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

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Compounds containing the epoxide or oxirane moiety are candidate insect control agents acting as juvenile hormone mimics and are photoproducts or metabolites of a variety of pesticide and pharmaceutical chemicals. They are also intermediates in many chemical reactions and biosynthetic pathways. A simple procedure for detection and colorimetric analysis of epoxides is therefore of interest.

There are several methods and reagents for detection and analysis of epoxides (FIORITI et al. 1966, PASTO and JOHNSON 1969, AKSENOV 1971, RUDENKO and ALEKSEEVA 1971), including the alkylation of 4-(p-nitrobenzyl)-pyridine (NBP) to form a blue derivative (BREWER and ARNSBERGER 1966, PREUSSMANN et al. 1969, SIMS 1972). NBP is also useful for detection and quantitative determination of ethylenimines, organophosphorus compounds and other alkylating agents (EPSTEIN et al. 1955, WATTS 1965, TURNER 1968, PREUSSMANN et al. 1969, BELLET and CASIDA 1974). We have examined the conditions for using NBP as a reagent for the detection and analysis of a variety of epoxides.

MATERIALS AND METHODS

Chemicals. The test compounds were either from commercial sources or were synthesized by m-chloroperoxybenzoic acid oxidation of olefins.

Detection of Epoxides on Thin-Layer and Paper Chromatograms. The epoxide as a 1 cm diameter spot on a silica gel thin-layer chromatoplate (0.25 mm gel thickness) or on Whatman No. 1 paper was subjected to chromatographic development with a solvent system appropriate for a Rf value of 0.3 to 0.8 unless the epoxide was known to be volatile. The NBP reagent used for epoxide detection on thin-layer chromatography (tlc) was a 2% (w/v) solution in acetone while that for paper chromatography (pc) was a freshly

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prepared 1% (w/v) solution in 50% aqueous acetone containing 0.5N potassium acid phthalate. The sprayed chromatograms were heated (110°C, 5 min), allowed to cool, and sprayed with a 10% (v/v) solution of tetraethylenepentamine in acetone to yield blue spots on a white background. The sensitivity is recorded as the amount of compound yielding a deep blue color which persists for at least 20 min; the lowest limit of detection as a transient light blue spot is generally less than 10% of this amount.

The aqueous acid NBP reagent is necessary for pc but not for tlc since the silica gel is sufficiently acidic. High levels of appropriate epoxides give a red color after spraying with NBP but the spot turns blue on treatment with base. Tetraethylenepentamine is a better base for color development than aqueous $NaHCO_3$, Na_2CO_3, or NaOH. The sensitivity limits are the same with or without development of the chromatograms based on comparative studies with 10 low volatility epoxides.

Colorimetric Analysis of Epoxides. The epoxide in 2 ml of acetone is mixed with aqueous potassium acid phthalate (1 ml, 0.1N) and NBP reagent (1 ml, 5% w/v in acetone) then a boiling chip is added, the mixture held at 105 to 110°C for 45 min, allowed to cool to 40-50°C, brought up to 9 ml with 50% aqueous acetone, shaken, and placed in an ice bath. Immediately before reading the absorbance at 600 nm, aqueous K_2CO_3 (1M, 1 ml) is added and the tube shaken. The response of different epoxides is compared on the basis of molar absorptivity (ϵ).

Several variables were examined in optimizing the NBP method for analysis of 4-ethylphenyl 6,7-epoxygeranyl ether (designated with an asterisk in Table I). The reaction of NBP with the epoxide is initiated only after essentially all of the acetone is evaporated at which point the attachment of a reflux condensor increases the reproducibility of the method. The color intensity increases only slightly on prolonging the heating period from 45 to 90 min. The reagent blank is colorless for up to 3 hr heating. The λ_{max} for the NBP-ethylphenyl epoxygeranyl ether reaction using either acetone or tetrahydrofuran as a solvent is 610 nm; however, there is little change in absorbance from 590 to 620 nm. Tetraethylenepentamine is less favorable as a base for color development than 1M K_2CO_3. TURNER's (1968) modification of the EPSTEIN et al. (1955) test for alkylating agents gives an ϵ value of 1700 for the ethylphenyl epoxygeranyl ether and 2700 for 1,2-epoxy-3,3,3-trichloropropane. The specificity of TURNER's procedure tested with seven substituted-phenyl epoxygeranyl ethers, three substituted-phenyl 2,3-6,7-diepoxygeranyl ethers and several compounds lacking epoxide substituents is similar

