Journal of Chromatography, 369 (1986) 97–103
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 984

GAS–LIQUID CHROMATOGRAPHY OF 3-CHLOROPROPANEDIOL

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(First received March 14th, 1986; revised manuscript received July 30th, 1986)

SUMMARY

To improve the gas chromatographic properties of 3-chloropropanediol, a phenylboronic acid derivative was prepared. This method appears to be suitable for trace analysis of the title compound. Epichlorohydrin, 1,2-propanediol, and 1,2-dichloropropanol were among the structurally related compounds shown not to interfere. The structure of the derivative was confirmed by gas chromatography–Fourier transform infrared spectroscopy utilizing a matrix isolation interface and gas chromatography–mass spectrometry.

INTRODUCTION

3-Chloropropanediol (3CPD) is a post-testicular male anti-fertility agent[1,2], which has been shown to be mutagenic in bacterial assays[3]. Animal testing of its carcinogenicity has given equivocable results[4]. In addition, 3CPD is nephrotoxic[5]. 3CPD is a hydrolysis product of epichlorohydrin[6], a widely used commercial chemical[7]. 3CPD, as well as several of its fatty acid esters, has been reported to be present in food[8–12].

The gas chromatography of underivatized 3CPD has been reported in several papers, but the reproducibility and linearity of response were not reported[9]. A variety of gas chromatographic columns have been utilized for the determination of 3CPD and other short chain dihydroxy compounds. Recommended columns for ethylene glycol, 1,2-propanediol, and 1,3-propanediol include 0.8% Theed on 80–100 mesh Carbopack C[13], 3% Carbowax 20M on Chromosorb 101[14], Chromosorb 101, 100–120 mesh or Non-Pakd Superox[15].

Although good separation, linearity, and a precision of 4% were reported using 0.8% Theed[13], in our hands the use of this packing material for the chromatography of 3CPD yielded poor reproducibility and chromatography. Maximum temperature before deterioration of column packing (for 0.8% Theed) is 125°C. Because of this low temperature, tailing of 3CPD on this column was a major problem.

Unsatisfactory results for the chromatography of ethylene glycol were demonstrated[14] on uncoated Chromosorb 101, 102, and Chromosorb 102 coated with Carbowax 20M. Super-pak 20M coated with Carbowax 20M gave marginal results, while

0021-9673/86/$03.50

better results were obtained using 3% or 6% Carbowax 20M on Chromosorb 101. Peak tailing, column bleed, ghost peaks, and the poor reproducibility with extended usage of these columns makes these columns undesirable for analysis of diol compounds.

Because of the lack of a satisfactory packed column for 3CPD, derivatization to facilitate gas chromatography was explored. N-Butylboronate has been used for gas chromatographic separation of the enantiomers of 3CPD[16]. The well-characterized reaction between phenylboronic acid and dihydroxy compounds[17,18] was utilized here to prepare a derivative for gas chromatographic determination of 3-chloropropanediol.

EXPERIMENTAL

Reagents and standards

Standards of 3CPD, epichlorohydrin, 1,3-dichloropropanol, 3-bromopropanediol, 3-chloro-2-propanol, 1,3-propanediol, 1,2-propanediol, 3-chloro-1-propanol, and ethylene glycol were obtained from the Aldrich (Milwaukee, WI, U.S.A.) and used as received. Phenylboronic acid was obtained from the Sigma (St. Louis, MO, U.S.A.). HPLC grade 2,2-dimethoxypropane was obtained from Fisher Scientific. The derivatization reagent consisted of 40 mmol/l phenylboronic acid in 2,2-dimethoxypropane. 2,2-Dimethoxypropane was added as a scavenger for water[19].

Derivatization procedure

A volume of 50 μl of sample solution in acetonitrile was added to 100 μl of phenylboronic acid derivatizing reagent. The solution was vortex mixed and a 1-μl aliquot was injected into the gas chromatograph.

