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NOTE

PREPARATION OF 4,4'-DIAMINODIPHENYLMETHANE-($^2H_4$) FOR USE AS INTERNAL STANDARD IN THE QUANTIFICATION OF 4,4'-DIAMINODIPHENYLMETHANE

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SUMMARY

A simple method has been developed for the synthesis of 4,4'-diaminodiphenylmethane-($^2H_4$) for analytical purposes in gas chromatography-negative ion chemical ionization mass spectrometry (GC-NICIMS). It consists in a proton-deuterium exchange reaction of the diamine using deutero-hydrochloric acid in deuterated methanol. The reaction was conducted at 60°C. Deuteration of the diamine in the ortho positions of the aromatic rings was observed after eight days in these conditions, using mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

Key words: 4,4'-diaminodiphenylmethane-($^2H_4$), proton-deuterium exchange reaction, gas chromatography-negative ion chemical ionization mass spectrometry.

INTRODUCTION

4,4'-Diaminodiphenylmethane (DDM) is an aromatic amine commonly used as an intermediate in the production of some polyurethane polymers. This compound is a carcinogen in laboratory animals, but no definitive data are available regarding its carcinogenicity in humans (1).

On account of its diffusion and toxicity, specific analytical methods are required for monitoring human exposure to this compound. Methods for its quantification in biological fluids using gas chromatography-mass spectrometry (GC-MS) have been developed, using electron impact (EI) (2-5) and negative ion chemical ionization (NICI) (6) in the selected ion recording (SIR) tecnique.

Some of these methods (2,3) employ a stable isotope-labelled DDM as internal

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standard for quantification. These are generally more accurate than methods with normal internal standards.

Here we describe a simple and cheap method preparing a tetradeuterated analogue of DDM (DDM-d_4) for its application as stable isotope-labelled internal standard in a GC-NICIMS analytical procedure for DDM.

MATERIALS AND METHODS

Reagents

DDM, deutero-hydrochloric acid (20.5 % in D_2O, 99.5 atom % D), and methanol-d_1 (>99.9 % atom D) were obtained from Fluka (Buchs, Switzerland). Pentafluoropropionic anhydride was purchased from Farmitalia-Carlo Erba (Milano, Italy). Sep-pak C18 cartridges were obtained from Waters Associates (Milford, MA).

Synthesis of DDM-d_4

DDM (0.5 g) was dissolved in a mixture of 15 g of methanol-d_1 and 6 g of deutero-hydrochloric acid, and heated at 60°C for four days. After evaporation of the solvent, the procedure was repeated again.

The purity of the compound was assessed by MS, on both the underivatized and the derivatized compound, by GC (on a 10 m methyl silicon column) with a FID detector, by NMR and TLC (on silica gel plates, eluent: ethyl acetate/methanol 9:1, indicators: UV light and iodine vapours). With GC-FID an impurity corresponding to the 3.2 % was detected. With all the other techniques the assessed chemical purity was better.

Standard solutions

Standard solutions of DDM and DDM-d_4 were prepared in methanol and stored at -4°C. These standards were used for preparing DDM and DDM-d_4 spiked pH 7 buffer solutions. Extraction of these solutions was made using Sep-pak C18 cartridges; DDM was eluted with 3 ml of methylene chloride.

The methylene chloride fractions from the cartridges were evaporated to dryness in a nitrogen stream and derivatized as described by Cocker et al. (4).

EI and GC-NICIMS

Instrumental analyses were done on a VG 70-250 mass spectrometer interfaced with a Dani 6500 gas chromatograph. Gas chromatographic conditions were as follows: column: CPSil8 CB (Chrompack, Middelburg, The Netherlands), 25 m lenght, 0.32 mm internal diameter; chromatographic program: 140°C initial for 1.5 min; then from 140 to 270°C programmed temperature, 20°C/min. For NICIMS, ammonia was used as the reagent gas. EI mass spectra of underivatized samples were recorded using the direct inlet system (DIS).

RESULTS

The DIS mass spectrum of the DDM-d_4 is shown in figure 1A.The mass spectrum of the DDM obtained in the same conditions is presented in figure 1B. The shift of four mass units of the molecular ion (at m/z 202) in the spectrum of the deuterated compound shows the introduction of four deuteria, two every phenyl group, as indicated by the ion at m/z 108 (m/z 106 in the protonated compound). Some DDM-d_3 and -d_2 appeared from the mass spectrum. The derivatized deuterated DDM, analysed in SIR with GC-NICIMS, was found to contain deuterium in the following atom % : d_0 (0.26 %), d_1 (0.34 %), d_2 (2.3 %), d_3 (13.1 %) and d_4 (84.0 %). So, this deuterated DDM does not give significative interferences when measuring DDM in SIR.

