3, CH_2 or CH) and have a similar chemical shift to the resonance of the component NOT observed in the ^{1}H spectrum. Use the molar ratio of the two components in the ^{13}C spectrum to calculate the mm/proton for the component unobservable in the ^{1}H spectrum. Do this for each compound observed in the ^{13}C spectrum but not observed in the ^{1}H spectrum.

(4) Record the mm/proton for each component detected by either ^{1}H or ^{13}C NMR. Add these together. Divide the mm/proton for each compound by this sum, and multiply by 100% to obtain the mole% for each component in the hydrolysate.

(5) Check the results by having a second spectroscopist repeat Steps 1-4.

2.13 References.

References pertinent to the use of NMR spectroscopy for the characterization of HD hydrolysates include:

(1) Leyden, D.E., and Cox, R.H. "Analytical Applications of NMR" in Volume 48 of Chemical Analysis, Elving, P.J. and Winefordner, J.D., eds. John Wiley and Sons, NY, NY. 1977.

(2) Sanders, J. K. M., and Hunter, B. K., Modern NMR Spectroscopy, Oxford University Press, NY, NY, 1987.

(3) Derome, A. E., Modern NMR Techniques for Chemistry Research, Pergamon Press, NY, NY, 1987.

(4) Yang, Y.-C., Szafraniec, L.L., Beaudry, W.T. and Ward, J.R. "Kinetics and Mechanism of the Hydrolysis of 2-Chloroethyl Sulfides," J. Org. Chem., 53(14), 3293-3297 (1988).

(5) Hovanec, J.W., Szafraniec, L.L., Albizo, J.M., Beaudry, W.T., Henderson, V.D., Yang, Y.-C., MacIver, B.K., Procell, L. "Evaluation of Standard and Alternative Methods for the Decontamination of VX and HD in Chemical Agent Disposal Facilities," ERDEC-TR-054, April 1993, Unclassified Report.

3. RESULTS AND DISCUSSION

The 400 MHZ ^{1}H NMR spectrum of a typical HD hydrolysate sample is shown in figure 1, and the spectrum with the vertical scale increased (x50) is shown in figure 2. In order to evaluate the spectrum, expansions of the various regions containing peaks of interest were plotted. Figure 3 shows the expanded region around the thiodiglycol (TDG) peaks; resonances from the other products present in the sample are labeled.

Figure 4 shows the $-SCH_2$ region of the ^{1}H NMR spectrum of the HD hydrolysate. The integral over this region was plotted, making sure that the integral was flat over the flat portions of the baseline (i.e., the beginning and the end of the plotted area). Regions of the spectrum that contained resonances from other products were expanded and plotted. The integral for the $-SCH_2$ resonance of TDG was measured ONLY over the three peaks constituting the $-SCH_2$ triplet. The small resonances from the

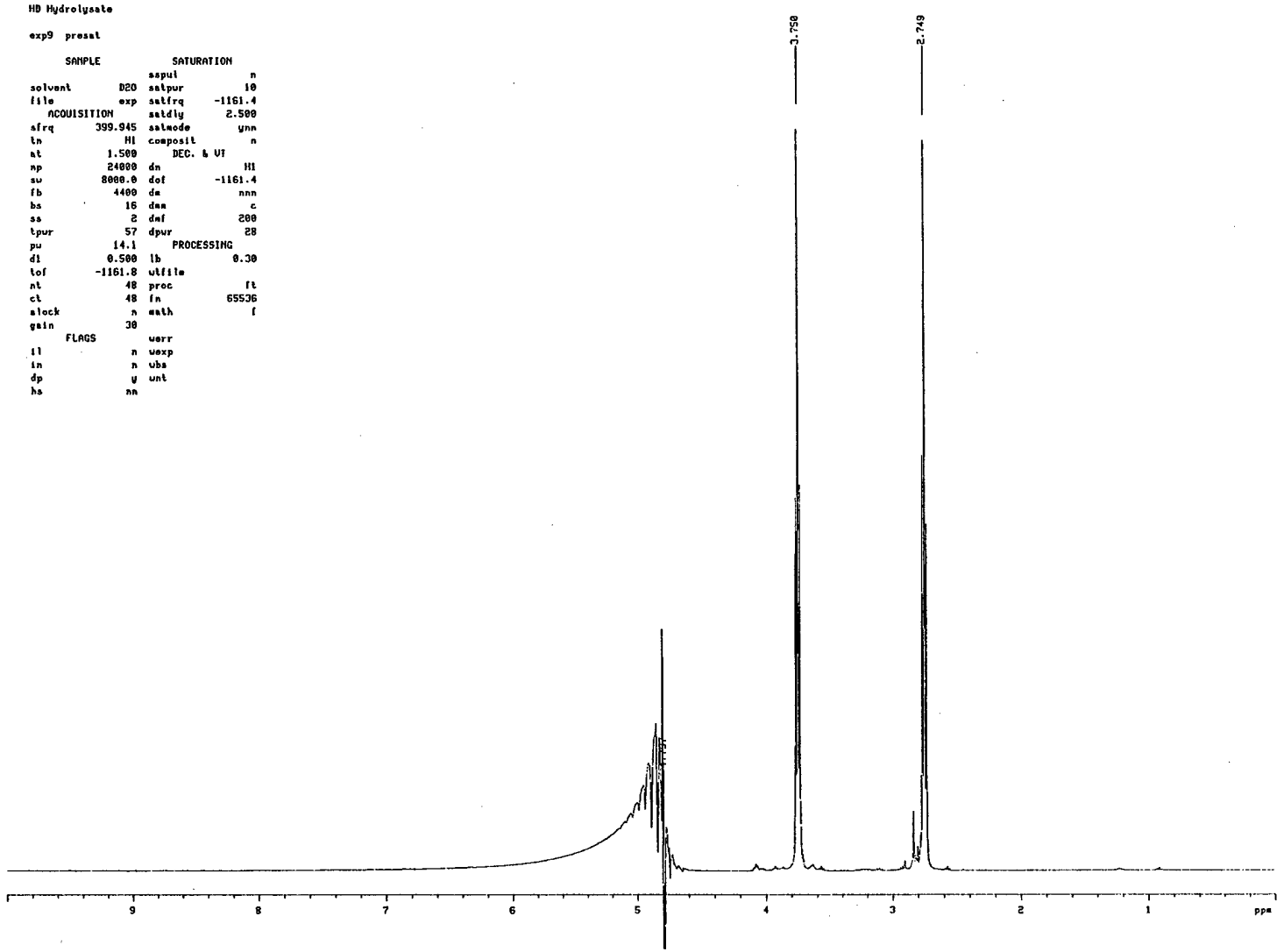


Figure 1. The 400 MHz ^{1}H NMR Spectrum of a Typical HD Hydrolysate Sample.

