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SYNTHESIS OF PERDEUTERATED ANALOGUES OF THE EPOXIDE METABOLITES OF BUTADIENE: 1,2-EPOXYBUT-3-ENE-d_6 AND 1,2,3,4-DIEPOXYBUTANE-d_6

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SUMMARY

Deuterated analogues of the carcinogenic metabolites of 1,3-butadiene (BD) 1,2-epoxybut-3-ene (EB) and 1,2,3,4-diepoxybutane (DEB), which are not commercially available, are conveniently synthesized in good yields by oxidation of BD-d_6 with appropriate quantities of dimethyldioxirane (DMDO). Both epoxides were characterized by proton and deuterium NMR and mass spectrometry; yields were quantitated by gas chromatography/mass spectrometry using samples of known concentrations of commercially available non-deuterated epoxides as standards. The synthesized DEB-d_6 was a mixture of the enantiomeric RR and SS enantiomers and the *meso* RS diastereomers.

Key words: 1,3-butadiene, 1,3-butadiene-d_6, 1,2-epoxybut-3-ene-d_6, 1,2,3,4-diepoxybutane-d_6, dimethyldioxirane, oxidation

INTRODUCTION

1,3-Butadiene (BD) is widely-used in manufacture of synthetic rubber and plastics, and has also been detected in automobile exhaust (1,2) and cigarette smoke (3). BD is a potent, multisite carcinogen in mice and a weak carcinogen in rats (4-6). It is therefore categorized as a class 2A carcinogen (probable human carcinogen) by the International Agency for Research on Cancer (7). A recent epidemiological study (8) has shown an increase in leukemia in workers exposed to BD. While metabolism of BD has been investigated in rodents, little is known about its metabolism in humans.

BD itself is not mutagenic, but is metabolized by cytochrome P450 2E1 (9-11) to 1,2-epoxybut-3-ene (EB) and subsequently to 1,2,3,4-diepoxybutane (DEB) which are direct-acting mutagens; DEB is about 2 orders of magnitude more toxic and mutagenic than EB in cultured

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human lymphoblasts (12). Both EB and DEB are also carcinogens (13,14). Species differences in susceptibility to butadiene-induced carcinogenicity have been ascribed to differences in metabolism (15), since mouse liver microsomes were more active in production of DEB than rat liver microsomes. EB and DEB are formed after inhalation exposure of rodents and primates to BD (16,17); identification, quantitation and dosimetry of hemoglobin and DNA adducts arising from reaction with EB and DEB would yield information on human metabolic pathways and exposure, and help elucidate the mechanisms of species differences in BD carcinogenicity, thus providing a better scientific basis for assessment of human health risk from butadiene exposure.

Recently we have identified and characterized EB and DEB adducts of purinic nucleobases (18). We have also developed a highly sensitive and specific assay using isotope dilution mass spectrometry for the detection and quantitation of hemoglobin adducts of epoxides formed from BD. This procedure requires isotopically-labelled synthetic EB and DEB adduct standards, which are not commercially available. We opted for deuterium labelling of the butadiene moiety, since the molecular weights of the perdeuterated analogs are six mass units higher than the natural abundance epoxide adduct isotopomers. DEB-d_6 has never been synthesized, and the previously reported synthesis of EB-d_6 (19) involves the cumbersome technique of cryogenic distillation for isolation of the required product. We therefore developed a more convenient route for preparation of deuterated epoxides EB-d_6 and DEB-d_6 based on oxidation of commercially available butadiene-d_6 (BD-d_6). We report here this synthesis and characterization of the products by NMR and mass spectroscopy.

Experimental

Instrumentation: ^{1}H NMR and deuterium NMR (^{2}H NMR) spectra were recorded on a Bruker AMX-500 spectrometer (Bruker Instrument Co., Billerica, MA). ^{1}H NMR spectra were obtained at 500 MHz, and chemical shifts are reported in ppm relative to the proton resonance of TMS, using the residual proton resonance of acetone- d_6 (2.04 ppm) as internal standard. ^{2}H NMR spectra of samples dissolved in acetone and H_2O were obtained at 76.77 MHz with shifts reported in ppm relative to TMS, calibrated by reference to the shift of the natural abundance deuterium resonance of acetone (2.04 ppm). Since shielding is not altered by substitution of deuterium for hydrogen,

chemical shifts of H and D coincide. Mass spectrometry was performed on a VG 70 250SEQ Mass Spectrometer (Vacuum Generators, Wythenshawe, UK) equipped with a Hewlett Packard 5890 Gas Chromatograph (Hewlett-Packard Instrument Co, Roseville, CA).

