Journal of Chromatography, 461 (1989) 35–44
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1511

DIOXANYL HYDROPEROXIDE IN PREPARATIVE LIQUID CHROMATOGRAPHY

ELLEN M. DERRICO*, HELGA BUSH, PATRICIA M. FINNEGAN
G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL 60077 (U.S.A.)

SUMMARY

A contaminant was discovered in the eluate from a preparative liquid chromatographic purification of a research drug. The contaminant was isolated by column chromatography and identified by spectral data as 1,4-dioxan-2-yl hydroperoxide (*p*-dioxanyl hydroperoxide). The hydroperoxide was characterized by NMR and IR spectroscopy and thin-layer and high-performance liquid chromatography. The conditions for safe removal and reduction were also investigated.

INTRODUCTION

Peroxides formed as a result of the autoxidation of ether solvents are known to be unstable and heat-, friction- and shock-sensitive compounds[1–5]. In the past, research has been carried out to characterize 1,4-dioxan-2-yl hydroperoxide (*p*-dioxanyl hydroperoxide), a peroxide found in dioxane. The single-crystal X-ray structure was determined by Nord and Lindberg[6], and IR and NMR characterization were carried out by Griere and Petterson (see ref. 6).

Safety issues regarding peroxide have been addressed in several publications[1–5,7–9], and the removal of peroxides from solvents by either scavenging with neutral alumina or reduction with metals, such as iron or silver, has been reported[10,11]. Peroxide formation in ether solvents when used in large volumes or in industrial applications has been acknowledged in several publications[10–12]. Verzele and DeWaele[12] suggested that the amount of peroxide generated is potentially hazardous.

Despite the problems, dioxane is a useful solvent in both normal and reversed-phase preparative chromatography for its unique selectivity and solubility properties. A peroxide of 1,4-dioxane, *p*-dioxanyl hydroperoxide, was isolated in our laboratory during normal-phase preparative liquid chromatography. This paper discusses the formation, isolation, identification, removal and hazards of *p*-dioxanyl hydroperoxide.

EXPERIMENTAL AND RESULTS

Materials and instrumentation

High-purity solvents (acetonitrile, dioxane, ethyl acetate, hexane, 2-propanol,

0021-9673/89/$03.50 © 1989 Elsevier Science Publishers B.V.

dichloromethane, methyl *tert.*-butyl ether, tetrahydrofuran and trimethylpentane) were obtained from Baxter, Burdick and Jackson (Muskegon, MI, U.S.A.). High-performance liquid chromatography (HPLC) grade water, concentrated sulfuric acid and potato starch were from J. T. Baker (Phillipsburg, NJ, U.S.A.). Acetic acid, granular iron(II) sulfate, phosphomolybdic acid (PMA) crystals, potassium iodide, resublimed iodine crystals and sodium hydroxide pellets were obtained from Mallinckrodt (Paris, KY, U.S.A.). Acculute standard volumetric hydrochloric acid was from Anachemia Chemicals (Champlain, NY, U.S.A.). Analytical reagent grade potassium permanganate and EM Quant peroxide test strips were obtained from EM Science (Cincinnati, OH, U.S.A.). N,N-Dimethyl-*p*-phenylenediamine (DMPD), 1% spray reagent, was from Sigma (St. Louis, MO, U.S.A.).

For preparative HPLC experiments, Bio-Sil A (200–400 mesh) silica gel was obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.). Woelm Super I neutral alumina was from Universal Scientific (Atlanta, GA, U.S.A.). ICN Adsorbents (formerly Woelm) 32–63-μm, 60-Å irregular silica gel was obtained from ICN Biomedicals (Cleveland, OH, U.S.A.). Partisil Prep 40 silica gel came from Whatman (Clifton, NJ, U.S.A.). For thin-layer chromatographic (TLC) analyses, Uniplate Woelm silica gel GF TLC plates were obtained from Analtech (Newark, DE, U.S.A.), and Merck silica gel $60F_{254}$ TLC plates were from EM Science. For the reduction experiments, analytical grade AG 50W-X4 H^+ (−400 mesh) cation-exchange resin was obtained from Bio-Rad Labs. The resin was prepared by washing with 1 *M* hydrochloric acid (Anachemia Chemicals), deionized water, 0.5 *M* iron(II) sulfate solution (Mallinckrodt), deionized water, 2-propanol and hexane.