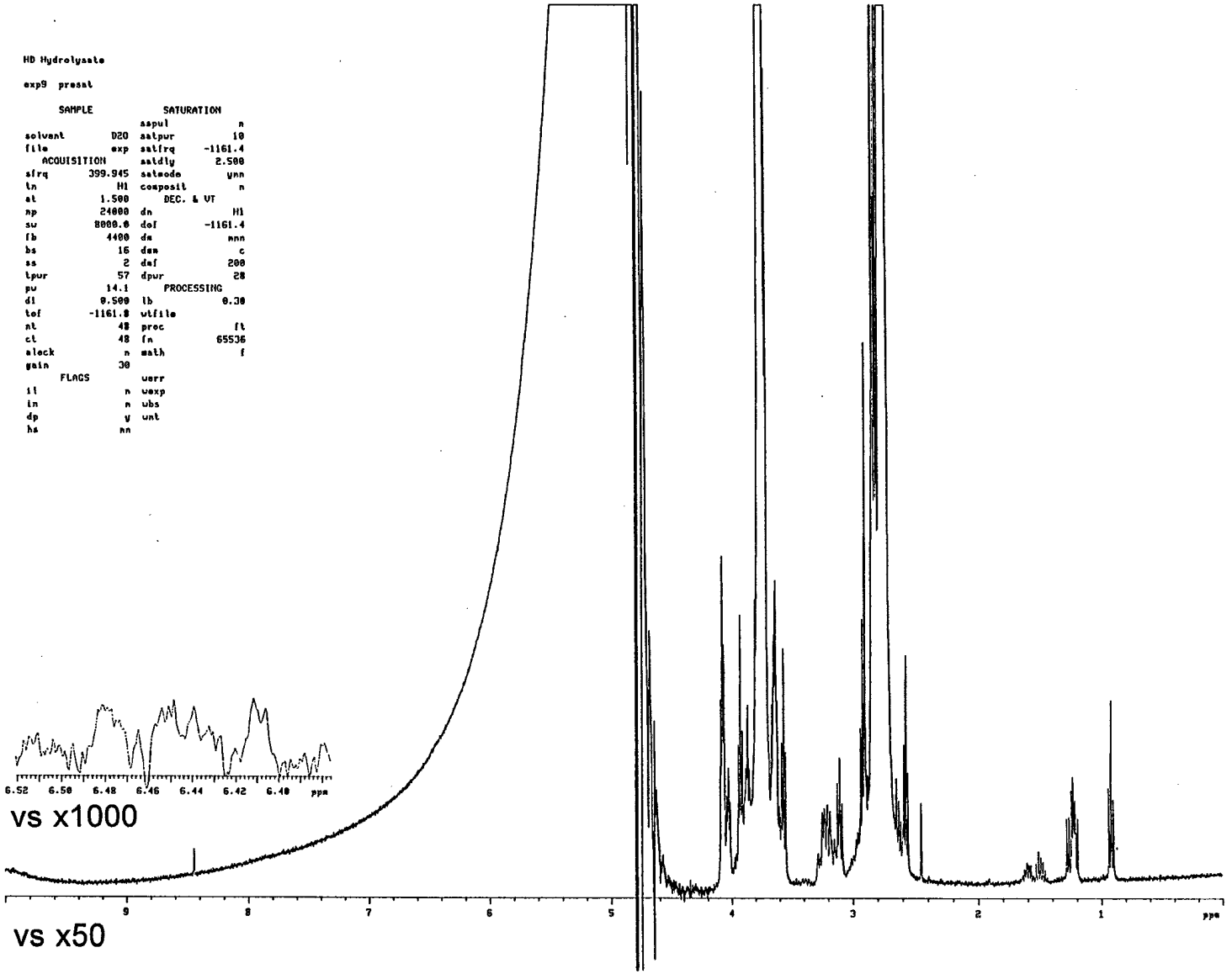


Figure 2. The 400 MHz ^{1}H NMR Spectrum: Increased Vertical Scale (x50).

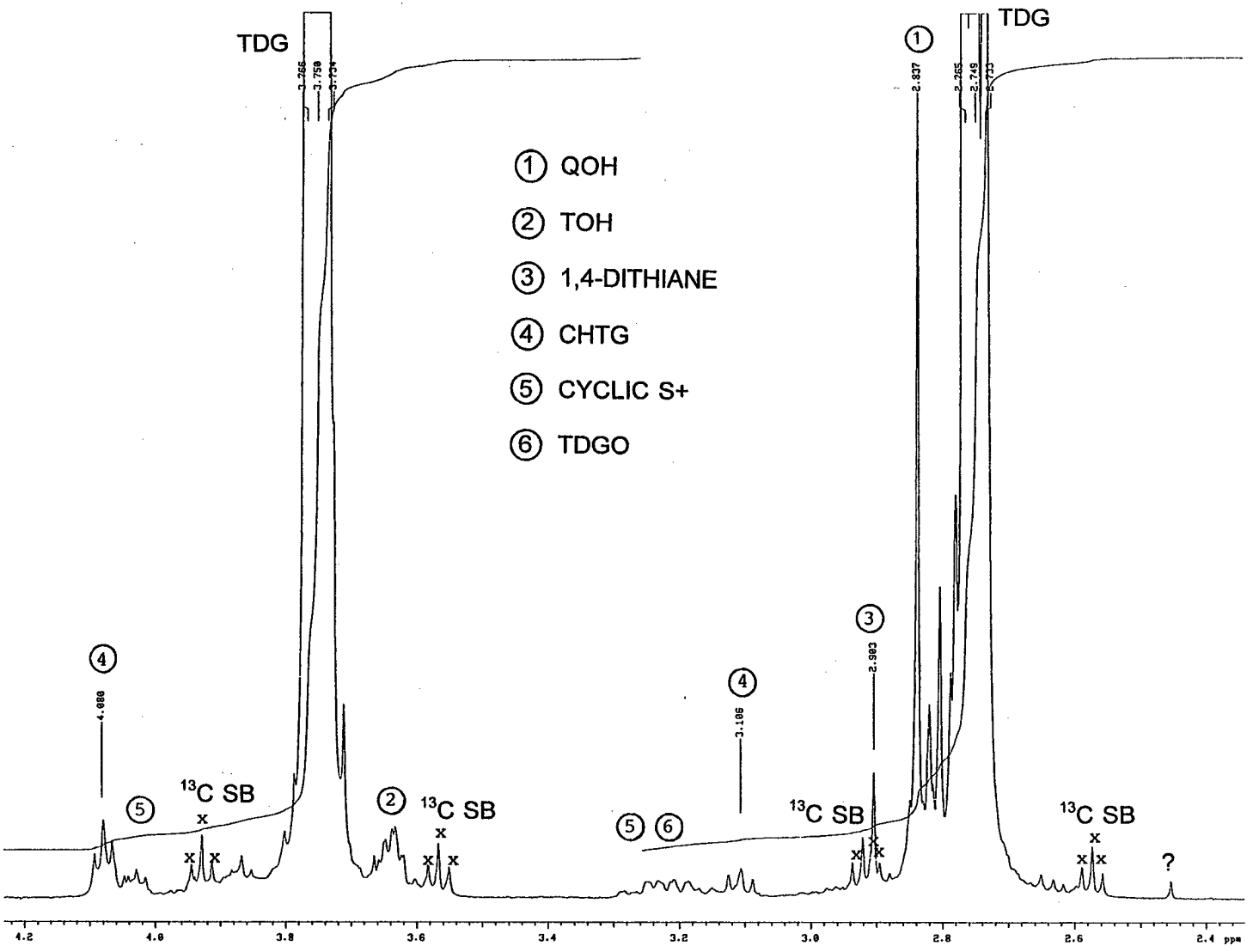


Figure 3. The 400 MHz ^{1}H NMR Spectrum: Expanded Plot, TDG Region.