TABLE I

Detection and Analysis of Epoxides on Chromatograms

and in Solution with 4-(p-Nitrobenzyl)-pyridine

Compound	Sensitivity on chromatograms, μmole tlc	pc	Molar absorptivity, ε, for solutions
$RCH\underset{\diagdown O \diagup}{-}CH_2$			
ϕ-	0.08	0.67	6,100
Cl_3C-	0.02	> 0.56	5,800
$H_2C{=}CHCH_2OCH_2$-	0.04	0.44	7,300
2-$CH_3\phi OCH_2$-	0.02	0.12	25,000
4-$CH_3O\phi OCH_2$-	0.01	0.02	21,000
Phthalimido-CH_2-	0.10	0.01	13,000
6',7'-Epoxyrotenone	0.01	0.01	15,000
$RCH\underset{\diagdown O \diagup}{-}C(CH_3)_2$			
4-$C_2H_5\phi OCH_2CH{=}\overset{t}{C}(CH_3)(CH_2)_2$-*	0.04	0.02	4,600
4-$Cl\phi OCH_2CH{=}\overset{t}{C}(CH_3)(CH_2)_2$-	0.04	0.02	4,600
$HOCH_2CH_2CH(CH_3)(CH_2)_2$-	0.35	0.58	1,800
Epoxyresmethrin	0.03	0.08	10,000
$RCH\underset{\diagdown O \diagup}{-}\overset{c}{C}(CH_3)C_2H_5$			
$CH_3OC(O)CH{=}\overset{t}{C}(CH_3)(CH_2)_2CH{=}\overset{t}{C}(C_2H_5)(CH_2)_2$-	0.07	0.1	3,700
Epoxycycloalkanes and related compounds			
1,2-Epoxycyclohexane	2	> 4	250
1,4-Epoxycyclohexane	> 13	> 3	< 17
1,2-Epoxycyclododecane	2	> 2	114
exo-2,3-Epoxynorbornane	> 12	> 6	7
3,4-Epoxytetrahydrothiophene-1,1-dioxide	> 1.5	> 3	13
Dieldrin, endrin and heptachlor epoxide	> 1	> 1	< 25

*This compound was used in optimizing the conditions for detection and analysis.

to that obtained with the standard procedure but the sensitivity for epoxides is lower. Tetrahydrofuran and acetone are suitable solvents whereas 2-butanone, dimethylsulfoxide, dimethylformamide, dioxane, ether, hexane and a variety of mono- and dihydric alcohols give little or no color development. Lewis acids ($SnCl_4$, $AlCl_3$ or BF_3) in organic solvents are not suitable replacements for the aqueous acid phthalate.

RESULTS AND DISCUSSION

Detection of Epoxides on Thin-Layer and Paper Chromatograms. As shown in Table I, the NBP reagent provides a sensitive test for epoxides derived from allyl, isobutenyl and closely related substituents. Other compounds with epoxidized isobutenyl groupings easily detected in amounts of 0.01-0.1 μmole are: 15 substituted-phenyl 6,7-epoxygeranyl ethers or 2,3-6,7-diepoxygeranyl ethers (HAMMOCK 1973, HAMMOCK et al. 1974) of interest as insect juvenile hormone mimics; 10 esters of epoxychrysanthemic and epoxyethanochrysanthemic acids derived from pyrethroid insecticides (UEDA et al. 1974). This method was useful in establishing that these hormone mimics and pyrethroids such as resmethrin undergo photoepoxidation under environmental conditions (GILL et al. 1974, UEDA et al. 1974). Epoxyrotenone, a photoproduct of rotenone (CHENG et al. 1972), also responds to NBP reagent. The method is less satisfactory for volatile epoxides and is insensitive for epoxycycloalkanes and related compounds.

The NBP reagent is not specific for epoxides since it also detects many other types of alkylating agents including organophosphorus compounds as noted in the introduction. However, it does not respond to thiaranes, oxetanes, furans, tetrahydrofurans, acyclic aromatic or aliphatic ethers, olefins, conjugated dienes, aldehydes, ketals, ketones, carboxylic acids, esters, alcohols, or phenols (HAMMOCK 1973).

WATTS (1965) found that olive oil, mineral oil, and safflower oil are unsatisfactory stationary phases for pc of organophosphorus insecticides because these oils give a blue background with NBP reagent. This is probably due to the presence of epoxides in the oils since we find that the reaction of safflower oil with NBP is increased by prior treatment with peracid and decreased by prior treatment with dilute aqueous H_2SO_4.

Analysis of Epoxides in Solution. The epoxides easily detected on chromatograms are those for which the NBP reagent provides a sensitive method of analysis (Table I). The standard conditions were optimized for the ethylphenyl

epoxygeranyl ether but may not be optimal for other epoxides. Lower reaction temperatures or shorter incubation periods are preferable for highly reactive epoxides such as 1,2-epoxy-3,3,3-trichloropropane, while lower temperatures and longer reaction times result in greater sensitivity for substituted-phenyl 2,3-6,7-diepoxygeranyl ethers. Alkylating agents are interfering materials in analysis of epoxides.

The NBP reagent is useful in monitoring enzymatic reactions with epoxide substrates. Incubation of the ethylphenyl epoxygeranyl ether with buffer, then pentane extraction and NBP analysis established that 0.2 μmole of this epoxide is detectable and that absorbance is proportional to the amount of epoxide up to a level of about 1.0 μmole; the reproducibility is ±3% for replicates of the same experiment and the ±10% between experiments. Appropriate cleanup procedures must be used with biological samples. Thus, with pentane extracts of enzyme incubation mixtures containing mouse microsomes (2 mg protein), a background color precludes accurate determinations at epoxide levels below 0.07 μmole prior to chromatographic removal of the interfering materials. The use of a microcuvette and smaller reagent volumes permit reproducible determinations at levels below 0.004 μmole.

SUMMARY

4-(p-Nitrobenzyl)-pyridine reagent provides a sensitive method for detection on paper and thin-layer chromatograms and for quantitative colorimetric analysis of many epoxides of biological and chemical interest and environmental significance.

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