Instrumentation

Gas chromatography–matrix isolation-Fourier transform infrared spectroscopy (GC–MI-FT-IR) was performed with a Varian 3700 gas chromatograph operating in the split mode interfaced to a Sirius 100 FT-IR spectrophotometer (Mattson Instruments). A matrix isolation interface (Cryolect, Mattson Instruments) was employed. With this instrumentation the effluent from a capillary gas chromatograph is split between a flame ionization detector (20%) and the matrix isolation device (80%). The effluent from the gas chromatograph which goes to the matrix isolation instrument is trapped in a frozen argon matrix on a rotating gold plated drum. The drum is kept under vacuum at 12 K. At the end of the chromatographic run, the FT-IR spectrophotometer is used to obtain spectra of the argon embedded GC effluent. The spectra of the peaks corresponding to the flame ionization detector response can readily be obtained. The flame ionization detector response is sent to the computer which controls the FT-IR spectrophotometer and the matrix isolation interface. The computer can then identify the area of matrix isolated effluent corresponding to a particular time in the GC run as characterized by the flame ionization detector. IR spectra were generated by co-addition of 100 scans with 4 cm^{-1} resolution.

GC–electron impact mass spectroscopy was performed using a Finnigan Model 4500 with a source potential of 70 eV. The gas chromatograph was operated with an initial temperature of 100°C for 4 min, programmed to 200°C at 20°C/min

and held at 200°C for 4 min. A 10M methylsilicone wide-bore capillary column (Alltech; RSL 150, 0.53 mm I.D., film thickness, 1.2 μm) was used.

RESULTS AND DISCUSSION

Direct chromatography without derivatization is attractive because of its simplicity. However, with 0.8% Theed on Carbopack C, 5% Carbowax 20M on Chromosorb W HP, Super Q (Alltech), and Tenax-GC, peak broadening, difficulties with low sensitivity and ghost peaks were observed with the chromatography of 3CPD. These results suggest that substantial retention of these compounds may occur on the chromatographic column. To overcome the undesirable chromatographic properties, a derivatization procedure was utilized. The chromatography of the phenylboronic acid derivative of 3CPD on two different types of columns is shown in Figs. 1 and 2.