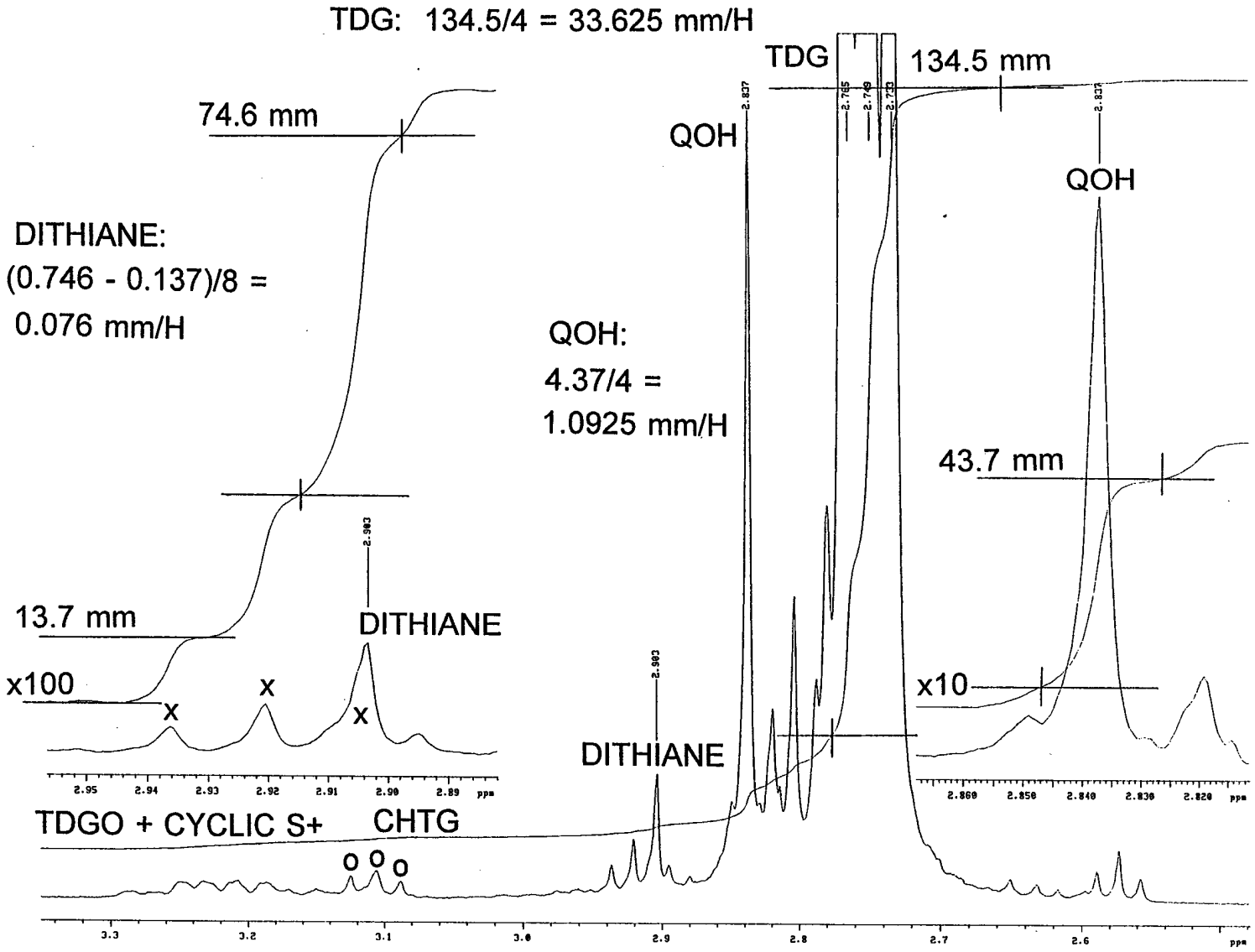


Figure 4. The 400 MHz ^{1}H NMR Spectrum: Expanded Plot, -SCH_2 Region.

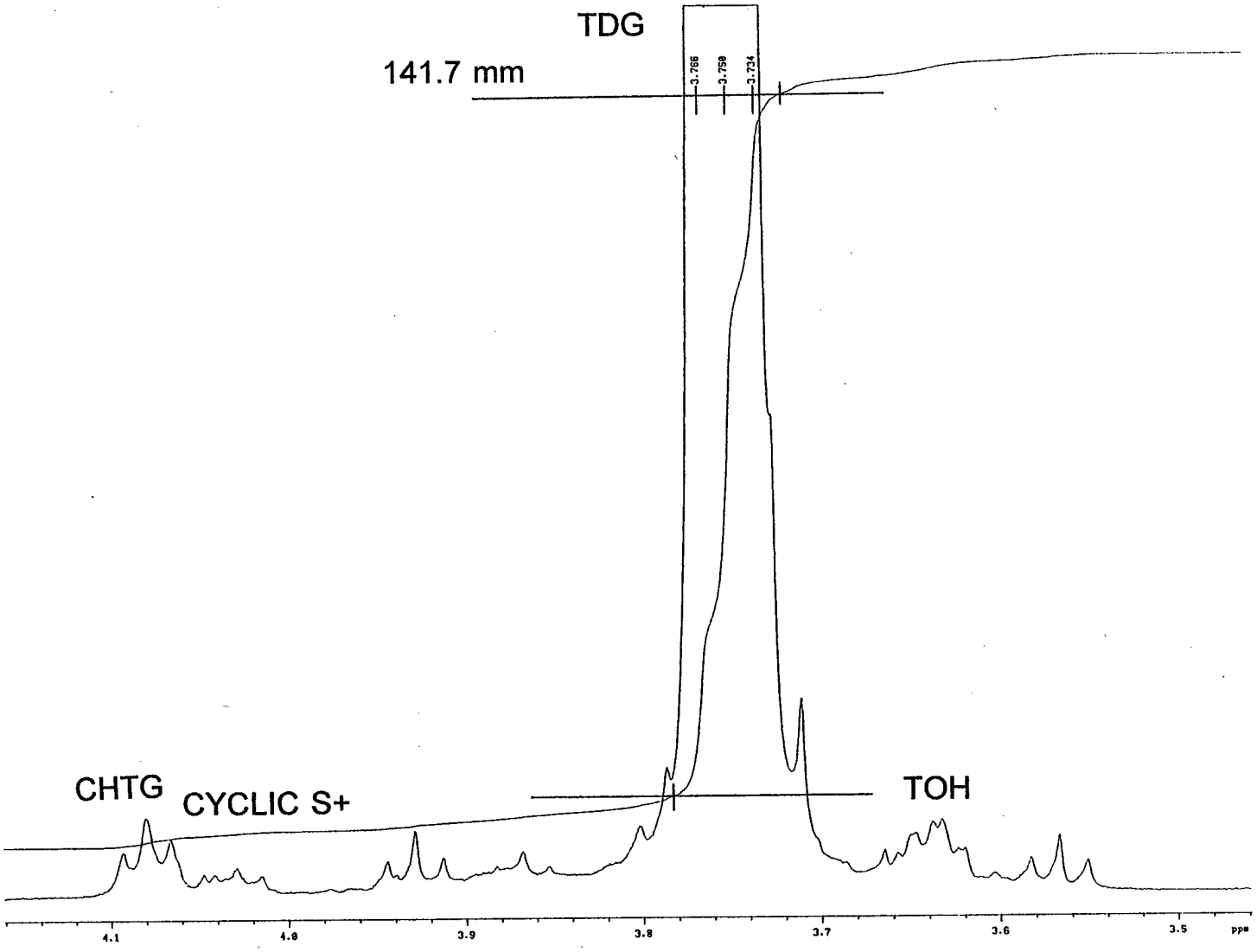


Figure 5. The 400 MHz ^{1}H NMR Spectrum: Expanded Plot, $-OCH_2$ Region.

other products were not included. Note that the area for this TDG triplet is less than the area of the TDG -OCH_2 triplet at δ3.75 (141.7 mm, figure 5). This is due to the overlap of other product peaks with the resonance at δ3.75. In other hydrolysate samples, the integral of the δ3.75 resonance may be the smaller;

Table 1. NMR Evaluation of HD Hydrolysate.

COMPOUND	mm/H	MOLE PERCENT
TDG	33.625	90.7
QOH	1.092	2.9
TOH	0.743	2.0
1,4-Dithiane	0.076	0.2
CHTG	0.343	0.9
C_5/C_6 Compounds	0.625	1.7
Other:		
TDGO	0.054	0.1
Ethers	0.187	0.5
Cyclic S^+	0.275	0.7 - 1.6
Vinyl Compounds	0.037	0.1
Unknown δ2.45	0.030	0.1

it depends on the nature of the other products present. The number of mm/proton (mm/H) for the TDG to be used to determine the mole percentages of each component in the sample was based on the triplet with the smallest integral. This integral (134.5 mm) was then divided by four (two -SCH_2 groups per molecule of TDG) to obtain the integral value per proton (see table 1).

The integral value per proton for each impurity uniquely observed in the ^1H spectrum was then ascertained:

(a) The integral for the -$S(CH_2)_2S$- singlet (δ2.84) from QOH ($[HOCH_2CH_2SCH_2-]_2$), the major hydrolysis of Q ($[ClCH_2CH_2SCH_2-]_2$), an impurity in HD, was measured on its expanded plot (figure 4) and excludes the small peaks on either side of the singlet. This integral (4.37 mm) was divided by four to obtain the integral value per proton for QOH (table 1).

(b) The integral for the 1,4-dithiane singlet (δ2.90) was measured on its expanded plot (figure 4). Since the dithiane resonance is coincident with the upfield peak of the triplet from the ^{13}C sideband of TDG (δ2.92), the integral from the sideband must be subtracted from the dithiane integral. Thus, the integral from the downfield peak of the sideband triplet (δ2.94) was measured and subtracted from the resonance at δ2.90 to give the

integral for the dithiane (0.609 mm). This value was then divided by eight to give the integral value per proton for dithiane (table 1).