The analytical system for the HPLC analyses of *p*-dioxanyl hydroperoxide consisted of an LC9533 ternary gradient liquid chromatograph from IBM Instruments (Danbury, CT, U.S.A.), a Schoeffel 770 variable-wavelength UV detector, obtained from Waters Chromatography Division, Millipore (Milford, MA, U.S.A.). an Erma ERC7510 refractive index detector, obtained from Anspec (Warrenville, IL, U.S.A.) and a Linear Instruments (Irvine, CA, U.S.A.) Model 585 recorder. The column used for the analyses was a μPorasil silica column (300 × 3.9 mm I.D.) from Waters Assoc.

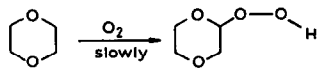
The formation of *p*-dioxanyl hydroperoxide occurred initially during a preparative HPLC purification in which a SepTech ST/800C system from Separations Technology (Wakefield, RI, U.S.A.) with a stainless-steel column (1 m × 4 in. I.D.) obtained from HT Chemicals (St. Louis, MO, U.S.A.) was used. To isolate *p*-dioxanyl hydroperoxide, a Whatman (Clifton, NJ, U.S.A.) Magnum 40 stainless-steel column was utilized.

NMR data were obtained with a Varian (Palo Alto, CA, U.S.A.) VXR200 spectrometer. For IR data, a Perkin-Elmer (Norwalk, CT, U.S.A.) 283B spectrometer was utilized. The UV absorbance spectrum of *p*-dioxanyl hydroperoxide was obtained using a Beckman (Irvine, CA, U.S.A.) DU-7HS UV–VIS spectrophotometer.

Peroxide formation in dioxane

A contaminant was discovered during the analysis of purified drug products from a preparative HPLC purification which utilized dioxane, trimethylpentane and acetonitrile in the mobile phase. The contaminant was identified as *p*-dioxanyl hydroperoxide. Several experiments were then performed to investigate the formation of *p*-dioxanyl hydroperoxide in the solvent and during preparative HPLC.

Dioxane is stored in 1- or 4-l brown glass bottles, under nitrogen. Once they are opened, air enters the bottles and autoxidation of dioxane begins, according to the following reaction[1,3,5,10,11,13–18]:



Dioxane *p*-dioxanyl hydroperoxide
(1,4-dioxan-2-yl hydroperoxide)

When a fresh 4-l bottle of dioxane was opened and immediately evaporated under vacuum at 40°C, 200 mg of fairly pure *p*-dioxanyl hydroperoxide were obtained. It was also demonstrated that amounts up to 200 mg/l could be generated when the dioxane was aerated with a stream of oxygen and irradiated with UV light in an open beaker for 100 h.

In experiments with preparative silica gel columns packed in the laboratory, a higher concentration of peroxide was found in the column eluate than in the initial solvent. Several brands of silica gel were investigated, and all produced an increase in peroxides (see Table I). In these experiments, the actual amount of peroxide produced during the chromatography and fraction work-up was determined by evaporating the solvent, weighing the peroxide residue and subtracting the amount contributed by the fresh dioxane from the bottle. The concentration of the peroxide present in the fresh solvent prior to chromatography was determined by evaporating the solvent and weighing the residual peroxide.

From these experiments with silica gel columns, one can expect the formation of peroxides during preparative HPLC. The amount produced can be minimized by using fresh dioxane, stored under nitrogen, and by limiting the exposure of the dioxane to oxygen during the preparation of mobile phases and the storage and work-up of fractions.