Under the described GC conditions (Fig. 1), concentrations of 3CPD-phen-

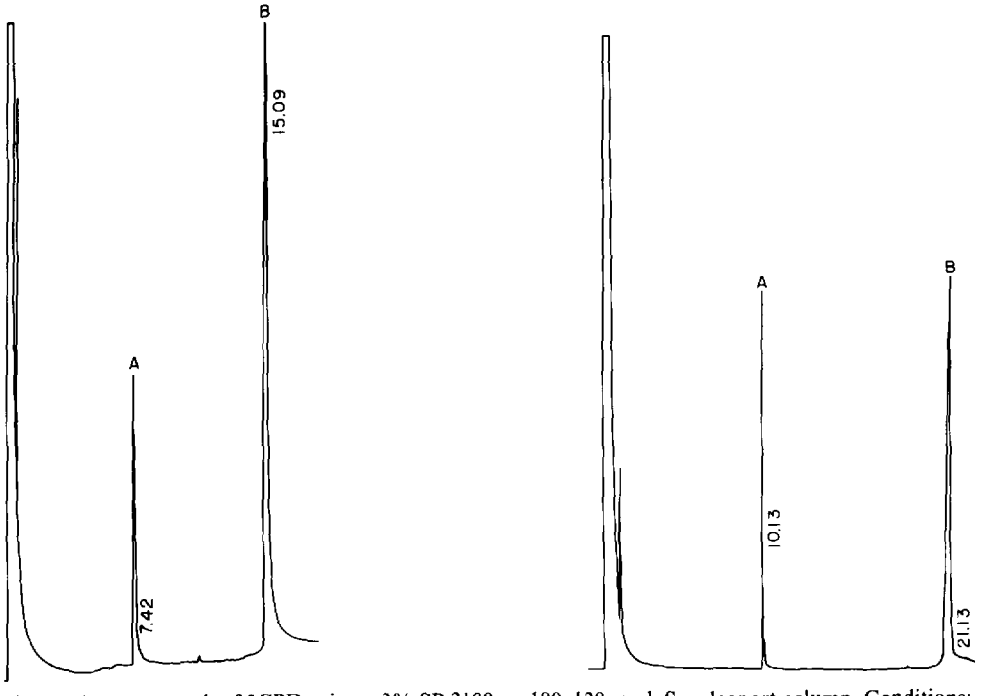

Fig. 1. Chromatograph of 3CPD using a 3% SP 2100 on 100–120 mesh Supelcoport column. Conditions: He = 40 ml/min, air = 300 ml/min, H_2 = 30 ml/min, FID sens. = 10^{11} (ATT = 1), inj. temp. = 250°C, det. temp. = 300°C, chart speed = 0.5 cm/min; ATT (integrator attenuation) = 64, program run = 100°C (4 min), program of 15°C/min to 280°C. Indicated retention times are in min. A = 3CPD-phenylboronate (98 ng); B = phenylboronic acid.

Fig. 2. Chromatograph of 3CPD on a DB5-30M (0.25 μ) fused-silica capillary column. Condition: He = 98.5% + Argon = 1.5% carrier, flow = 1.0 ml/min, make-up = 29 ml/min, split ratio = 1:10, air = 300 ml/min, H_2 = 30 ml/min, FID sens. = 10^{-11} (ATT = 2), inj. temp. = 250°C, det. temp. = 300°C, program run = 100°C (4 min), program of 10°C/min to 280°C, chart speed = 0.5 cm/min. ATT = 16. Indicated retention times are in minutes. A = 3CPD-phenylboronate (2 ng); B = phenylboronic acid.

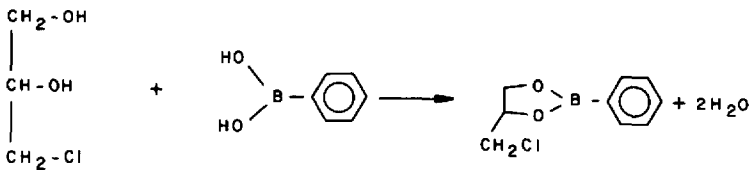

Fig. 3. 3-Chloropropanediol reaction with phenylboronic acid.

ylboronate ranging from 2.6 ng/μl to 133 ng/μl were analyzed for reproducibility and linearity. Concentrations of 17 (n = 2), 33 (n = 2), 66 (n = 3), and 133 (n = 4) ng/μl, using 1-μl injections, showed coefficients of variation (C.V.) of less than 2.5%. A C.V. of 11% was observed with injections at a concentration of 133 ng/μl over 2 days. Even with the large C.V. seen with injections made over a period of days, injections of 2.6–133 ng/μl over three days were linear (r = 0.99). Linearity and reproducibility for the derivative of 3CPD were not tested on the DB-5 30M fused-silica capillary column, although a sizeable response was seen for the 2 ng/μl on-column concentration seen in Fig. 2.

The proposed structure of the 3CPD derivative is shown in Fig. 3. This type of derivatization has been utilized for determination of ethylene glycol[20], and is shown to be a general reaction for diols[19,17]. It is felt that the derivative is formed in the gas chromatographic injection port, although this has not been documented. Derivatization using the phenylboronic acid reagent gave distinct peaks for 3-bromopropanediol, 1,3-propanediol, 1,2-propanediol, and ethylene glycol which did not interfer with 3CPD. Compounds which were found to give no peaks with derivitization were 1,2-dichloropropanol, 1,3-dichloropropanol, 2-propanol, methanol, and epichlorohydrin. Although it is likely that reaction occurs with these compounds, either the conversion is too poor for these compounds to be detected, or they do not appear with the chromatographic conditions utilized. No interference occurred with the solvent blank (acetonitrile).