(c) The unique resonance for the linear sulfonium ion CHTG ($HOCH_2CH_2$-S-$CH_2CH_2S^+[CH_2CH_2OH]_2$) is observed at δ3.11. The integral over this triplet was measured on its expanded plot (0.686 mm, figure 6), and the value was divided by two (-S$\underline{CH_2}$-CH_2S^+-) to obtain the integral value per proton for CHTG (table 1). The presence of CHTG or any sulfonium ion containing a -CH_2OH moiety beta to the sulfonium ion center can be confirmed by the ^{13}C NMR data; a peak between δ59.0 and 59.5 will be observed.

(d) Most HD samples contain C_5 and C_6 homologs of HD which hydrolyze to give the corresponding hydroxy compounds. The combined integral for these C_5 and C_6 compounds present in the sample was obtained from the expanded plot of the methyl region of the ^1H spectrum (figure 7). The integrals for the methyl triplet (δ0.92) and the methyl doublets (δ1.18 - 1.29) were added (0.670 mm + 1.206 mm), and the sum (1.876 mm) divided by three to obtain the integral value per proton for all C_5/C_6 compounds (table 1). GC/MS data from our laboratory indicates that there is one C_5 compound and three C_6 compounds having the molecular formulas $C_5H_{12}O_2S$ and $C_6H_{14}O_2S$, respectively. Since the exact structures of these compounds are not known, NMR must assume the worst case: Each compound is represented by one methyl group. Therefore, the measured integral is divided by three.

(e) In this spectrum, the single resonance at δ2.45 cannot be assigned to any known compound. The integral was measured on its expanded plot (0.091 mm, figure 7) and divided by three (since the resonance is in the methyl region of the ^1H spectrum) to obtain the integral value per proton for the unknown (table 1).

(f) The final product uniquely identified in this ^1H spectrum of HD hydrolysate is represented by a vinyl resonance at δ6.44 (-$\underline{CH}$=C, figure 8). The C=$\underline{CH_2}$ resonance of the vinyl moiety was not observed in this spectrum because the resonance is very small and obscured by the large residual water peak. GC/MS data from our laboratory has detected several CH_2=CH-R compounds in HD hydrolysate samples (R = -SCH_2CH_2OH, -SCH_2CH_2SCH=CH_2, -$SCH_2CH_2OCH_2CH_2SCH$=CH_2, and -$SCH_2CH_2SCH_2CH_2OH$). Since the exact structure of the vinyl product in this sample is not known, NMR must assume the worst case: One vinyl moiety is present per molecule. Thus, the integral value per proton is the same as the measured integral (0.037 mm, table 1).

In order to determine integral values for other products which do not have unique resonances that can be measured in the ^1H spectrum, data from the ^{13}C spectrum was used. The 100 MHZ ^{13}C NMR spectrum of the same HD hydrolysate sample is

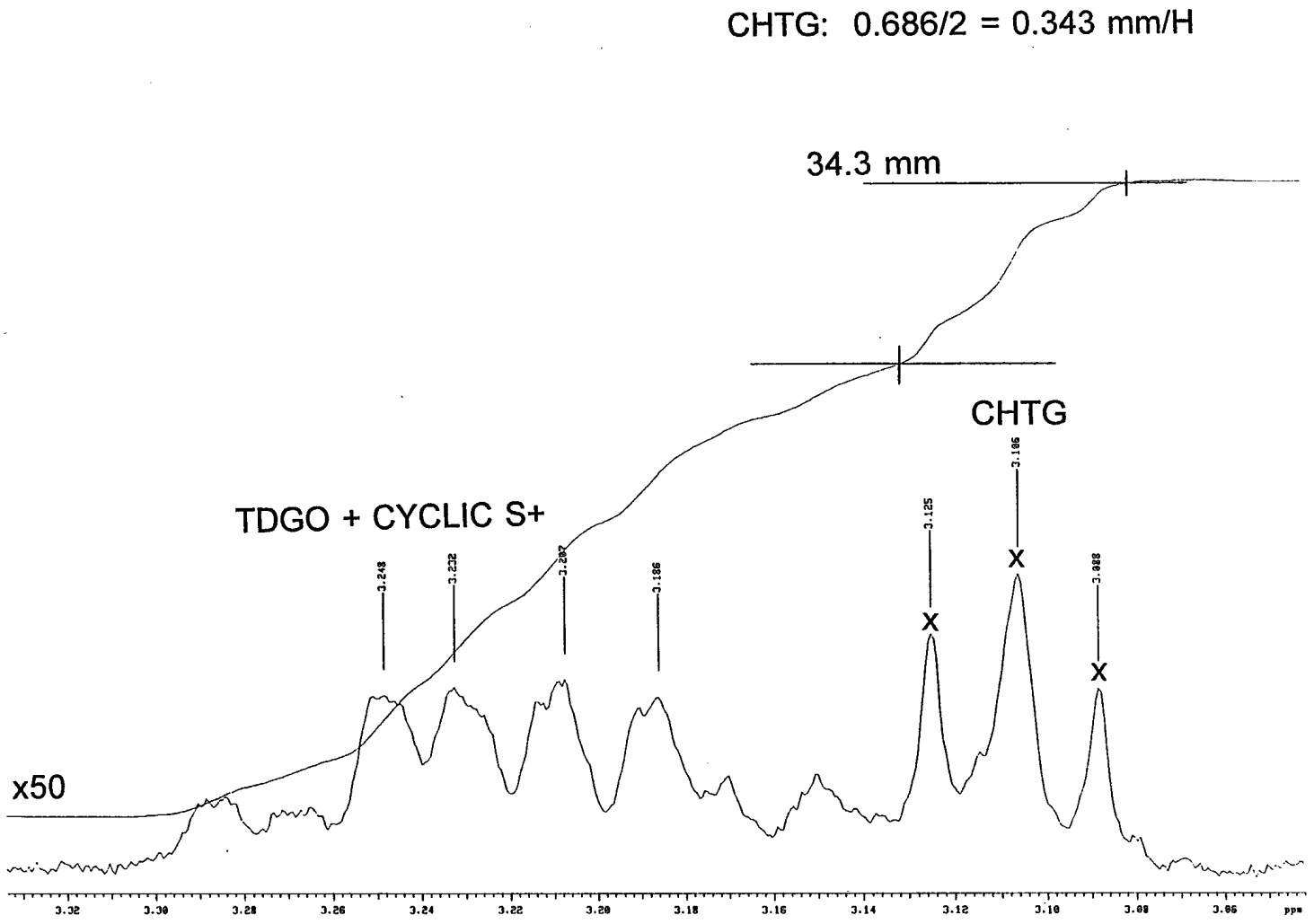


Figure 6. The 400 MHz ^{1}H NMR Spectrum: Expanded Plot, CHTG Region.

