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Detection of Epoxides with 4-(*p*-Nitrobenzyl) pyridine

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Epoxides are alkylating agents which have been recognized as potent mutagens and carcinogens (BOOTMAN et al. 1979, VAN DUUREN 1969, WADE et al. 1979). Many epoxides are used as intermediates in organic syntheses, e.g. high energy fuel plastics and hydroxyethylated cellulose of fibers (ethylene epoxide), surfactants, urethane elastomas, cosmetics (propylene epoxide), curing of polymers, pharmaceuticals (1,2,3,4-diepoxybutane) and could escape directly into the environment. In addition to industrial pollution, spontaneous or photochemical oxidation reactions of aliphatic and aromatic hydrocarbons in the atmosphere may also lead to the formation of carcinogenic and mutagenic epoxides along with other oxidation products (NATIONAL ACADEMY OF SCIENCES 1972, VAN DUUREN 1972). A sensitive analytical method for their detection will therefore be of significant interest.

There are several methods and reagents for detection and analysis of epoxides. We have used 4-(p-nitrobenzyl)pyridine (NBP) as the reagent of choice because of its versatility in the determination of wide variety of alkylating and acylating agents (EPSTEIN et al. 1955, HAMMOCK et al. 1974, PREUSSMAN et al. 1969, SAWICKI et al. 1963, SWAISLAND et al. 1973). SWAISLAND et al. (1973) had reported the determination of epoxides using 2% of NBP reagent. SAWICKI et al. (1963) had developed autocatalytic method for the determination of alkylating agents with NBP in which the reaction mixture in acetophenone is heated at 180°C. Epoxides and some other alkylating agents may be unstable under these conditions. Modification of the above procedures by using an excess of NBP reagent under milder conditions greatly improved the sensitivity for the determination of epoxides.

MATERIALS AND METHODS

Chemicals. Epoxides were obtained from commercial sources or synthesized according to the literature procedures.

Procedure. A 20% solution of the reagent was prepared by warming 80 mg NBP in 0.4 mL ethylene glycol and then cooling to room temperature. A solution of the compound to be tested in 0.1 mL acetone was added followed by 0.2 mL 0.2M tris-HCl

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buffer, pH 7.4. The mixture was incubated at 50°C in a water bath for 2 h and then cooled at room temperature. The absorbance at 570 nm was taken immediately after color development by the addition of 0.5 mL 50% (v/v) triethylamine in acetone against a reference which contained a solution of 0.7 mL acetone, 0.4 mL tris buffer and 0.8 mL ethylene glycol. The net absorbance was obtained by subtracting the absorbance of the blank. The blank usually had some absorbance due to a slight pale yellow color of the commercial NBP.

RESULTS AND DISCUSSION

The method chosen for analysis of epoxides involved reaction of the substrate with an excess of NBP. The absorbance of the product at wavelength maximum can be measured and correlated with the concentration of epoxides. Acetone was chosen as the solvent in conjunction with ethylene glycol and tris-HCl buffer since most of the epoxides are soluble and remain fairly stable in this solvent mixture. The wavelength maxima of absorbance shifted with different substrates from 562 nm for 9,10-epoxyphenanthrene (aromatic epoxide) to 576 nm for 1,2,5,6-diepoxyhexane and 1-ethyleneoxy 3,4-epoxycyclohexane (aliphatic epoxides). A intermediate wavelength of 570 nm was therefore selected for the comparison of molar absorptivity (ε) of different epoxides (Table 1).

TABLE 1. Molar Absorbance of Various Epoxides in the 4-(p-Nitrobenzyl)pyridine Procedures

Compound	λmax nm	Molar absorbance (ε) at 570 nm
Propylene epoxide	576	7300
1,2-Butylene epoxide	574	6900
1,2,5,6-Diepoxyhexane	576	23700
Styrene epoxide	570	13800
1-Ethyleneoxy 3,4-epoxycyclohexane	574	8700
1,2-Epoxycyclohexane	580	2000
1,2,3,4-Diepoxycyclohexane	575	1500
9,10-Epoxyphenanthrene	562	21900
4,5-Epoxy-4,5-dihydropyrene	566	10700
5,6-Epoxy-5,6-dihydro-dibenz(a,h)anthracene	562	15500