Isolation and detection of p-dioxanyl hydroperoxide

The original *p*-dioxanyl hydroperoxide contaminant was formed during a preparative HPLC purification in which a mobile phase consisting of dioxane–trimethylpentane–acetonitrile (25:75:0.5, v/v) was used. A total of 90 l of mobile phase was passed through a column containing 3.9 kg of silica gel. The peroxide was first observed when the column fractions were analysed by TLC on Analtech Woelm silica gel plates developed with 100% ethyl acetate (R_F = 0.55). The peroxide was rendered

TABLE I

COLUMN CHROMATOGRAPHY EXPERIMENTS

Experiment No.	*Silica gel brand*	*Volume of mobile phase (l)*	*Peroxide produced during chromatography (mg)*
1	Bio-Rad Bio-Sil A	2.7	8.7
2	ICN Adsorbents	2.7	27.0
3	Whatman Partisil Prep 40	2.5	43.7
4	Whatman Partisil Prep 40	2.6	38.2

visible by two techniques: (1) the plates were sprayed with 50% sulfuric acid and heated at 300°C for 3–5 min and then viewed under long-wavelength UV light (366 nm); (2) the same plates were then sprayed with 10% phosphomolybdic acid (PMA) solution and heated at 300°C for 1–3 min to produce blue spots against a yellow background. The peroxide contaminant was visible by both techniques and was present in every chromatographic fraction.

The contaminated drug product was purified and 400 mg of the peroxide were isolated by the following method. A Whatman Magnum 40 preparative column packed with silica gel was eluted with 2 l of ethyl acetate–hexane (3:2) followed by 4 l of ethyl acetate. The *p*-dioxanyl hydroperoxide was eluted in the first 2400 ml, completely resolved from the main drug product (Fig. 1).

HPLC analysis

HPLC was utilized to analyze samples and solvent for *p*-dioxanyl hydroperoxide. The initial system was similar to the method for the preparative HPLC isolation of the *p*-dioxanyl hydroperoxide. A μPorasil column was eluted with ethyl acetate–hexane (1:1) at a flow-rate of 2 ml/min. Both UV (265 nm, 0.2 a.u.f.s.) and refractive index (RI) (temperature 35°C, range = 1) detection were utilized. The retention time of *p*-dioxanyl hydroperoxide was *ca.* 3.9 min. The system was useful for separating *p*-dioxanyl hydroperoxide from impurities with RI detection. Owing to the high UV cut-off of ethyl acetate, UV detection was hindered.

The following system was developed to accommodate both UV and RI detection. A μPorasil column was eluted with 2-propanol–hexane (1:9) at a flow-rate of 2 ml/min. UV detection was effected at 210 nm, 0.4 a.u.f.s. and RI detection at a temperature of 40°C and range = 1. The retention time of the peroxide was *ca.* 4.6 min. The RI response is greater than the UV response, as there are no strong UV chromophores in dioxane or *p*-dioxanyl hydroperoxide. Typical chromatograms are shown in Fig. 2. All of the lots of *p*-dioxanyl hydroperoxide, dioxane and the original peroxide contaminant isolated by preparative HPLC were analyzed by HPLC.

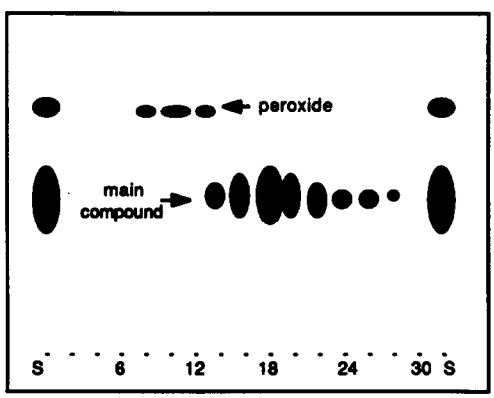


Fig. 1. TLC analysis of fractions for the preparative isolation of *p*-dioxanyl hydroperoxide. Mobile phase, ethyl acetate; detection, 10% PMA after 50% sulfuric acid. S is the sample prior to chromatography. Only the even-numbered fractions from the isolation were spotted on the TLC plate.