C_5/C_6 COMPDS: (1.206 + 0.670)/3 = 0.625 mm/H

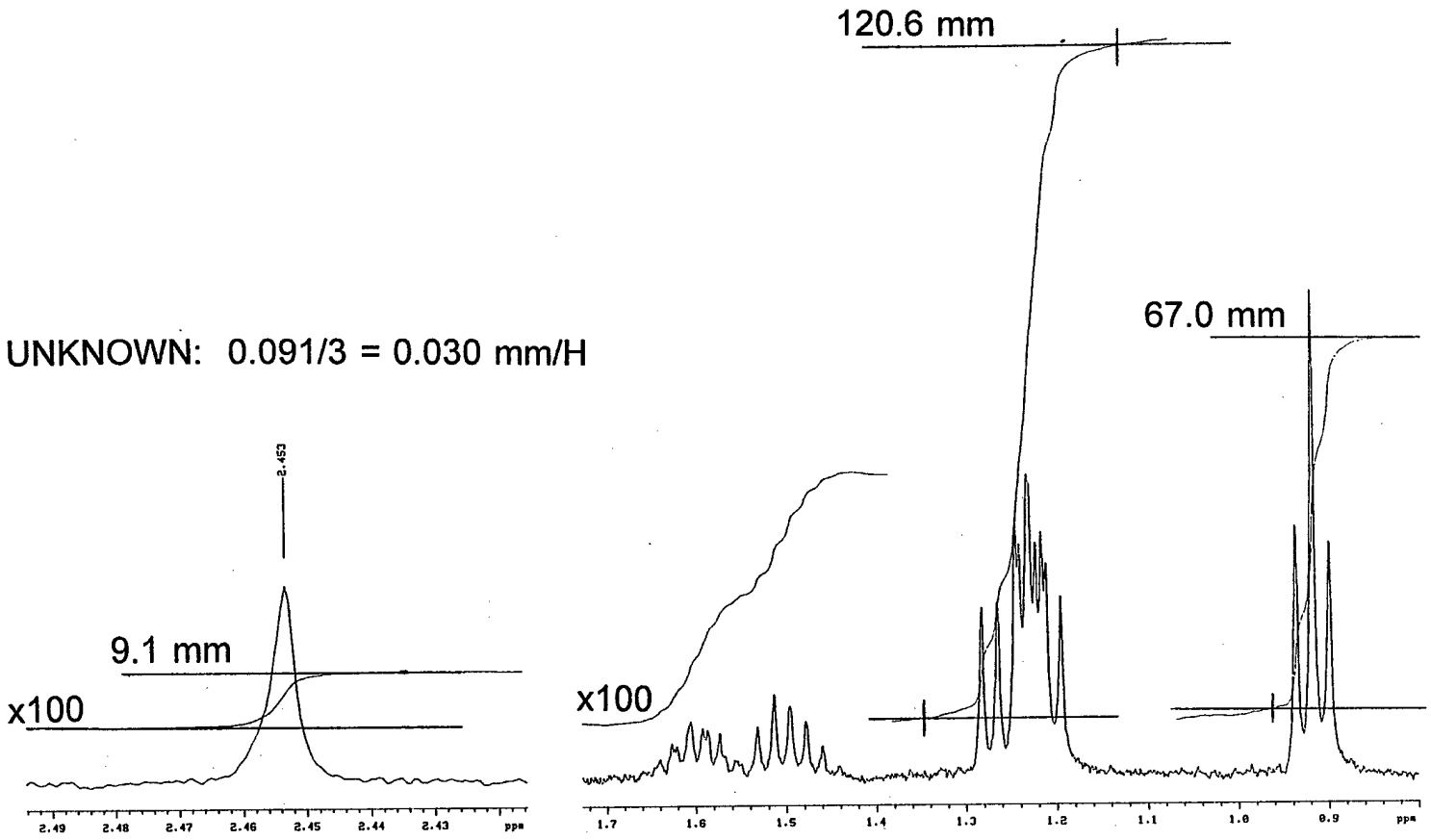


Figure 7. The 400 MHz ^{1}H NMR Spectrum: Expanded Plot , Methyl Region.

VINYL COMPD (R-CH=CH_2): 0.037 mm/H

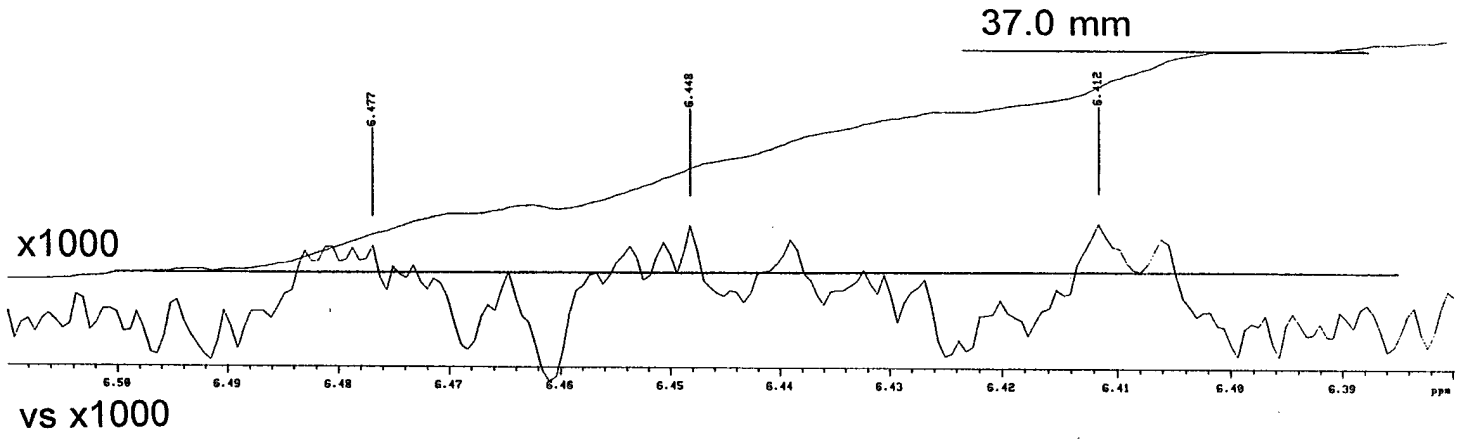


Figure 8. The 400 MHz ^{1}H NMR Spectrum: Expanded Plot, Vinyl Region.

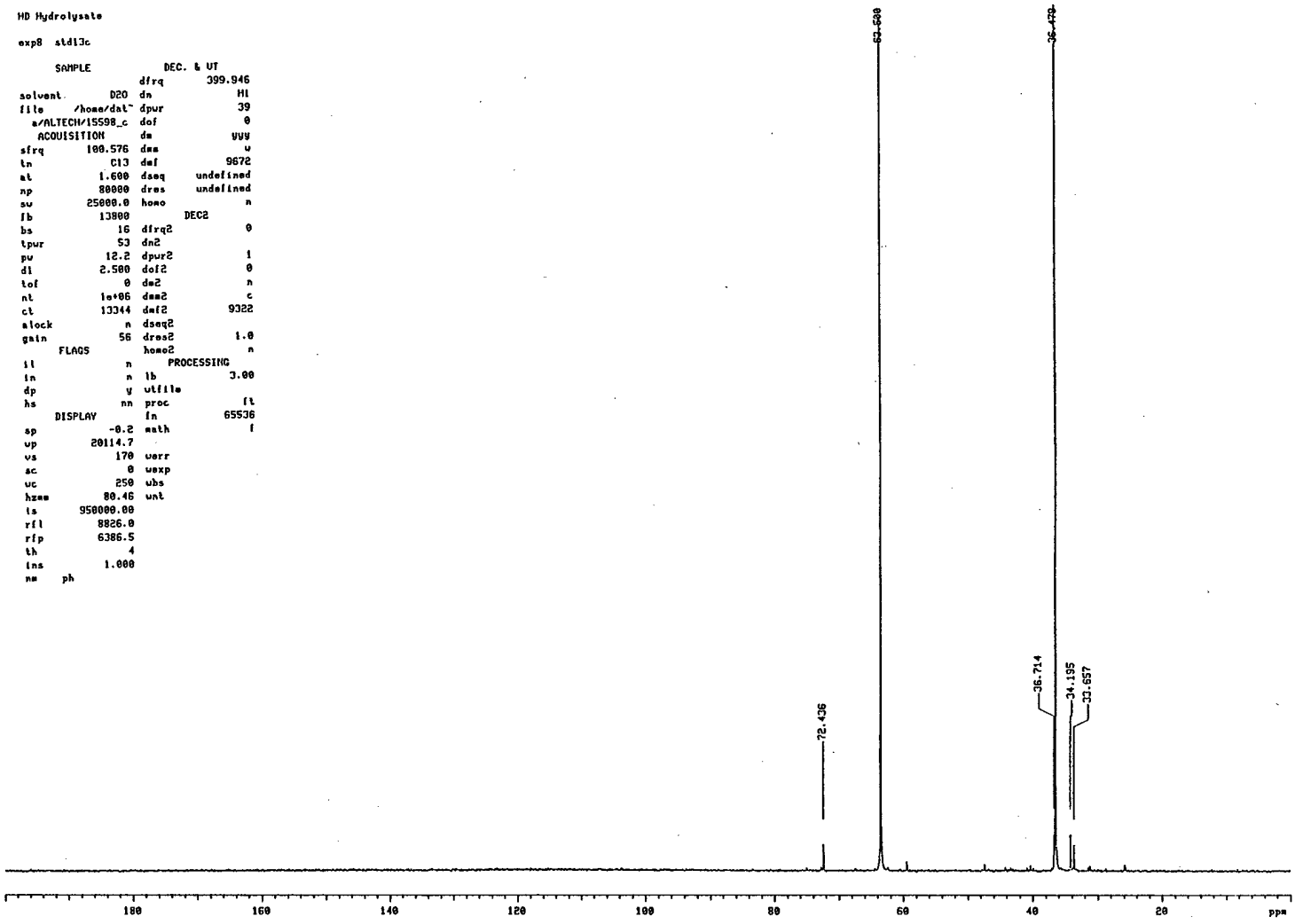


Figure 9. The 100 MHz ^{13}C NMR Spectrum of a Typical HD Hydrolysate Sample.