Materials: BD (99%), EB (98%), DEB (97%) and oxone were purchased from Aldrich Chemical Co. (Milwaukee, WI). BD-d_6 (98.73% enriched in deuterium) was obtained from Cambridge Isotopes Laboratories, Inc. (Andover, MA).

Dimethyldioxirane (DMDO). A solution of DMDO (~0.1 M) in acetone was prepared from commercially available oxone according to a published procedure (20).

Synthesis of 1,2-Epoxybut-3-ene-d_6: Acetone (2 mL) was placed in a 50 mL two-necked flask fitted with a rubber septum and a cold finger condenser which was attached to an argon bubbler by a three-way connector. The cold finger condenser and the reaction flask were cooled with dry ice-acetone. BD-d_6 (0.2 mL, 3.0 mmol), condensed into a graduated tube by cooling in a dry ice-acetone bath, was transferred to the reaction flask by means of a cannula using pressure generated by warming the tube. A solution of DMDO in acetone (10 mL, 1.0 mmol) was injected into the reaction flask with stirring. The reaction mixture was allowed to warm to –15 °C and maintained at that temperature for 1 h. Removal of excess BD-d_6 by bubbling argon gas through the solution for 30 min at 0 °C gave a solution of 1,2-epoxybut-3-ene-d_6 in acetone. The identity of the product was confirmed by analysis of the reaction product in acetone by EI mass spectrometry (ions observed: m/z 42, 48,74 and 76) and ^{2}H NMR spectroscopy. For detailed NMR data, see Figure 2. The isotopic purity of the synthesized EB-d_6 was determined to be 98.5% by GC/MS analysis.

Synthesis of 1,2,3,4-Diepoxybutane-d_6: The synthesis of DEB-d_6 was carried out by following the above procedure, but reversing the order of addition and proportion of BD to DMDO. BD-d_6 (0.1 mL, 1.5 mmol) was added to the solution of DMDO (40 mL, 4.0 mmol) in the reaction flask. The reaction mixture was maintained at –15 °C for 2 h, then argon was bubbled through the solution at 0 °C for 30 min to remove excess DMDO. The resulting solution of deuterated diepoxide in

acetone was characterized by spectroscopic methods. Diepoxide formation was confirmed by EI mass spectrometry which indicated the presence of two products with identical mass spectra (ions observed: m/z 42, 58, 62, 74, 90 and 92). The ^2H NMR spectrum (Figure 3) of the reaction product showed the presence of DEB-d_6 as a mixture of enantiomeric and *meso* diastereomers. For recording the ^2H NMR spectrum in H_2O, an aliquot of the acetone solution cooled in ice was evaporated with argon gas to remove acetone and redissolved in H_2O.

Quantitation by ^2H NMR: 1.0 mL of acetone solution of the product was spiked with 5 mL of $CDCl_3$ and the ^2H NMR spectrum was recorded. The concentration of EB-d_6 was calculated by comparing the integral of the deuterium signals from EB-d_6 to the deuterium signal from the $CDCl_3$ standard. The same method was used to determine the concentration of DEB-d_6 solution in acetone.

Analysis by GC/MS: EB-d_6 and DEB-d_6 were analyzed on a DB-5 column (60m x 0.25 μm film thickness, J&W Scientific, Folsom, CA), with 10 psi He head pressure, 250 °C injector temperature. Injections were made in the splitless mode with the following column temperature program: 50 °C (held for 1 min) to 150 °C at 15 °C/min, then 300 °C at 30 °C/min. The MS was operated at 70 eV with a source temperature of 250 °C and the emission current at 600 μA. Full scan electron impact (EI) mass spectra were obtained at a resolving power of 1000 ppm. The corresponding commercially available non-deuterated epoxides were used as internal standards for quantitation. Quantitative selected-ion monitoring experiments were performed at a resolving power of 10000 ppm. For analysis of EB-d_6, ions monitored were m/z 76/70 (Md_6^+/M^+) and 74/69 ($[Md_6–D]^+/[M–H]^+$), and calibration curves showed linearity of response (R^2 = 0.998) over 6 concentration ratios up to 5:1 EB-d_6/EB-H_6. For quantitation of DEB-d_6, the ions monitored were m/z 90/85 ($[Md_6–D]^+/[M–H]^+$), and the calibration curve showed a linear response (R^2 = 0.997) to a concentration ratio of 20:1 DEB-d_6/DEB-H_6.

Modeling. Models of EB and DEB were constructed with Alchemy (Tripos Associates, St. Louis, MO). Optimal geometry configurations were determined with the energy minimization routine included with the program.