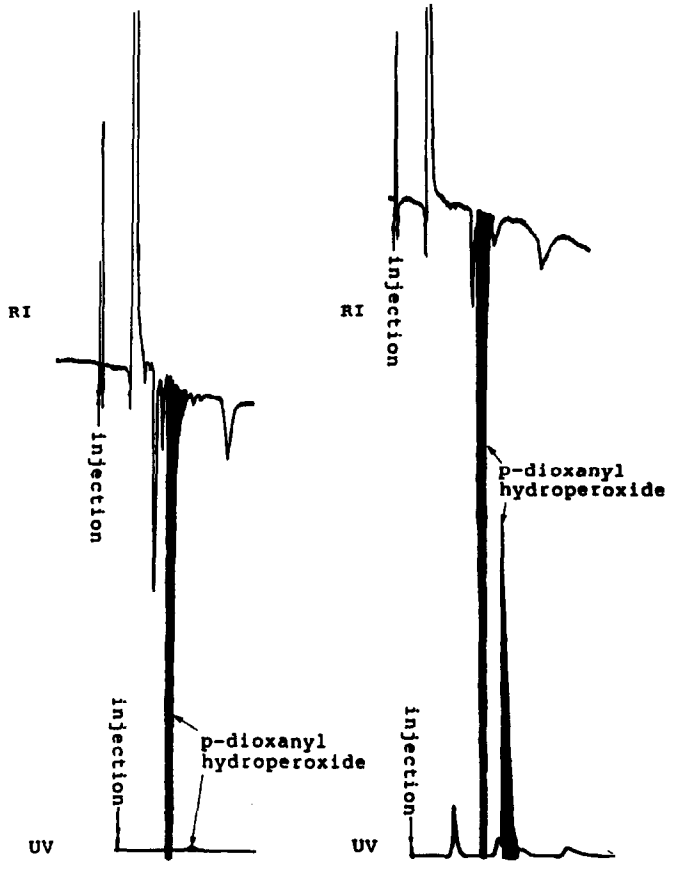


Fig. 2. HPLC methods for *p*-dioxanyl hydroperoxide. System (A): mobile phase, ethyl acetate–hexane (1:1); UV detection, 265 nm, 0.2 a.u.f.s.; RI detection at 35°C, range 1. System (B): mobile phase, 2-propanol–hexane (1:9); UV detection, 210 nm, 0.04 a.u.f.s.; RI detection at 40°C, range 1. Sample, 100 μl of 2 mg/ml *p*-dioxanyl hydroperoxide dissolved in the mobile phase.

This system is also suitable for determining the peroxide content in old bottles of dioxane and other ethers, such as tetrahydrofuran and methyl *tert.*-butyl ether. Both UV and RI detection are sufficiently sensitive to detect the ether peroxides at low levels when a 5-μl aliquot of the solvent is injected.

TLC analysis

A TLC method for analyzing samples containing *p*-dioxanyl hydroperoxide was developed to provide a quick and cost-effective method of analysis. The optimal system, with respect to convenience and R_F value, was 100% ethyl acetate on silica gel TLC plates, with the following detection techniques. (1) N,N-dimethyl-*p*-phenylenediamine (DMPD): the plates are sprayed with a 1% solution of DMPD in acidified methanol and heated very briefly at 300°C. A positive response is a purplish pink spot against a white background. The plates can be viewed in reflected or transmitted white light. This test is specific for organic peroxides and can be used to detect 1 μg. (2)

TABLE II

COMPARISON OF TLC DETECTION METHODS

Scale: 1 = best; 5 = worst.