The IR spectra of the phenylboronic acid derivatives of 1,2-propanediol and 3-chloropropanediol are shown in Figs. 4 and 5 respectively. Both IR spectra dem-

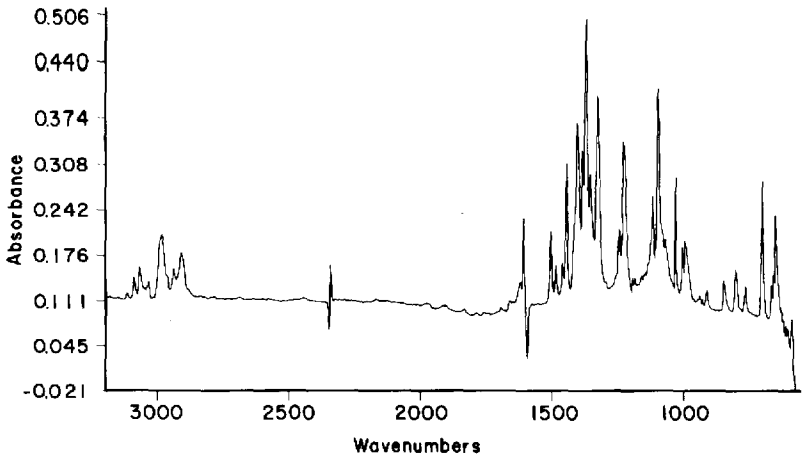

Fig. 4. GC–MI-FT-IR spectra of 1,2-propanediol phenylboronate.

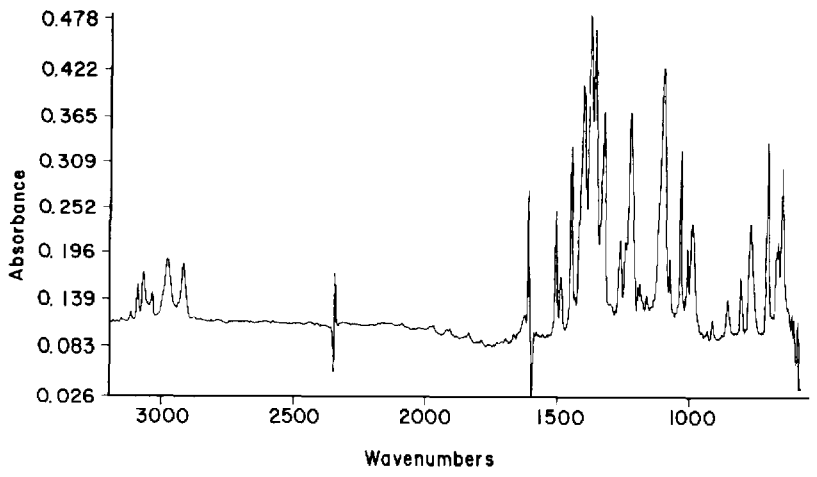

Fig. 5. GC–MI-FT-IR spectra of 3-chloropropanediol phenylboronate.

onstrate similar aromatic CH stretches above 3000 cm^{-1}. The methyl CH bending absorbances near 1370 cm^{-1} and 1440 cm^{-1} are nearly identical for the two compounds as are the methylene C–H stretch absorbances near 2940 cm^{-1}. The aromatic disubstituted ring bending occurs at 764 cm^{-1} for 1,2-propanediol derivative. For the 3CPD derivative this is obscured by the strong C–Cl absorbance at 763 cm^{-1}.

The mass spectra of the phenylboronic acid derivatives of 1,2-propanediol and 3CPD are shown in Figs. 6 and 7 respectively. In each mass spectrum we see a small