RESULTS AND DISCUSSION

We have developed simple and convenient syntheses of deuterated analogues of EB and DEB by oxidation of BD-d_6 with DMDO, as shown in Figure 1. Although numerous examples of alkene epoxidations with this powerful oxidant have been reported in the literature (21), BD has not previously been used as a substrate. Since DMDO is a gas at ambient temperature, is used as an acetone solution, and is converted to acetone in the course of the oxygen transfer, unexpended oxidant and many by-products can conveniently be removed from the reaction mixture. The reaction conditions and work up procedures were optimized by pilot reactions with non-deuterated BD.

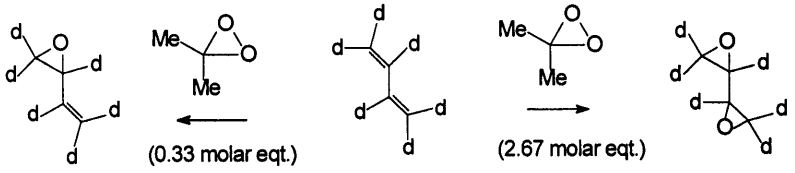

Figure 1. Synthesis of EB-d_6 and DEB-d_6.

To minimize diepoxide formation during the synthesis of the monoepoxide, we added DMDO in acetone to a 3-fold molar excess of BD-d_6 in acetone. The reaction product was confirmed as EB-d_6 by MS analysis (15,19) and by comparison of the ^{2}H NMR spectrum (Figure 2A) with the ^{1}H NMR spectrum of commercially available proteo-EB (Figure 2B). In the ^{2}H NMR trace, three broad

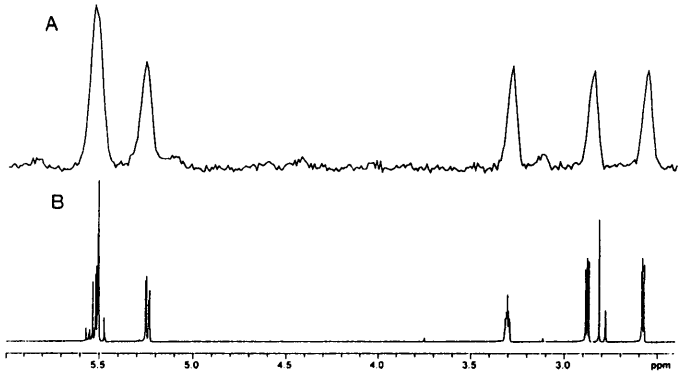

Figure 2. ^{2}H NMR spectrum (76.77 MHz) of EB-d_6 in acetone (A) and ^{1}H NMR spectrum (500 MHz) of commercial sample of EB in acetone-d_6 (B).

singlets at 2.55, 2.84 and 3.27 ppm were attributable to the deuterium label at epoxide carbons C_1 and C_2, while the three vinylic deuterium atoms gave rise to signals at 5.26 and 5.53 ppm. By ^2H NMR, the concentration of EB-d_6 in acetone was calculated to be 3.7×10^{-2} M, consistent with the concentration determined by GC/MS analysis. The yield indicated complete consumption of DMDO in the presence of a three-fold excess of BD-d_6. Absence of DEB-d_6, as an impurity in the EB-d_6, was confirmed by both NMR and MS analyses.

Conditions favoring oxidation of both BD - double bonds to obtain DEB-d_6 were established using excess DMDO and reversing the order of addition of reagents. GC/MS analyses of the product showed the presence of two peaks of similar size at 8.56 and 9.03 min with identical mass spectra suggesting resolution of two. The later-eluting peak (9.03 min) co-chromatographed with commercially available DEB, which consists of the (±) enantiomers. The same set of two peaks, with matching retention times, was observed by GC/MS analysis of the unlabelled DEB synthesized in pilot reactions. We infer from these data that the DMDO oxidation gave rise to an isomer which is not present in the commercially available (±) DEB. By proton and deuterium NMR (Figure 3), the isomer was assigned as the *meso* DEB isoform shown in figure 4. For analysis of DEB, NMR spectra were recorded in D_2O or H_2O, because spectra of commercially available DEB and the pilot reaction products in acetone-d_6 did not resolve into assignable signals. The ^2H NMR spectrum of