No.	*Test*	*Detection limit (μg)*	*Contrast*	*Response*	*Ease of mechanics*	*Stability*
1	DMPD	1.0	3	1	1	2
2	DMPD–heat	5.0	1	1	2	2
3	DMPD–heat–LWUV* (366 nm)	1.0	1	1	2	2
4	Potassium permanganate–sodium hydroxide	0.5	2	1	2	2
5	50% sulfuric acid–LWUV*	5.0	2	4	2	1
6	50% sulfuric acid–10% PMA	5.0	4	5	2	2

* Long-wavelength UV detection.

DMPD with heating: the plates from technique 1 are heated for 5–10 min at 300°C and produce better contrast of the spots against the background. The sensitivity is decreased to 5 μg. The plates can be viewed under reflected white light. (3) DMPD with heating and long-wavelength UV detection: the plates from technique 2 are viewed under long-wavelength UV light (366 nm). The spots appear dark with a bright corona against a dark background. The sensitivity is 1 μg. (4) Potassium permanganate–sodium hydroxide: the TLC plates are sprayed with a solution of 0.5 g of potassium permangenate dissolved in 1 *M* sodium hydroxide solution and heated for *ca.* 20 s at 300°C. A positive response is a yellow spot against a magenta background. The plates can be viewed under reflected or transmitted white light. This test is not specific for peroxides, but can be used to detect 0.5 μg. (5) Sulfuric acid with long-wavelength UV detection: the plates are sprayed with 50% sulfuric acid and heated at 300°C for 3–5 min, then viewed under long-wavelength UV light (366 nm). The spots appear as light spots against a dark background. The sensitivity is 5 μg. (6) Sulfuric acid–PMA: After technique 5, the plates are sprayed with a 10% solution of PMA and heated at 300°C for 1–3 min. The peroxide gives blue spots against a yellow background when viewed under white light. The sensitivity is 5 μg.

In Table II, these detection methods are compared on the basis of limit of detection, contrast, response, ease of mechanics and stability of the spray reagent solution.

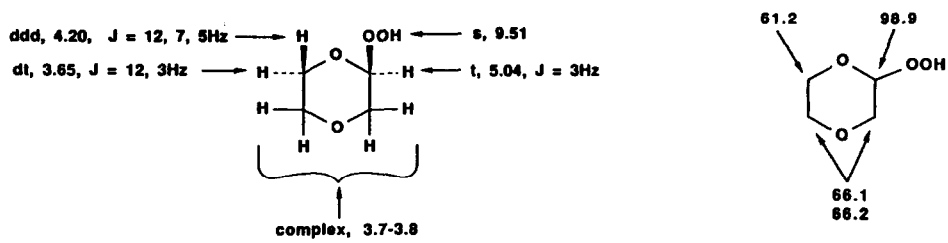


Fig. 3. (Left) Proton and (right) carbon-13 NMR assignments for *p*-dioxanyl hydroperoxide.