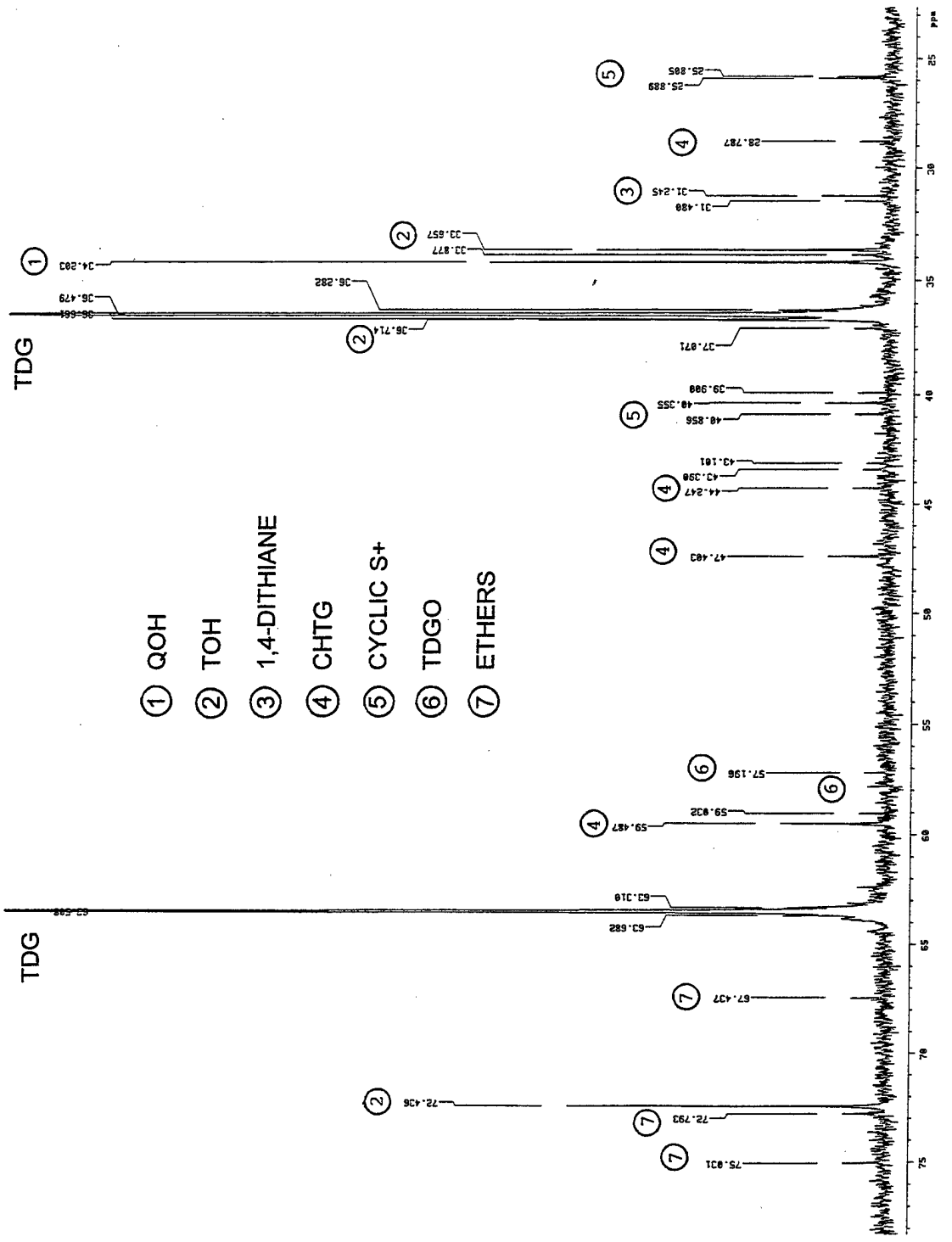


Figure 10. The 100 MHz ^{13}C NMR Spectrum: Increased Vertical Scale (x10).

shown in figure 9, and the spectrum with the vertical scale increased (x10) and the TDG and other product peaks labeled is shown in figure 10.

To determine the integral value per proton for TOH ($[HOCH_2CH_2\text{-}S\text{-}CH_2CH_2]_2\text{-}O$), the-$SCH_2$ resonances for both QOH (δ34.2) and TOH (δ33.7) were expanded and integrated (figure 11). The integral value per carbon for each was obtained by dividing each integral (QOH: 56.6 mm; TOH: 38.5 mm) by two. The mole ratio observed in the ^{13}C spectrum was then used to calculate (by direct proportions, figure 11) the integral value per proton for the TOH (0.743 mm) to be used in the final calculation (table 1).

The integral value per proton for each of the products observed only in the ^{13}C spectrum was determined in the same way:

(a) Two cyclic sulfonium ions having the $S(CH_2CH_2)_2S^+$ moiety were observed in the sample (δ25.8 and 25.9). Evidence for the presence of cyclic sulfonium ions was also observed in the ^1H spectrum (figure 3); however, because of overlapping peaks, no unique resonance was present that could be used to obtain the integral value per proton for these species. Using the integral of the expanded ^{13}C plot (14.3 mm, figure 11), divided by two (since these resonances represent the two ring -SCH_2 carbons beta to the sulfonium ion center), and using the mole ratio of QOH to the cyclic sulfonium ions from the ^{13}C spectrum, the integral value per proton (0.275 mm) for the sulfonium ions was calculated by direct proportions (figure 11 and table 1).

(b) The integral value per proton for the thiodiglycol sulfoxide (TDGO) was obtained by adding the integrals for the two TDGO resonances (δ57.2, 2.60 mm and δ57.8, 2.95 mm, figure 12) and dividing the sum by four. The mole ratio of QOH to TDGO observed in the ^{13}C spectrum was then used to calculate (by direct proportion, figure 12) the integral value per proton for TDGO (0.054 mm) to be used in the final calculation (table 1).

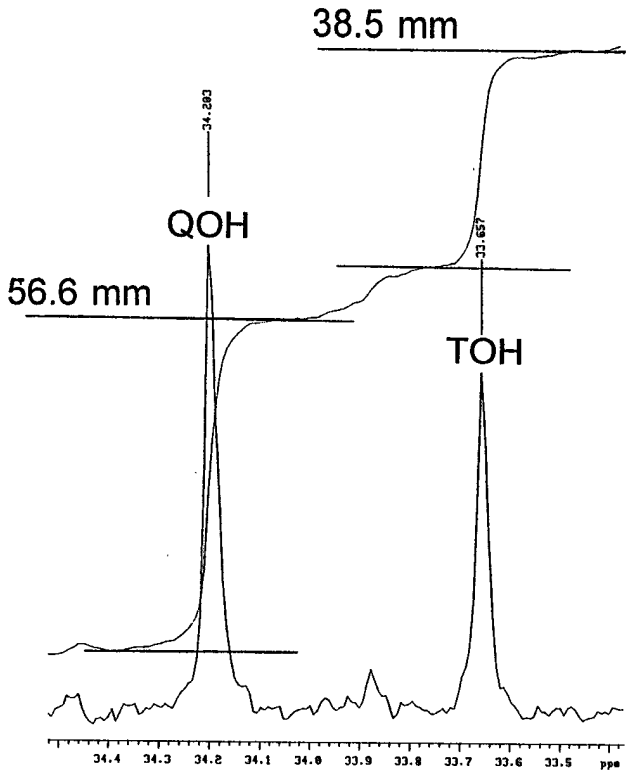
(c) In addition to TOH, other resonances were observed in the ^{13}C spectrum that indicate the presence of other ether compounds (δ67.4, 72.8 and 75.0). Indeed, the peak at δ67.4 suggests that this ether may even be a sulfonium ion since the ether -OCH_2 carbon beta to a sulfonium ion center resonates at this chemical shift. To determine the total amount of these other ethers, the integrals of these peaks on the expanded plot (figure 13) were added together (2.34 mm + 3.20 mm + 5.60 mm), and the sum divided by two (since each ether must have two carbons attached to the ether oxygen). The mole ratio of TOH to these ethers observed in the ^{13}C spectrum was then used to calculate (by direct proportion, figure 13) the integral value per proton (0.187 mm) to be used in the final mole percent calculation (table 1).

