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Studies on the molecular toxicology of buta-1,3-diene and isoprene epoxides

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Abstract

Reactions of ethenyloxirane with amino (RNH_2) and thiol (R'SH) nucleophiles occur by an S_N2 mechanism involving competing ring cleavage at C-2 and C-3. In contrast, 2-ethenyl-2-methyloxirane reacts with amino (RNH_2) and thiolate ($R'S^-$) nucleophiles in methanol by regioselective S_N2 attack at C-3 ("*neo*-pentyl" position). However, in pure water or methanol S_N1 reaction occurs mainly at C-2.

Keywords: Ethenyloxirane; 2-Ethenyl-2-methyloxirane; Nucleophile; Regioselectivity

1. Introduction

We wish to define the molecular basis of the detoxification and intoxification processes for buta-1,3-diene and 2-methylbuta-1,3-diene (isoprene), and therefore provide information for rational risk assessment. These studies will benefit from a better understanding of the mechanisms of reactions of the epoxides of butadiene and isoprene with biologically relevant nucleophiles, and require reference samples of adducts derived from reactions of the epoxides with amino acids and nucleosides.

For the mono-epoxides of butadiene (ethenyloxirane, Fig. 1) and isoprene [2-ethenyl-2-methyloxirane, Fig. 2, and (1'-methylethenyl)-oxirane, Fig. 3] it is important to quantify the relative extents of nucleophilic attack at C-2 and C-3 (oxirane ring) and C-2' (S_N2' reaction). On the basis of Frontier Molecular Orbital analysis it has been suggested (Jaime et al., 1988) that the preferred site of attack with ethenyloxirane should be at C-2 with a hard nucleophile, with C-2' becoming relatively more favourable with a soft nucleophile. The additional methyl group in the isoprene mono-epoxides compared to ethenyloxirane is expected to influence strongly the outcome of reactions through steric and electronic effects. It has been suggested that the high rate of hydrolysis of 2-ethenyl-2-methyloxirane (half-life 75 min at 37°C; Gervasi et al., 1985) will preclude its reaction with biomolecules. We have compared the reactivity of ethenyloxirane and 2-ethenyl-2-methyloxirane with oxygen, nitrogen and sulfur nucleophiles. We have discovered that 2-ethenyl-2-methyloxirane unexpectedly suffers

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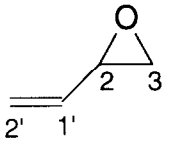


Fig. 1. Structure of ethenyloxirane.

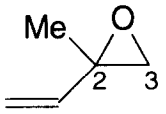


Fig. 2. Structure of 2-ethenyl-2-methyloxirane.

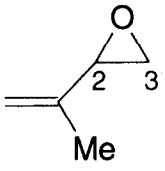
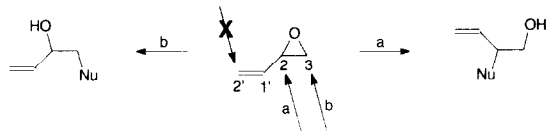
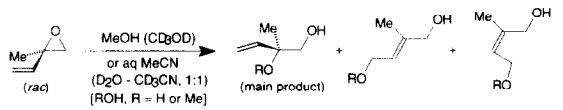


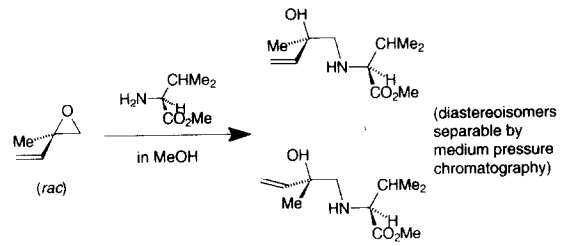
Fig. 3. Structure of (1'-methylethenyl-)-oxirane.



Scheme 1. Outcome of reactions of ethenyloxirane with nucleophiles Nu (RNH_2 and R'SH; for R and R' see text).



Scheme 2. Reaction of 2-ethenyl-2-methyloxirane with methanol or water (reaction conditions: 50°C for methanol, 60°C for water/overnight).



Scheme 3. Reaction of 2-ethenyl-2-methyloxirane with (*S*)-valine methyl ester (reaction conditions: 144 h/50°C).

cleavage by nitrogen (e.g. L-valine methyl ester) and sulfur (e.g. 2-methoxybenzenethiol) nucleophiles preferentially at the "*neo* pentyl" position C-3. The resulting adducts have been obtained as homogeneous compounds, fully characterised by spectroscopic studies (note that racemic epoxides have been used in these studies).