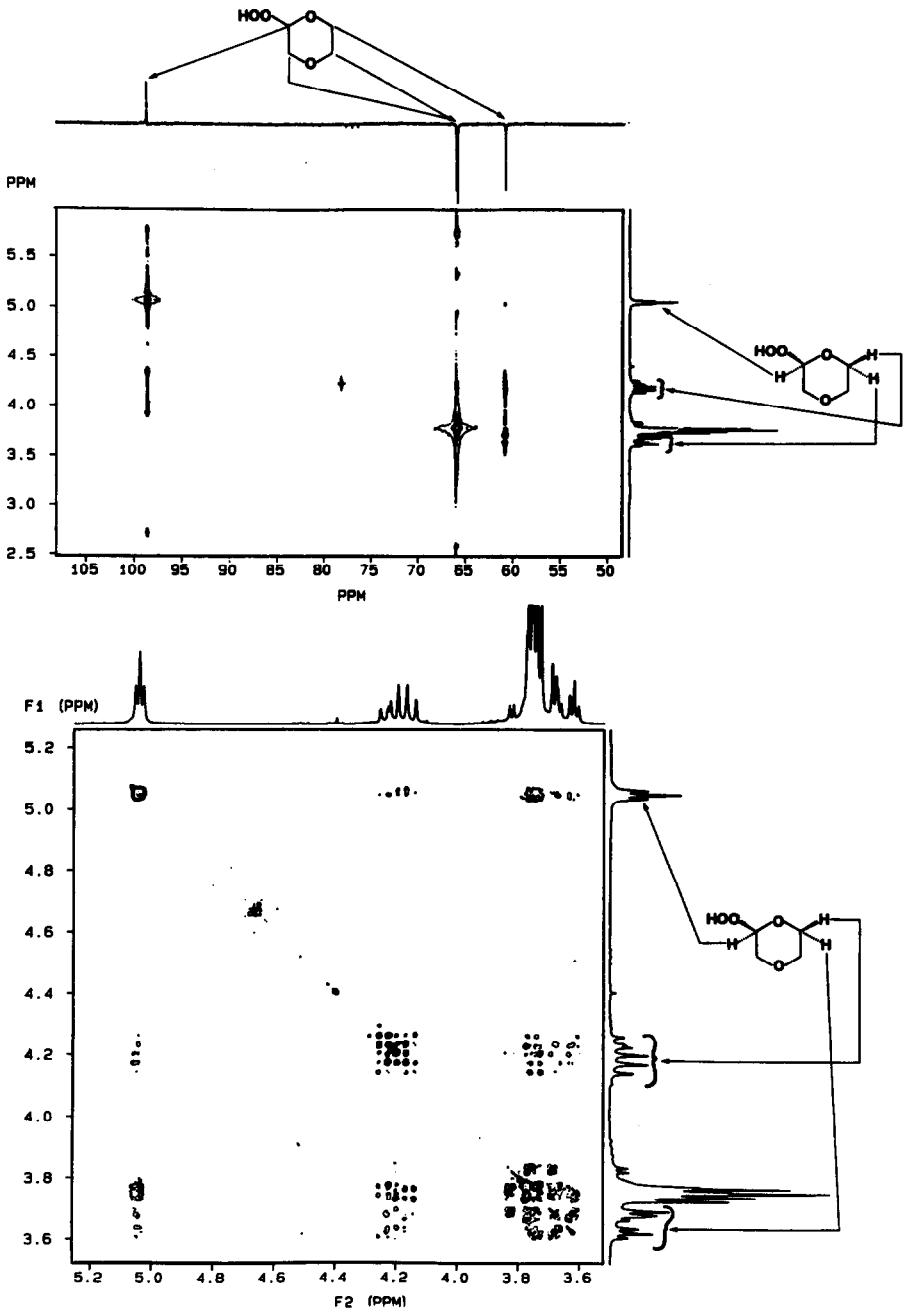


Fig. 4. (Top) 200 MHz proton–carbon heterocorrelation and (bottom) proton–proton homocorrelation spectra of *p*-dioxanyl hydroperoxide in deuterochloroform solution.

