

Identification of Components in Iodinated Glycerol

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Received May 11, 1988, from the ^{*}Midwest Research Institute, 425 Volker Boulevard, Kansas City, MO 64110, and [†]Carcinogenesis and Toxicologic Evaluation Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. Accepted for publication August 22, 1988.

Abstract □ Iodinated glycerol (CAS no. 5634-39-9), therapeutically used as an expectorant and source of organically bound iodine, was analyzed to determine the purity and composition of the chemical samples used in carcinogenicity and toxicity studies. The manufactured product is described by the patent and chemical literature as a mixture of two isomeric iodopropylideneglycerols (structures 1 and 2). The results of our studies, however, indicate that the two principal components of the product were 3-iodo-1,2-propanediol (IPD) and glycerol (GLY). Analyses from GC-MS (full scan electron impact) and carbon-13 nuclear magnetic resonance spectrometry provided conclusive identification of these components. The quantification of IPD and GLY in one of two samples of commercial product using GC-flame ionization detection indicated concentrations of 33 and 17%, respectively (Sample A). Similar concentrations were determined for a second sample from the same source (Sample B), which was a gratis sample procured approximately nine years after Sample A. Numerous minor components were also observed in these two samples. These components were tentatively identified as condensation products of glycerol and iodine-containing analogues. The iodopropylideneglycerol compounds, described in the patent, were not observed in either of the two samples.

Pharmaceutical formulations containing iodinated glycerol (IG) are used as mucolytic agents in the treatment of various respiratory disorders. While the medicinal applications of IG have been evaluated,¹ and bioavailability and distribution studies have been reported,²⁻⁴ the composition of the material remains to be clearly identified.⁵ Analyses have been inconclusive^{6,7} and have relied primarily on the structural information claimed in the patent.⁸ The patent and all subsequent chemical literature describe the material as a mixture of 2-(1-iodoethyl)-1,3-dioxolane-4-methanol (1) and 2-(2-iodoethyl)-1,3-dioxolane-4-methanol (2). The purity and composition of two unformulated samples of commercial IG were analyzed as part of the chemistry support to in vivo toxicity studies performed by the National Toxicology Program.

Experimental Section

Materials—Iodinated glycerol [Sample A: lot no. 276-YY-30 (procured in 1979), Sample B: lot no. WM1454 (procured gratis in 1987)] was obtained from Carter-Wallace Laboratories (Cranbury, NJ). These samples were stored at -20 °C from the time of their procurement. 3-Iodo-1,2-propanediol (IPD, CAS Registry no. 5634-39-9) was

obtained from Custom Chem Labs (Rivermore, CA). *n*-Butylboronic acid (BBA) and "100.0 atom % D" acetone-d₆ were purchased from Aldrich Chemical, (Milwaukee, WI). Other reagents and materials were commercially available and were ACS reagent grade or of the highest purity available.

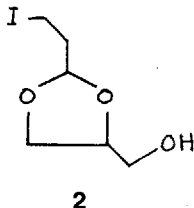
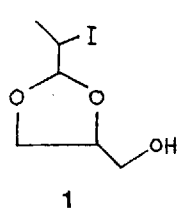
Chemical Analyses—Carbon-13 nuclear magnetic resonance spectroscopy (carbon-13 NMR) analyses were performed on a Varian VXR-300 Fourier transform spectrometer at ambient temperatures. Proton decoupled spectra of iodinated glycerol (IG), IPD, and glycerol (GLY) samples were obtained from solutions prepared at approximate concentrations of 25% in acetone-d₆. Chemical shifts were referenced to the 29.2 ppm acetone heptet. Quantitative NMR analyses were performed by adding known quantities of *p*-dichlorobenzene as an internal standard to the sample solutions.

Gas chromatography-mass spectrometry analyses were performed using electron-impact (70 eV) ionization. A Hewlett-Packard 5890 gas chromatograph (170 °C inlet temperature) was interfaced to a Finnigan 4000 mass spectrometer. Sample components were separated using a 60 m DB-5 capillary column (0.25 mm I.D., 0.25-μm film thickness; J and W Scientific, Folsom, CA). The column temperature was programmed to 50 °C isothermal for 5 min and then to 250 °C at a rate of 10 °C/min. The carrier gas was helium at a linear velocity of 30 cm/s. Linear centroid data was handled by an Inco 2400 data system. The scan range was 35 to 450 amu. The IG (~2 mg/mL), IPD (~2 mg/mL), and GLY (~1 mg/mL) samples were prepared in acetonitrile for analysis. Aliquots of these solutions (1 mL) were further diluted with BBA (1 mL of a 10 mg/mL solution in acetonitrile) to prepare the cyclic boronate diester derivative (IPD-BBA). The reaction of BBA with the 1,2-glycol function of IPD was complete within 30 min using these procedures.