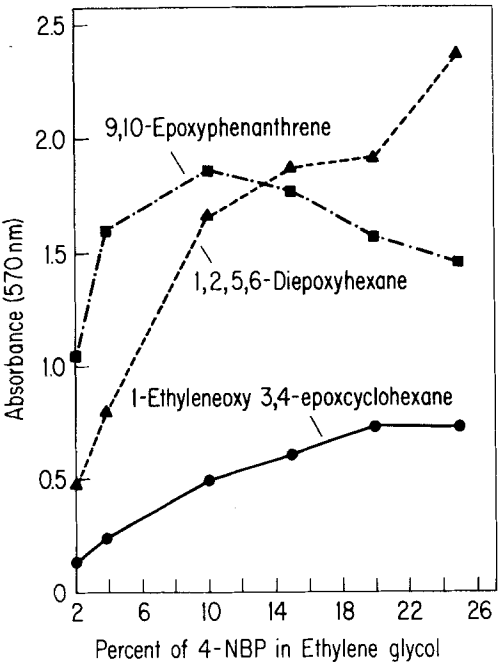

Fig. 1 - Effect of increased concentration of NBP on absorbance. Concentration of substrate was 0.1 μmole/0.1 mL.

Increasing the concentration of NBP from 2% to 25% increased the absorbance in determination of most aliphatic and aromatic epoxides thus lowering their detection limit (Figure 1). This may be explained if the reaction is not kinetically favored and excess of the reagent is probably required to drive the reaction to completion. In the case of 9,10-epoxyphenanthrene and 1-ethyleneoxy 3,4-epoxycyclohexane, the absorbance was maximum with 10 and 20% concentration of the reagent respectively, and then decreased when the concentration of the reagent was further increased. However, in case of 1,2,5,6-diepoxyhexane the absorbance continued to increase with increased amount of the reagent. Increased concentration of acetone had a marked quenching effect on absorbance in this assay as shown in Figure 2. In all these determinations the absorption maxima was at 570 nm and there was no red (or blue) shift with increase in the concentration of acetone. Based on these observations we chose a 20% concentration of the reagent and 2 h incubation time at 50°C as optimal. It is important that the absorbance be read immediately after the addition of base. In some cases the color is quite unstable and the intensity starts to fade very rapidly with time.

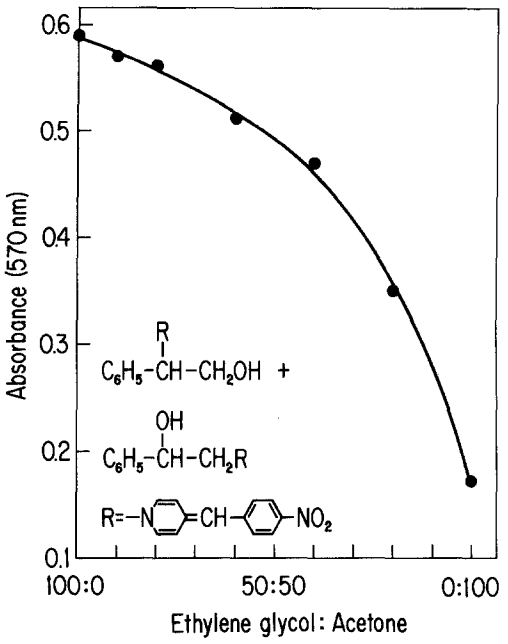

Fig. 2 - Effect of increased concentration of acetone on absorbance. Concentration of styrene epoxide was 0.05 μmole/0.1 mL acetone.

NBP reagent is not specific for epoxides alone and that it has been used to detect many other types of alkylating agents as mentioned in earlier references. However, several aliphatic and aromatic N-nitrosamines along with some peroxides and hydroperoxides did not react with NBP under the conditions employed.

Ten epoxides have been investigated by this method. Unlike the previous methods (HAMMOCK et al. 1974, SAWICKI et al. 1963) which gave negative results with epoxycycloalkanes and related compounds, the present analytical procedure is sensitive and furnished fairly high molar absorbance values (ε) for such compounds (Table 1). Beer's law was obeyed in this range of concentration. The precision of the method was determined by running samples in triplicate; the reproducibility is ± 3% for replicates of the same experiment and ± 3 to 10% between experiments. The molar extinction coefficients of most epoxides (Table 1) tested are sufficiently high to allow 0.1 - 0.01 μ mole level detection.

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