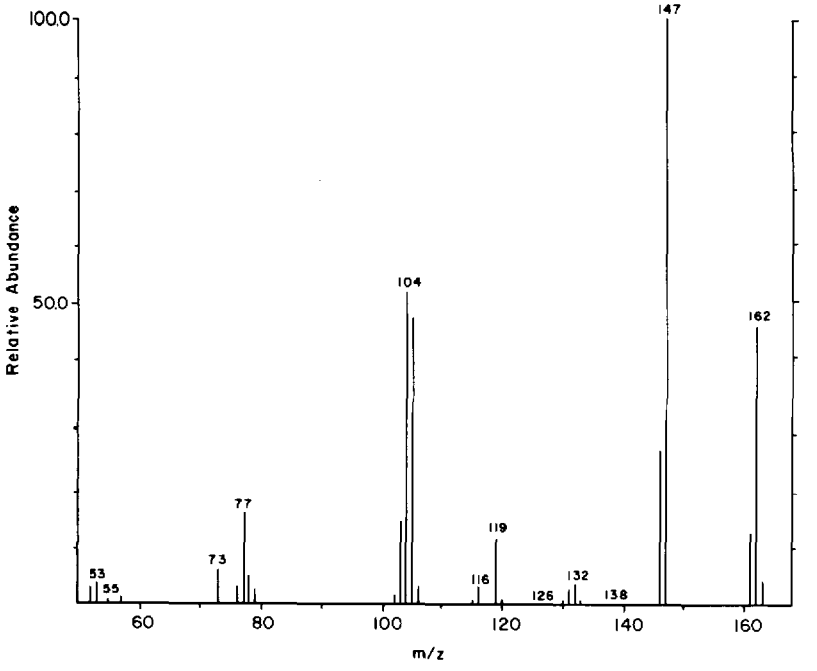

Fig. 6. Mass spectra of 1,2-propanediol phenylboronate.

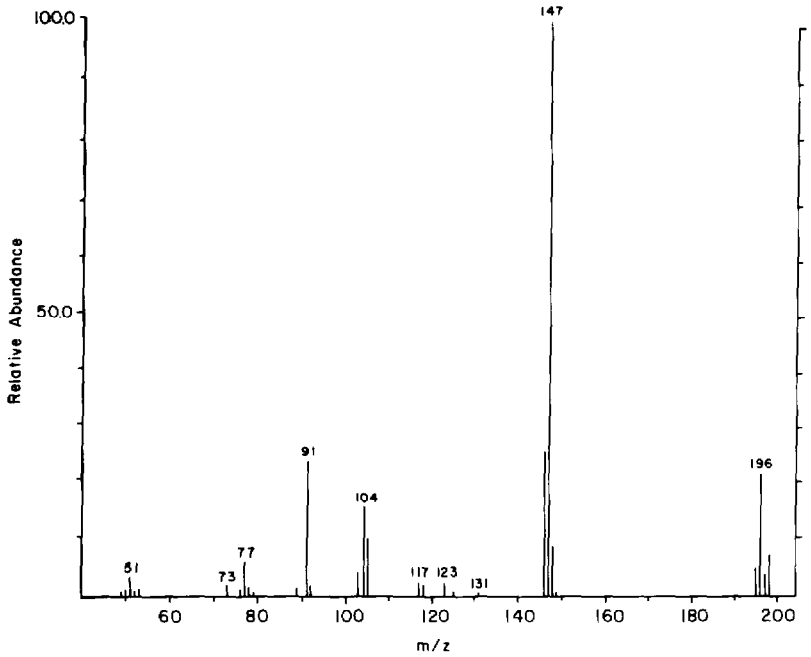

Fig. 7. Mass spectra of 3-chloropropanediol phenylboronate.

molecular ion. For 1,2-propanediol the base peak is the loss of CH_3 peak. Similarly the base peak for 3CPD is the peak corresponding to the loss of CH_2Cl. Both spectra demonstrate a $C_6H_5BO^+$ peak.

Because of the sensitivity and reproducibility, this procedure is suitable for use in applications where trace determination of 3CPD is necessary.

ACKNOWLEDGEMENTS

This work was supported in part by a Basic Science Research Grant 2SO7RRO5374. Dr. Jerry Blake (Department of Veterinary Science, College of Agriculture, University of Kentucky) provided assistance with the GC–MS determinations. Technical assistance was provided by R. G. Robinson and S. Koury.

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