Gas chromatography-flame ionization detection (GC-FID) quantification of IPD was performed using a Varian 3700 gas chromatograph (Varian Associates, Palo Alto, CA), with direct injection (170 °C injector temperature) of solutions onto a 30-m DB-5 Megabore capillary column (0.53 mm I.D., 1.0-μm film thickness). An initial column temperature of 50 °C for 5 min was followed by a ramp to 250 °C at 10 °C/min. The carrier gas was helium, and the flow rate was 10 mL/min. A Nelson 4400 data system was used for integration and data handling. Solutions of IG (~5 mg/mL) and IPD (~3.8, 2.1, and 1.4 mg/mL) were prepared in methanol. These solutions also contained 2-butoxyethanol as internal standard (~1 mg/mL).

Quantification of GLY was performed by reversed-phase high-performance liquid chromatography (HPLC). A 5-μm Beckman Ultrasphere ODS C18 column (250 × 4.6 mm) and a 10-μm Waters Guard-pak guard column were used. The mobile phase was 5% acetonitrile in water (premixed), and the flow rate was 1 mL/min. Detection was by refractive index using a Shodex RI SE-51 detector. Sample A was prepared in water (20 mg/mL) and GLY was quantified using external standards at concentrations of 2, 3, and 4 mg/mL.

The IG samples were also analyzed using normal-phase HPLC, and IPD quantification was performed using a Resolvex Sil normal-phase column (250 × 4.6 mm). The mobile phase was tetrahydrofuran:methylene chloride (10:90, premixed), and the flow rate was 1.0 mL/min. Detection was by 254 nm wavelength absorption. Solutions of Samples A and B were prepared in tetrahydrofuran (5 mg/mL) which also contained the 2-phenoxyethanol internal standard (3 mg/mL). The IPD standards were also prepared in tetrahydrofuran (1, 2, and 5 mg/mL) containing the same concentration of internal standard. Chromatographic profiles were obtained using the same instrument system and parameters except that a solvent gradient



the 1,3-dioxolane ring and the carbons of the 1- or 2-iodoethyl substituent were not observed. The five major absorbances in the IG spectra were consistent with a mixture of GLY and IPD. The spectrum obtained for Sample A is illustrated in Figure 5 and the chemical shifts for the carbon nuclei assigned to IPD and GLY are shown in Table I. Chemical shifts for carbon nuclei assigned to GLY were consistent with the literature.¹⁰ No reference spectra were found for either the IG or the IPD standard.

Quantification of IPD in Samples A and B using GC-FID indicated 33 and 34% (w/w), respectively. The calibration curve and the quantitation analyses of IPD utilized the internal standard method. The linear correlation coefficient was >0.9999 and the relative standard deviation for triplicate determinations was <2%. Additional confirmation of the identity of IPD was obtained by retention time comparison and fortified sample analyses. Representative chromatograms from the GC-FID quantitation of IPD are illustrated in Figure 6. The concentration of IPD in the two IG samples was corrected for an IPD standard purity of 86%, as determined from quantitative carbon-13 and proton NMR analyses.

Glycerol (GLY) was quantitated at a concentration of 17.4% (w/w) in Sample A by reversed-phase HPLC. The concentration of GLY in Sample B was not determined. However, comparison of the peak areas for GLY recorded during the IPD quantitation analyses (GC-FID and carbon-13 NMR) indicated that the concentration in Sample B was

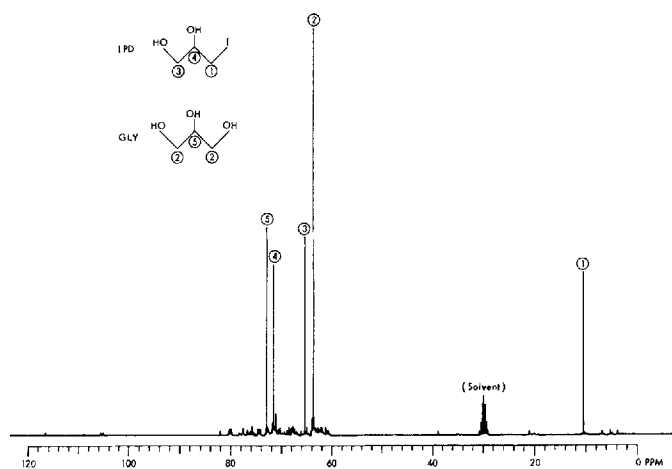


Figure 5—Carbon-13 nuclear magnetic resonance spectrum of iodinated glycerol (Sample A).

