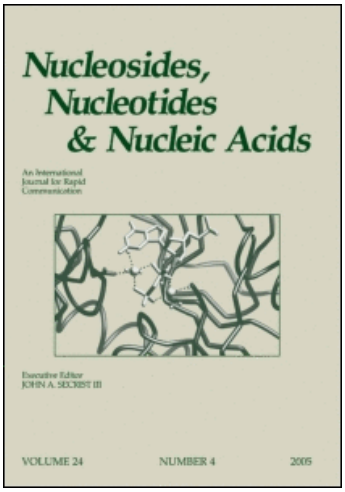

Nucleosides, Nucleotides and Nucleic Acids

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CHROMATOGRAPHY AND CHARACTERIZATION OF PHENYLGLYCIDYL ETHER NUCLEOSIDE ADDUCTS

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ABSTRACT

The HPLC separation and structure elucidation (UV, NMR, MS) of the nucleoside adducts formed between 2'-deoxyadenosine, thymidine, 2'-deoxycytidine and 2'-deoxyguanosine with phenylglycidyl ether is described.

INTRODUCTION

Mono -and di-functional glycidylethers are widely used for the production of epoxy resins. Phenylglycidyl ether (PGE) belongs to this group. It is generally believed that reactions of electrophilic reactants, - such as epoxides -, with sites in DNA, RNA or proteins are fundamental to the induction of mutations (1-3). Characterization of the formed reaction products between epoxides and DNA bases is of the utmost importance in the understanding of the mutation process. Our interest in the structure-mutagenicity relationship for aliphatic epoxides (4) and in the reactivity of some of these epoxides with 2'-deoxynucleosides and DNA prompted us to extend the limited literature (5-6) on the reactivity and identification of adduct formation between phenylglycidyl ethers and 2'-deoxynucleosides.

Here we report on the HPLC and TLC separation and structure elucidation of the nucleoside adducts formed between 2'-deoxyadenosine, thymidine, 2'-deoxycytidine and 2'-deoxyguanosine with phenylglycidyl ether. The importance of photo diode array detection is stressed as a powerful preliminar tool towards final characterization by NMR and mass spectrometry of the formed adducts.

EXPERIMENTAL

Products.

Thymidine (Thy), 2'-deoxyadenosine (dAdo), 2'-deoxyguanosine (dGuo), 2'-deoxycytidine (dCyd) (Sigma) and 2,3-epoxypropylphenylether (PGE) (Janssen Chimica, Belgium) were used.

Reaction of 2'-deoxynucleosides with PGE.

The nucleoside (2 mg) was dissolved in 2 ml methanol. Then 1 ml 1M PGE in methanol was added. The compounds were allowed to react for 24 hours (or 48 hours) at 37°C in

tightly sealed test tubes equipped with a teflon lined screw cap. For preparative purposes 50 mg of 2'-deoxynucleoside in methanol was used.

Chromatography.

Analytical HPLC was on a reverse phase column 10 RP 18 (25 cm x 4,6 mm, Alltech) with mixtures of 0.01 M $HCOONH_4$ (pH 5,1) - MeOH, and photo diode array detection (Hewlett Packard 1040A, equipped with 8290 M flexible disk drive and Hewlett Packard 85 computer). Detection wavelength : 260 nm. Details for individual separations are given in results and discussion.

Preparative HPLC was on a reverse phase column 10 RP 18 (25 cm x 2,2 cm, Alltech). Same instrumentation as described under analytical HPLC. Details for individual separations are given in the section results and discussion.

Preparative centrifugal circular thin layer chromatography with Chromatotron (Harrison Res., Palo Alto, Ca), silicagelplates, mobile phase CH_2Cl_2 - THF / 30-70, flow rate 7.0 ml/min, detection UV at 254 nm, was used for the for Thy-PGE adduct.

NMR.

Details published elsewhere (7).

Mass spectrometry.

See abstract "Fast atom bombardment and tandem mass spectrometry for the identification of nucleoside adducts with phenylglycidylether", J. Claereboudt et al.).