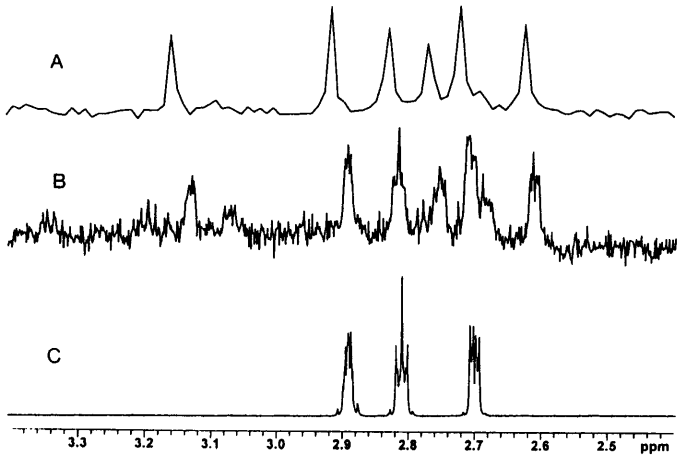

Figure 3. ^2H NMR spectrum (76.77 MHz) of synthesized DEB-d_6 in H_2O (A), ^1H NMR spectra (500 MHz) of synthesized DEB (B) and commercial sample of DEB in D_2O (C).

deuterated product resolved into 6 distinct signals in H_2O (Figure 3A). Three of the resonances, at 2.91, 2.82 and 2.72 ppm were also present in the pilot reaction product (Figure 3B) and in the commercial sample of (±) DEB (Figure 3C); these were therefore assigned to the (±) form of DEB. The three additional resonances, at 3.15, 2.75 and 2.63 ppm, in the ^2H NMR (Figure 3A) and the ^1H NMR (Figure 3B) spectra of our synthetic products, were assigned to the *meso* form of DEB.

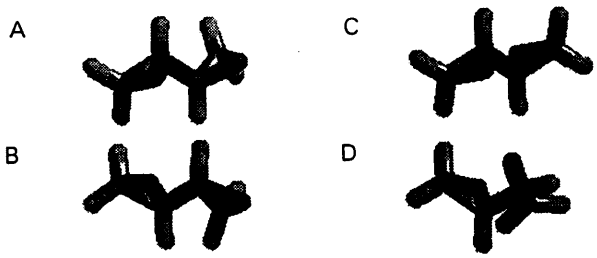

Figure 4. Energy minimized configurations of DEB isoforms: **RR** (A), **SS** (B), **RS** (C) and **SR** (D).

Examination of the energy-minimized configurations of the DEB isoforms (Figure 4) suggests an explanation for differences in chemical shifts between the (±) and *meso* forms. In the optically active enantiomers (RR and SS), both epoxy oxygens reside on one side of the plane of the carbon chain, minimizing interaction with the protons on the other side of the plane, whereas in the optically inactive *meso* form (RS and SR) one epoxy oxygen is located on each side of the plane of the carbon chain. Thus the protons on carbons 2 and 3 are closer to the deshielding cones of oxygens at C3-C4 and C1-C2, respectively, resulting in a downfield shift of the signals to 3.15 ppm. By ^2H NMR spectrometry the DEB-d_6 solution was 6.52×10^{-3} M and the racemic and *meso* forms were present in approximately equal proportions; this concentration and composition were consistent with those determined by GC/MS analysis.

Stepwise formation of DEB can be inferred from the diastereomeric mixture of racemic and *meso* products of DEB-d_6. A racemic mixture of R-EB and S-EB (or R- and S-EB-d_6) would be formed initially. Transfer of a second oxygen from DMDO to EB-d_6 can then occur from either side of the plane of the double bond of EB-d_6 (22) to give a mixture of RR-, SS-DEB-d_6, and the *meso* (RS)-DEB-diastereomers. Commercially available DEB is prepared from 1,4-dibromobut-2-ene (23), and consists of the racemic diastereomer (RR and SS mixture) alone. There are currently no data available regarding the stereoselectivity of monooxygenases toward mono- or diepoxidation of BD.

In principle, both enantiomers of EB and the diastereomeric mixture of DEBs could be formed as metabolites. Moreover, it can be anticipated that the pure stereoisomers may differ with respect to macromolecular binding; the (±) form was both more toxic and more carcinogenic than the *meso* form in mouse skin painting assays (13,14). Thus, the availability of the complete array of perdeuterated epoxides of BD not only provides synthons for isotope dilution mass spectrometric standards for quantitation of BD-derived adduct levels, but also, through the inherent resolvability of the diastereomeric adducts, provides a potential means to investigate the biological activity of the various stereoisomeric BD metabolites. The synthesis of EB-d_6 described here provides convenient and efficient methodology applicable to the preparation of deuterated epoxides of many other volatile olefin substrates. A particularly attractive aspect of this synthetic procedure is that it yields deuterated epoxides in a form suitable for direct use with minimal work-up.

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