Table I—Carbon-13 Chemical Shifts (ppm) of Iodinated Glycerol Major Components

Carbon	Sample		Standard	
	A	B	IPD	GLY
1	10.3 ^a 9.9 ^b	9.5 ^b	10.3 ^a	—
2	63.5 ^a 63.1 ^b	62.6 ^b	—	63.8 ^b
3	65.0 ^a 64.7 ^b	64.4 ^b	65.0 ^a	—
4	71.5 ^a 71.1 ^b	70.7 ^b	71.5 ^a	—
5	72.6 ^a 72.3 ^b	71.8 ^b	—	72.8 ^b

^aQuantitative (long relaxation delay). ^bQualitative (short relaxation delay).

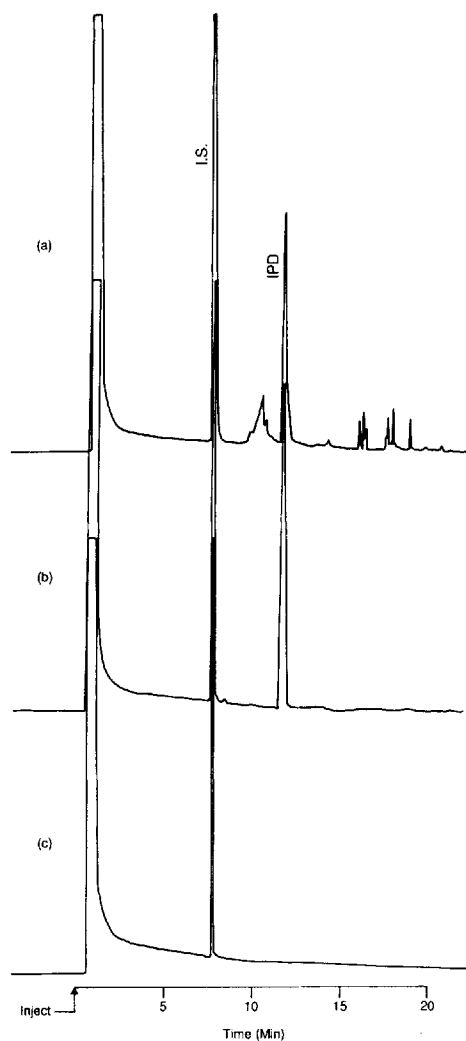


Figure 6—The GC-FID chromatograms from IPD quantitation in iodinated glycerol. (a) Sample A (5.0 mg/mL); (b) IPD standard (3.8 mg/mL); (c) I.S. (2-butoxyethanol) blank (1 mg/mL). Retention times were 7.9 and 11.7 min for I.S. and IPD, respectively.

similar to that of Sample A. The retention times for GLY and IPD were 2.5 and 7.5 min, respectively.

Although IPD was quantitated in the IG samples using other analytical methods (HPLC and carbon-13 NMR), the results were inconclusive because of interferences from the additional components in the samples. However, no interferences were observed in the GC-MS data, as evidenced from the high degree of component separation and the coincidence of the ion intensity maxima in the IPD peak. Therefore, the results from GC-FID analysis, obtained using similar chromatographic conditions, are considered the most reliable measure of the IPD concentration in the IG samples. Results from the other analyses, however, did reconfirm the IPD identity (fortification and retention time experiments). Chromatograms typical of that observed for normal-phase HPLC analyses are illustrated in Figure 7.

Numerous additional components were evident in the GC-FID, HPLC, and GC-MS profiles, as well as in the carbon-13 NMR spectra. Preliminary data indicated that they were polymers of glycerol and iodine-containing analogues. Specifically, isomers of bis(hydroxymethyl)-*p*-dioxane (MW = 148), diglyceryl ether (MW = 166), glyceryl iodoglyceryl ether (MW = 276), (hydroxymethyl)-(iodomethyl)-*p*-dioxane

was used. After a 20-min initial hold, the tetrahydrofuran:methylene chloride mobile phase (10:90) was programmed linearly to 100% tetrahydrofuran in 10 min.