2. Materials and methods

Reactions of epoxides (typically 0.3 M) for ^{1}H-NMR observations (200 or 300 MHz) were done in [$^2H_4$]methanol or 1:1 (v/v) deuterium oxide-[$^2H_3$]acetonitrile. Preparative scale reactions were typically 1 M in epoxide in methanol, which was present in 20% excess over any added nucleophile, and were conducted at a temperature in the range 45–60°C (except for reactions of 2-ethenyl-2-methyloxirane with thiols which were done at 20°C, and with thiolates which were done at −10°C). Products were isolated by removal of solvent(s) in vacuo. Diastereoisomeric adducts, e.g. those from 2-ethenyl-2-methyloxirane and (*S*)-valine methyl ester (Scheme 3), were separated by medium pressure chromatography on silica [elution with 1:1 (v/v) petrol-diethyl ether].

3. Results

3.1. Reactions of ethenyloxirane with nucleophiles

With (*S*)-valine methyl ester, (*R*)-α-methylbenzylamine, and *N*-acetylcysteine methyl ester mixtures of products derived from nucleophilic attack at C-2 and C-3 were obtained (see Scheme 1). No products from S_N2' attack were observed (> 5% would have been detected). Similar results

have been reported by Elfarra and his co-workers for reactions of ethenyloxirane with *N*-acetylcysteine and glutathione (Elfarra et al., 1995).

3.2. Reactions of 2-ethenyl-2-methyloxirane with nucleophiles

With methanol or water, 2-ethenyl-2-methyloxirane yields mainly the product from attack at C-2, but also ca. 10% total yield of the (*E*)- and (*Z*)-isomers derived from apparent attack at C-2′ (Scheme 2). All of these products are presumed to arise by an S_N1 process involving capture of an intermediate carbocation derived by cleavage of the C-2/O bond of 2-ethenyl-2-methyloxirane. The identity of (*Z*)-2-methylbut-2-ene-1,4-diol was confirmed by synthesis of an authentic sample by reduction of citraconic anhydride with lithium aluminium hydride in diethyl ether.

With 2-methoxybenzenethiol, *N*-acetylcysteine methyl ester and (*S*)-valine methyl ester, 2-ethenyl-2-methyloxirane gives products from S_N2 attack at C-3 (i.e "*neo*-pentyl" attack occurs) (see Scheme 3). With 2-methoxybenzenethiol at pH 12 and (*S*)-valine methyl ester the reactions occur almost exclusively at C-3. With 2-methoxybenzenethiol in neutral methanol there is a competition between attack at C-3 (S_N2 pathway) and attack at C-2/C-2′ (S_N1 pathway). In kinetic experiments we have found that ethenyloxirane, 2-ethenyl-2-methyl-oxirane and methyloxirane are similar in reactivity towards 2-methoxybenzenethiolate. Methyloxirane reacts with N and S nucleophiles by an S_N2 mechanism subject to steric control (predominant attack — ca. 95% at C-3; Ellis et al., 1984). The greater relative extent of internal (C-2) attack with ethenyloxirane compared to terminal attack (C-3) with methyloxirane can be ascribed to a stereoelectronic effect (stabilisation of the S_N2 transition state by the ethenyl group in ethenyloxirane; no comparable effect from the methyl group in methyloxirane). The remarkable propensity for C-3 attack in 2-ethenyl-2-methyl-oxirane can be partly understood by considering the trajectories for S_N2 attack at C-2 and C-3. Assuming that the approach of the nucleophile is along or near to the axis of the C—O bond undergoing cleavage, then attack at C-2 should be preferred on steric grounds.

4. Discussion

Studies of the reactions of butadiene and isoprene epoxides with nucleophiles enable the relative reactivities of the epoxides to be determined, reference standards to be generated, and both intoxification and detoxification pathways to be assessed. Gervasi et al. (1985) stated that "the methyl substitution in the oxirane ring (of 2-ethenyl-2-methyloxirane) causes a steric hindrance..... shifting the substitution towards an S_N1 type" and that "(2-ethenyl-2-methyloxirane) did not show any mutagenicity because (it is) specifically and highly reactive towards water". However, we have found a relatively high S_N2 reactivity of epoxide 2-ethenyl-2-methyloxirane at C-3 ("*neo*-pentyl" position), leading to efficient adduct formation, despite the ease of hydrolysis and methanolysis of 2-ethenyl-2-methyloxirane by an S_N1 mechanism. In future studies, we plan to develop a human dose monitor for the environmental carcinogen, butadiene, and natural product, isoprene, and define the nature of damage to DNA caused by butadiene and isoprene epoxides.

Acknowledgements

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