TOH: 38.5/2 = 19.25 mm/C

28.3 mm/C / 1.092 mm/H = 19.25 mm/C / x mm/H

x = 0.743 mm/H

QOH: 56.6/2 = 28.3 mm/C



CYCLIC S+: 14.3/2 = 7.13 mm/C

28.3 mm/C / 1.092 mm/H = 7.13 mm/C / x mm/H

x = 0.275 mm/H

CYCLIC S+ (2 TYPES)

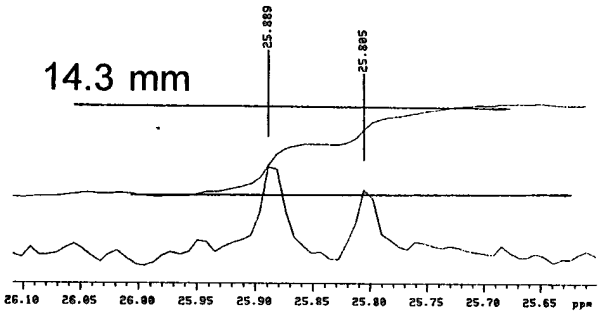


Figure 11. The 100 MHz ^{13}C NMR Spectrum: Expanded Plots, TOH and Sulfonium Ion Regions.

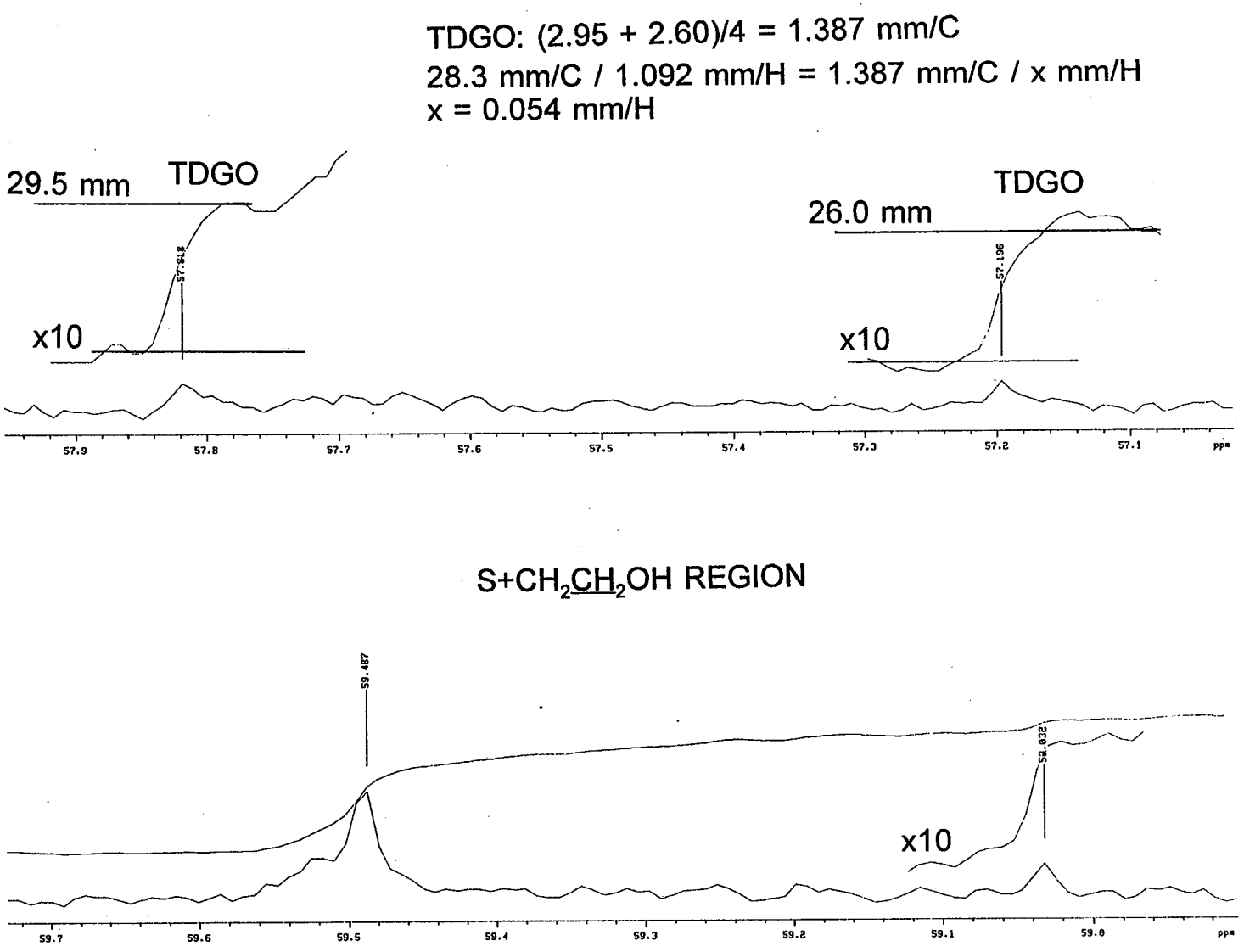


Figure 12. The 100 MHz ^{13}C NMR Spectrum: Expanded Plots, TDGO and Sulfonium Ion Regions.