Results and Discussion

The reconstructed ion-current chromatogram (RIC) from GC-MS analysis of Sample A indicated two major components and numerous smaller components (Figure 1). The largest component observed in this sample had a retention time of 17 min, identical to that of the IPD standard. This component was enhanced when IG (Sample A) was fortified with IPD. The mass spectrum, shown in Figure 2, was also identical to that of the IPD standard. The retention time of the second largest component was consistent with that of a GLY standard. Chromatographic conditions were not optimized for GLY; therefore, this component was poorly eluted from the column. However, the retention time for this peak (14 to 15 min) was consistent with that of a GLY standard and was also enhanced when IG was fortified with GLY. The resulting mass spectrum was virtually identical to that of the GLY standard and was also consistent with a literature reference.⁹

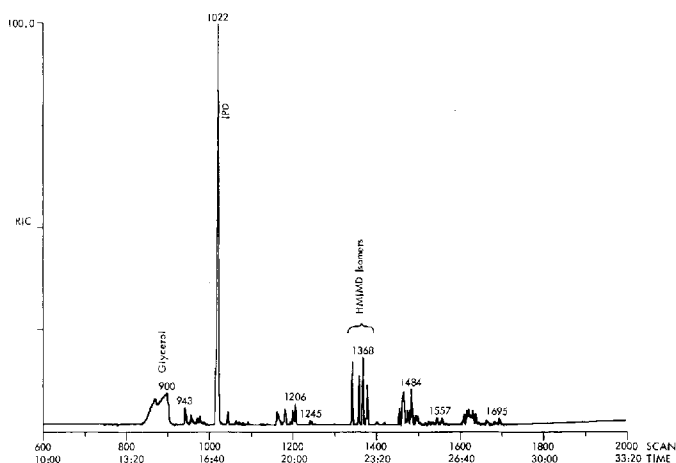


Figure 1—The GC-MS reconstructed ion-current chromatogram for iodinated glycerol (Sample A).

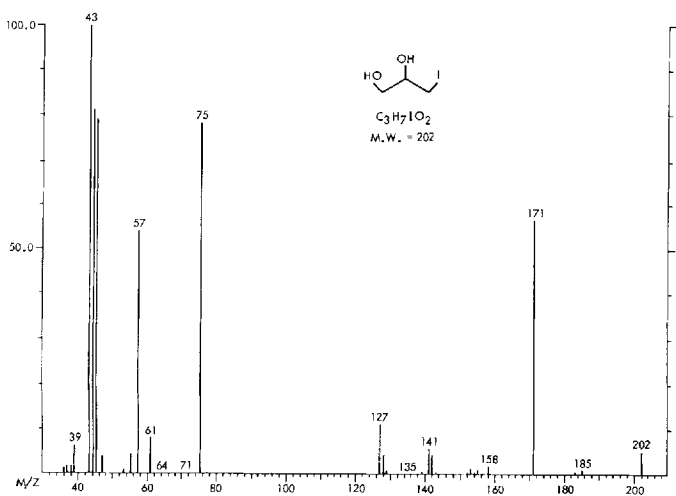


Figure 2—Electron-impact mass spectrum of iodinated glycerol major component 3-iodo-1,2-propanediol (IPD).

The GC-MS analyses of *n*-butylboronic acid (BBA) derivatives of IG (Sample A) and IPD reconfirmed the identity of the major component. The RIC is illustrated in Figure 3. The retention time (21 min) and mass spectrum (Figure 4) of the BBA derivative of this component (MW = 268, C₇H₁₄BIO₂) were virtually identical to the BBA derivative of the IPD standard. No reference spectra were found for the derivative. Excess BBA reagent eluted at 22.1 min.

Gas chromatographic analysis of the BBA derivative of GLY was not successful. Peaks attributable to either the BBA derivative or unreacted GLY were not observed in the RICs of the derivatized IG or a derivatized GLY standard up to 12 min past the elution point of the excess BBA reagent. The HPLC analyses of the BBA derivatives were not performed.

The carbon-13 NMR spectra of the IG samples (A and B) were not consistent with the iodopropylidene glycerol structures contained in the patent⁸ (1 and 2). The spectra of compounds with these structures would be expected to contain at least six lines (from six nonequivalent carbons and optical centers). The spectra obtained for both samples of IG contained five major and numerous smaller absorbances. Absorbances characteristic of the carbon at the 2 position of

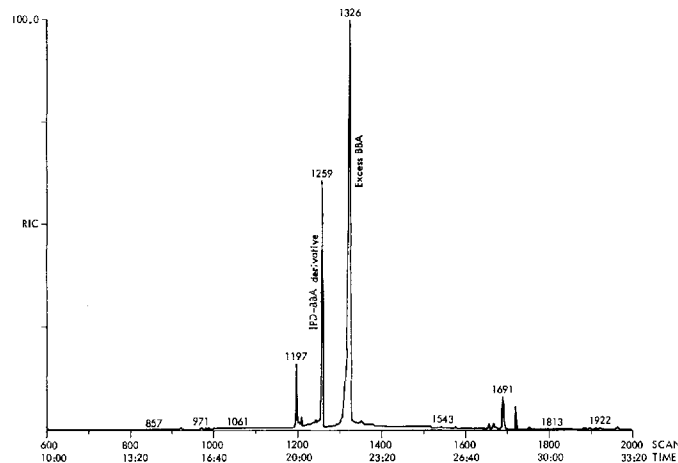


Figure 3—The GC-MS reconstructed ion-current chromatogram for BBA-derivatized iodinated glycerol (peak no. 1259).