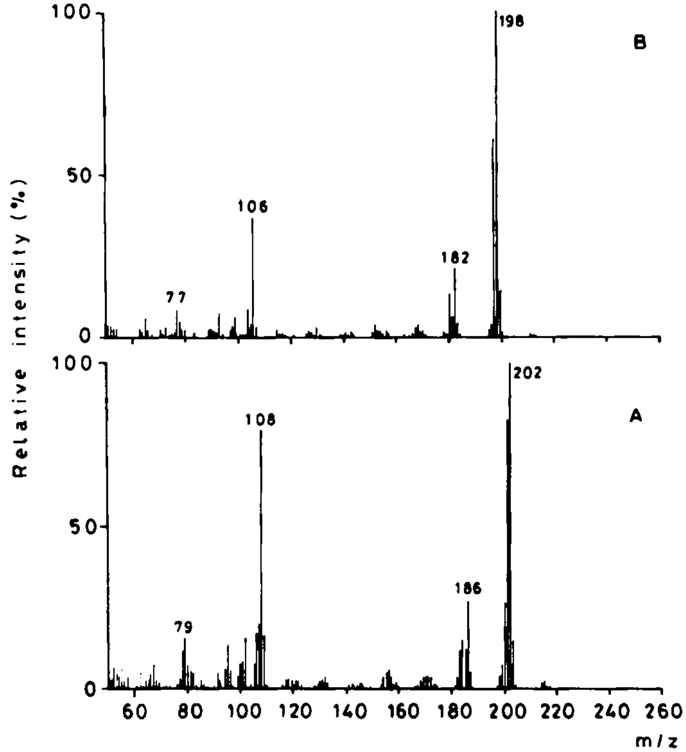

Figure 1: DIS mass spectra of DDM-d_4 (A) and DDM (B).

The $^1H_2$-NMR spectrum of the deuterated DDM presents in the aromatic ring the signal at 6.98 ppm due to the m-aniline positions (7); a signal at 6.5-6.7 ppm due to DDM not completely deuterated in ortho is less than 8 % of the peak at 6.98 ppm.

The GC-SIR profile relative to the injection of an extracted pH 7 buffer solution containing both the protonated and the deuterated amine at the nanogram level is reported in figure 2.

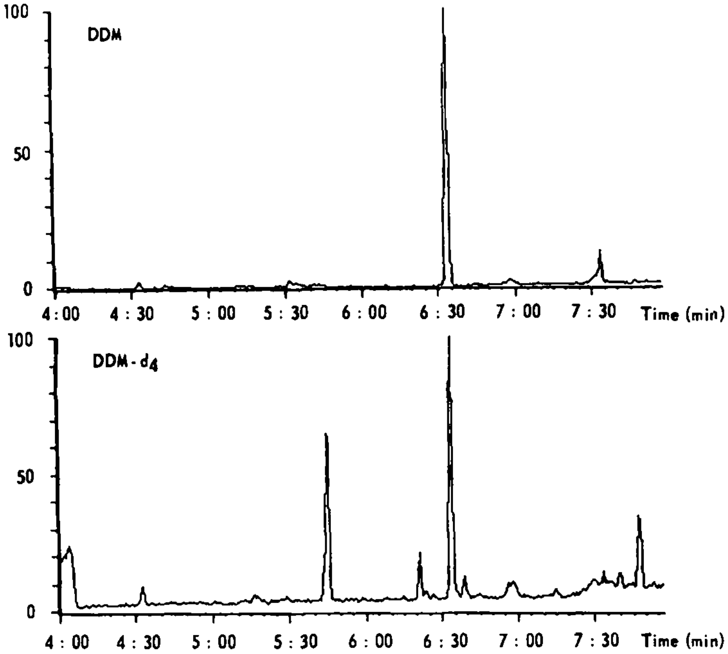

Figure 2: GC-SIR traces of a pH 7 buffer solution spiked with DDM (upper panel) and DDM-d_4 (lower panel). (DDM retention time: 6:33 min; the other peaks are due to impurities of the buffer and the derivatizing reagent).

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