ETHERS: (2.34 + 3.20 + 5.60)/2 = 5.57 mm/C

22.1 mm/C / 0.743 mm/H = 5.57 mm/C / x mm/H

x = 0.187 mm/H

TOH: 44.2/2 = 22.1 mm/C

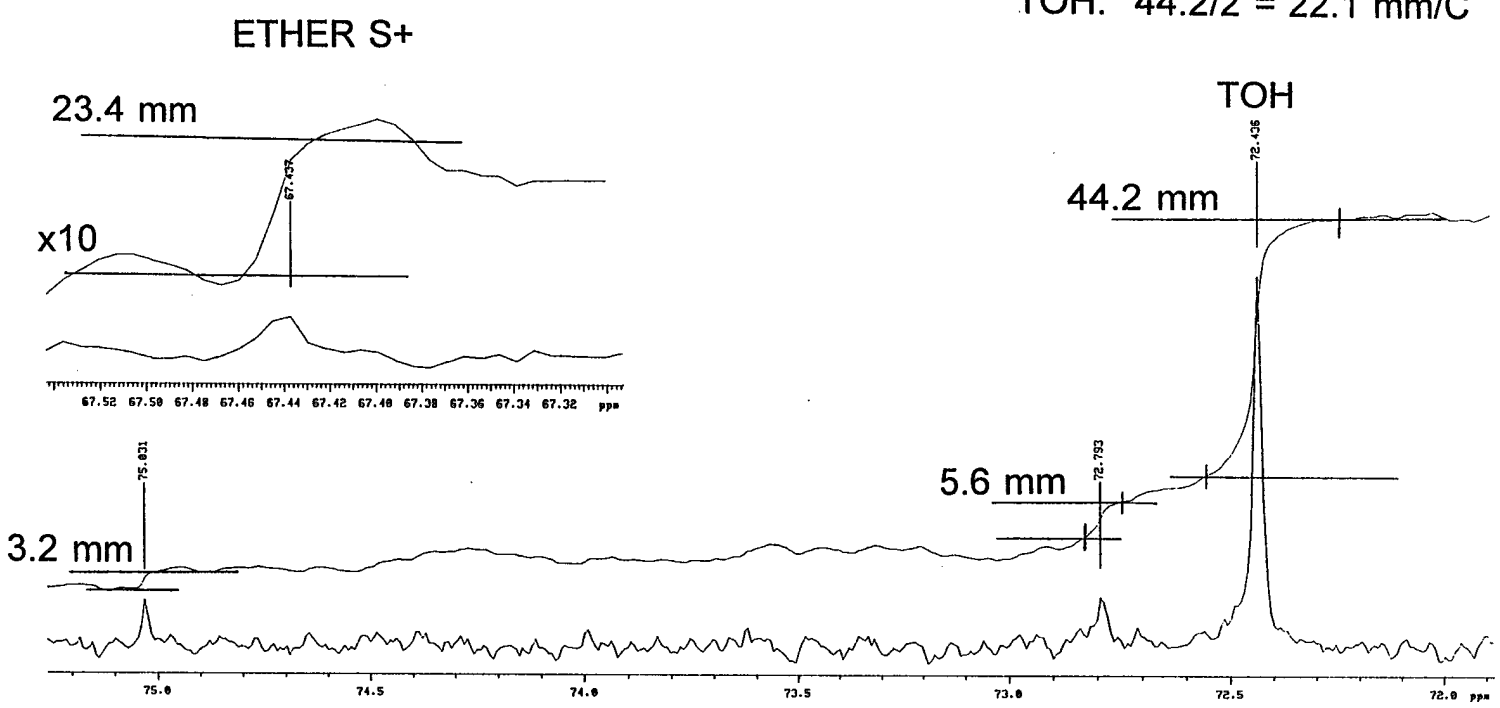


Figure 13. The 100 MHz ^{13}C NMR Spectrum: Expanded Plot, Ether Region.

After all of the products that could be observed in either the ^{1}H or the ^{13}C spectrum of the HD hydrolysate were identified and the integral value per proton determined for each, the integral values for all components were added together. Each

Table 2. Multiple NMR Analyses for the Same Sample of HD Hydrolysate (Mole%).

COMPOUND	RUN 1*	RUN 2	RUN 3	RUN 4	RUN 5
	^{1}H: 96 ^{13}C: 57136	^{1}H: 64 ^{13}C: 6000	^{1}H: 96 ^{13}C: 13632	^{1}H: 96 ^{13}C: 6000	^{1}H: 48 ^{13}C: 13632
TDG	92.4	91.7	92.4	91.6	91.4
QOH	2.6	2.8	2.7	3.2	3.1
TOH	1.7	1.9	1.7	1.8	2.0
1,4-Dithiane	0.4	0.4	0.4	0.4	0.4
CHTG/$S^{+}CH_2\underline{CH_2}OH$	0.1	0.1	0.1	0.1	0.1
C_5/C_6 compounds	1.3	1.3	1.4	1.4	1.4
Other:					
TDGO	0.1	0.2	0.2	0.2	0.2
Vinyl compound	0.4	0.5	0.3	0.3	0.3
Ethers	0.3	0.5	0.2	0.3	0.3
Glycol	0.5	0.6	0.6	0.7	0.7
Unknown δ3.86	<0.1	N.O.	N.O.	N.O.	N.O.
Unknown δ1.92	<0.1	<0.1	<0.1	<0.1	<0.1

N.O. = Not Observed

* The number of transients used to obtain each spectrum is given.

integral value was then divided by this sum (37.087 mm) and multiplied by 100% to obtain the mole percent for each compound observed in the sample.

To determine the reproducibility of the NMR analyses, a sample of HD hydrolysate was characterized five separate times. The same sample in the same NMR tube was used. Five separate sets of ^{1}H and ^{13}C spectra were acquired using the parameters in Section 2.11.4, above. Between each set of experiments, the sample was physically removed from the spectrometer; the probe frequency and magnetic field homogeneity were tuned each time. Each set of spectra was plotted, integrated and evaluated separately as described above; the results are shown in table 2. The

standard deviation for all components was calculated to be <0.5 mole%.

As is the case with most samples evaluated using NMR, the analysis of the HD hydrolysates is highly subjective since the results depend directly on the ability of the spectroscopist to interpret the spectra (i.e., recognize all the compounds represented) as well as on his/her ability to obtain the quantitative data necessary for the analysis (i.e., correctly expand and integrate the resonances of interest). With a little practice, however, these problems can be overcome; and reproducible information can be obtained.

4. CONCLUSION

A useful and facile NMR method for characterizing HD hydrolysates has been developed. The method uses both ^{1}H and ^{13}C NMR to identify the major components in the hydrolysate (>0.1 mole%), and, to determine the mole ratios of these compounds in solution. The NMR method is especially useful since no sample preparation is required, and all soluble compounds, including the polar and ionic products from HD hydrolysis, are easily observed. To date, NMR is the only analytical method that can give quantitative information on the amounts of the various sulfonium ions present in the HD hydrolysates.

APPENDIX A

REPRESENTATIVE NMR PARAMETERS FOR COMPOUNDS OBSERVED IN HD HYDROLYSATES

COMPOUND	δ ^{1}H, ppm (J in Hz)*	δ ^{13}C, ppm (J in Hz)*
TDG:		
SCH_2	2.75 (t, 6.3)	36.5
OCH_2	3.75 (t, 6.3)	63.5
QOH:		
SCH_2	2.84 (s)	34.2
CH_2S	2.80	36.5 (N.O.)
OCH_2	3.75 (N.O.)	63.5
TOH:		
SCH_2	(N.O.)	33.7
CH_2S	(N.O.)	36.7
CH_2OH	3.75 (N.O.)	63.5 (N.O.)
OCH_2	3.64 (m)	72.4
1,4-Dithiane:		
SCH_2	2.90 (s)	31.2
CHTG:		
SCH_2	3.11 (t, 7.2), 2.85 (t, 5.7) (N.O.)	28.9, 36.7
$S^+(CH_2)$	3.7-3.8 (N.O.)	44.6
$S^+(CH_2)_2$	3.7-3.8 (N.O.)	46.7
$O\underline{C}H_2CH_2S$	3.84 (N.O.)	63.4
$O\underline{C}H_2CH_2S^+$	4.11 (t, ~5.4)	59.3
H2TG:		
SCH_2	3.20 (t, 7.2)	28.9
$S^+(CH_2)$	3.8 (N.O.)	44.3
$S^+(CH_2)_2$	3.78 (N.O.)	46.9
OCH_2	4.11 (t,~5.4)	59.3
TDGO:		
SCH_2	3.10 (d, 14.0; t, 4.4) 3.17 (d, 14.0; d, 8.0; d, 5.6)	57.1
OCH_2	4.03 (m)	57.8
Glycol:		
OCH_2:	3.66 (s)	65.7
Cyclic Sulfonium Ions:		
$S(CH_2)_2$	3.20 (m), 3.25 (m)	25.7
$(CH_2)_2S^+$	3.63 (m), 3.94 (m)	40.0
S^+CH_2	3.96 ("t")	45.7
CH_2Cl	4.07 ("t")	40.5
$O(CH_2)_2$	4.13 (m), 4.35 (m)	65.1
$(CH_2)_2S^+$	3.49 (m), 3.75 (m)	37.3
S^+CH_2	4.07 (m)	45.8
CH_2Cl	4.14 (m)	40.6
Vinyl Product:		
CH_2	5.25 (d, 17), 5.32 (d, 10.0)	115.6
CH	6.44 (d, 17; d, 10.0)	134.0
1,4-Thioxane:		
SCH_2	2.60 ("t")	27.0
OCH_2	3.92 ("t")	68.5

s = singlet; t = triplet; m = multiplet; N.O. = Not Observed

* ^{1}H chemical shift values *vs* internal TSP (sodium 3-trimethylsilylpropionate-2,2,3,3-d_4); ^{13}C chemical shifts *vs* external TSP in D_2O.

Blank

APPENDIX B

REPRESENTATIVE LONGITUDINAL RELAXATION TIMES (T_1's) AT 9.4T FOR COMPOUNDS PRESENT IN THE HD HYDROLYSATES

RESONANCE	^1H T_1	^{13}C T_1
	sec	sec
TDG:		
SCH_2	2.3 ± 0.1	3.1 ± 0.1
OCH_2	2.3 ± 0.1	3.1 ± 0.1
QOH:		
$S(CH_2)_2$	1.2 ± 0.4	2.0 ± 0.5
Dithiane:		
SCH_2	0.8 ± 0.1	N.O.
Glycol:		
OCH_2	3.0 ± 0.5	N.O.

N.O. = Not Observed