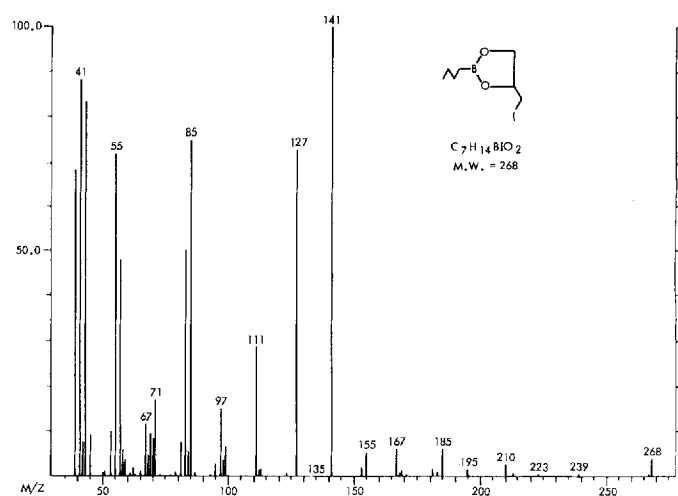


Figure 4—Electron-impact mass spectrum of BBA derivative of iodinated glycerol major component (Sample A).

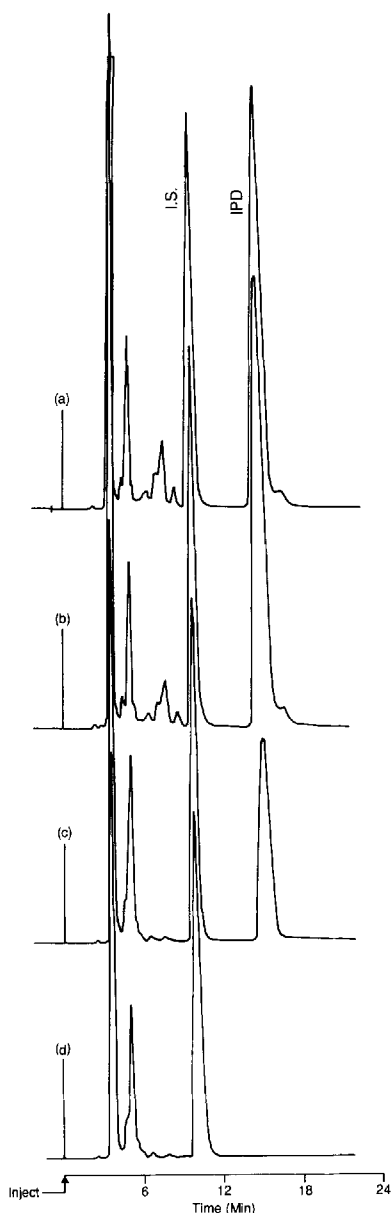


Figure 7—Normal-phase HPLC chromatograms obtained from IPD quantitation in IG samples (detection wavelength 254 nm). (a) Sample A (5 mg/mL); (b) Sample B (5 mg/mL); (c) IPD standard (2 mg/mL); (d) I.S. (2-phenoxyethanol, 3 mg/mL). Retention times were 9.5 and 14.0 min for I.S. and IPD, respectively.

(HMIMD; MW = 258), and trioxobicyclononane (MW = 130) were identified from GC-MS data (unpublished results). We have identified four HMIMD isomers in iodinated glycerol.¹¹

Conclusions

The principal components of the IG samples (procured approximately nine years apart) were IPD and GLY. The two iodopropylideneglycerol isomers described in the patent were not detected during these studies. The use of nonthermal (carbon-13 NMR) and thermal (GC) methods of analysis clearly confirmed the structures of the major components of IG. The concentration of IPD in Samples A and B were 33 and 34%, respectively, based on an assigned IPD standard purity of 86%. Glycerol (GLY) was quantitated at 17% in Sample A. Although not specifically determined for Sample B, the concentration of GLY was estimated to be comparable to that of Sample A. Additional components of the samples were not quantified because of the lack of suitable standards.

References and Notes

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