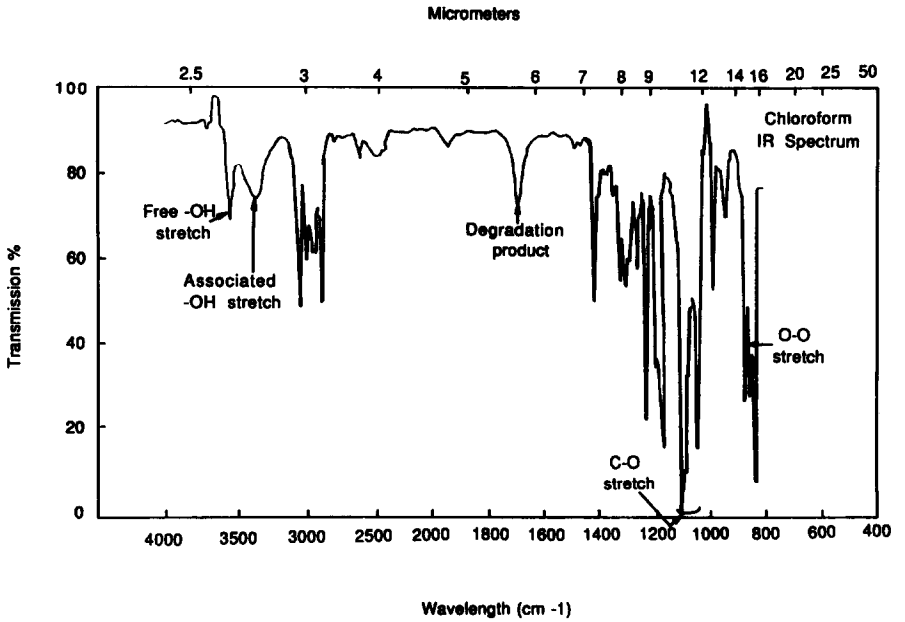


Fig. 5. Chloroform IR spectrum of isolated *p*-dioxanyl hydroperoxide.

Identification

The contaminant isolated by preparative HPLC was determined to be *p*-dioxanyl hydroperoxide based on elemental analysis (found, C 40.2, H 6.8; required for $C_4H_8O_4$, C 40.0, H 6.7%), spectral data and a positive response to EM Quant peroxide test strips.

The proton and carbon-13 NMR spectra obtained by using deuterated chloroform and a tetramethylsilane reference are fully consistent with the structure of *p*-dioxanyl hydroperoxide. Assignments shown in Fig. 3 were confirmed by double resonance, HOMCOR and HETCOR experiments. HOMCOR and HETCOR spectra are given in Fig. 4.

The IR spectrum shown in Fig. 5 contains bands due to free and associated –OH stretching and a band due to O–O stretch. The band at about 1725 cm^{-1} is probably due to a degradation product of the hydroperoxide, as no evidence of major impurities was present in the chromatographic data or other spectral data.

The UV spectrum of a concentrated solution of *p*-dioxanyl hydroperoxide, shown in Fig. 6, contains end absorption beyond 210 nm. Therefore, UV detection in HPLC can be utilized.

Removal of peroxide from dioxane

Several methods of removing *p*-dioxanyl hydroperoxide from dioxane were investigated. When *p*-dioxanyl hydroperoxide is present at concentrations greater than 5 mg/ml, preparative HPLC can be utilized to remove the hydroperoxide from the solvent. In this procedure, the peroxide is concentrated and is not chemically reduced and there exists a potential explosion hazard. Woelm states[10] that alumina scavenges peroxides while letting other compounds pass through unaltered. With *p*-dioxanyl

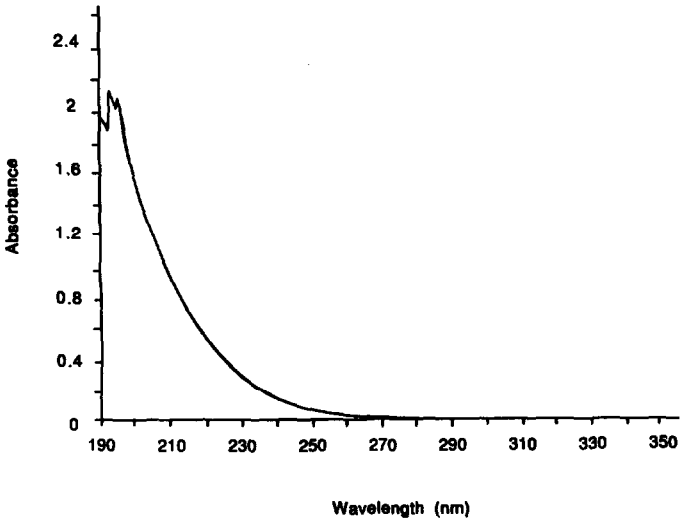


Fig. 6. UV absorbance spectrum of *p*-dioxanyl hydroperoxide, dissolved in acetonitrile (UV cut-off = 190 nm) at a concentration of 11.9 mg in 2.3 ml, 504.2 mg-%, 0.1-cm cell.

hydroperoxide, it has been reported[2,10,14,17] that alumina has been found to cause decomposition of this peroxide.