RESULTS AND DISCUSSION

Analytical HPLC on reverse phase coupled to photo diode array detection revealed one adduct for the Thy-PGE and dAdo-PGE mixtures and two adducts for dCyd-PGE and dGuo-PGE mixtures.

Preparative centifugal circular thin layer chromatography was first used for preparative isolation of the formed adduct, but gave only satisfactory results for the Thy-PGE adduct, which was identified by UV, NMR and MS as N-3-PGE-Thy.

Preparative HPLC on reverse phase coupled to photo diode array detection was used to isolate the adducts of dAdo-PGE, dGuo-PGE and dCyd-PGE mixtures. Chromatograms will be published elsewhere (7-8).

For the dAdo-PGE adducts, mass spectral analysis revealed next to a major N-1-dAdo-PGE adduct, the presence of N-1,N^6-dAdo-PGE dialkylated material. The dCyd-PGE adducts were identified as N-3-dCyd-PGE isomers. For the dGuo-PGE mixture, mass spectral analysis revealed 2 isomers which lost their sugar moiety, probably alkylated on N-7.

U.V.spectra and absorbance ratio's at 254/280 obtained on line in the HPLC analysis gave in most of the cases already a good indication of the alkylation site. By comparing our UV data as presented in table I, with existing data in the literature for other nucleoside adducts, the alkylation site could be attributed, which was then confirmed by MS and/or NMR.

TABLE I :

		max (nm)	ratio 254/280
N-3 PGE - thymidine	H_2O	269	1.0
	pH 1	269	
Thymidine	H_2O	267	1.2
	pH 1	267	
N-1-PGE - deoxyadenosine	H_2O	261	2.4
	pH 1	261	2.4
Deoxyadenosine	H_2O	260	5.9
	pH 1	257	4.4
N-7-PGE - deoxyguanosine	H_2O	259	1.4
	pH 1	260	1.4
Deoxyguanosine	H_2O	253	1.5
	pH 1	254	1.4
N-3-PGE - deoxycytidine	H_2O	279	0.40
	pH 1	280	0.40
N^4-PGE - deoxycytidine	H_2O	272	0.85

CONCLUSION

Preparative circular chromatography on silicagel coupled with UV, NMR and mass spectral analysis led to the identification of N-3 PGE-thymidine as only adduct formed in the reaction between thymidine and phenylglycidyl ether.
Preparative reversed phase HPLC on RP-18 coupled to photo diode array detection proved to be a powerfull tool towards the structure elucidation of adducts formed between PGE and dAdo, dGuo and dCyd. Proposed structures N-1-PGE-dAdo, N-1,N^6-PGE-dAdo, N-3-PGE-dCyd and N-7-PGE-dGuo were confirmed by mass spectral analysis. A thorough NMR analysis was performed for N-1-PGE-dAdo (7).

REFERENCES

(1) J. Hopkins, Food Chem. Toxicol., 1984, 22, 780-783.
(2) M.M. Manson, Br. J. Ind. Med., 1980, 37, 317.
(3) L. Ehrenberg, S. Hussain, Mutat. Res., 1981, 86, 1.
(4) S.W. Frantz, E. Van den Eeckhout, J.E. Sinsheimer, M. Yashihara and M. Koreeda, Toxicol. Letters, 1985, 25, 265
(5) R. Lauwereys, M. Mercier and M. Roberfroid (Eds.), Mechanism of toxicity and hazard evaluation, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, p. 241-243.
(6) K.Sugiura, M. Goto, Chem. Biol. Interactions, 1983, 45, 153.
(7) E. Van den Eeckhout, A. De Bruyn, H. Pepermans, E. Esmans, I. Vreyens, J. Claereboudt, M. Claeys, J.E. Sinsheimer, J. Chromat., in press.
(8) E. Van den Eeckhout, J. Claereboudt, J. Coene, E. Esmans, A.P. De Leenheer, J.E. Sinsheimer, J. Chromat., in preparation.