To investigate the chemical reduction of peroxides by alumina, two experiments were performed with columns containing Super I neutral alumina. In the first experiment, 0.2 g of *p*-dioxanyl hydroperoxide was injected into a column containing 4 g of alumina. The first mobile phase was dichloromethane. To elute the peroxide, a stepwise gradient with increasing amounts of 2-propanol in the mobile phase was applied. The peroxide was not eluted by 100% 2-propanol. Water is recommended for eluting the peroxide from alumina[10], and therefore an additional stepwise gradient from 100% 2-propanol to 100% water was used. No peroxide was eluted. In a second experiment, the maximum amount of peroxide effectively decomposed was calculated. The same experiment was repeated using *ca.* 0.6 g of *p*-dioxanyl hydroperoxide. A total of *ca.* 0.27 g was successfully decomposed. From this experiment, the maximum loading ratio for peroxide decomposition was calculated to be 1 part of peroxide on 17 parts of alumina.

An alternative method for the decomposition of *p*-dioxanyl hydroperoxide by chemical reduction was also investigated. In this method, a strong cation-exchange resin with iron in the 2+ oxidation state was slurried with the sample solution[11,14,19]. It was found that 10 mg of peroxide in 1 ml of solvent were decomposed in 1.5 h when in the presence of 120 mg of iron resin. For small amounts of hydroperoxides, at concentrations less than 5 mg/ml of peroxide in solvent, the solution can be disposed of as for general waste solvents.

CONCLUSION

Peroxides are known to be potentially unstable, explosive and friction-sensitive compounds[1–5,12,20]. Our work has demonstrated that *p*-dioxanyl hydroperoxide is

formed during preparative HPLC and chromatographers using dioxane need to be aware of the potentially hazardous situations when this peroxide is passed through packed columns or concentrated during fraction work-up. The detection and analysis procedure we have described can be useful in monitoring the peroxide content of solvents, samples and solutions during preparative HPLC. Precautions to minimize the exposure of dioxane or dioxane-containing mobile phases to air and UV radiation need to be implemented throughout the preparative chromatographic process.

ACKNOWLEDGEMENTS

The following assisted in the investigations by performing additional analyses and participating in helpful discussions: Clifford J. Seul, Paul W. Schoen, Arthur L. Campbell, Kevin A. Babiak, Elisabeth Hajdu and Lilian Garcia.

REFERENCES

1 *"CHAS" Notes*, American Chemical Society, Division of Chemical Health and Safety, Washington, DC, No. 1(4), 1982, p. 3.
2 H. L. Jackson, W. B. McCormack, C. S. Rondestvedt, K. C. Smeltz and I. E. Viele, *J. Chem. Educ.*, 47 (1970) A175.
3 M. C. Nagel, *J. Chem. Educ.*, 61 (1984) 250.
4 D. A. Pipetone and D. D. Hedberg, *J. Chem. Educ.*, 59 (1982) A159.
5 J. Varjavandi and O. L. Mageli, *J. Chem. Educ.*, 48 (1971) A451.
6 A. G. Nord and B. Lindberg, *Acta Chem. Scand.*, 27 (1973) 1175.
7 *Data Sheet*, No. 655, National Safety Council, Chicago, IL, 1976.
8 *B & J Solvent Applications Update*, Baxter, Burdick and Jackson, Muskegon, MI, 1986, p. 3.
9 *Fisher Methyl tert.-Butyl Ether (MTBE)*, Fisher Scientific, Pittsburgh, PA, Bulletin No. 625/0-1020-07, 1981.
10 *Purification of Solvents by Adsorbents Woelm, 1 and 1a*, Woelm Pharma., Eschwege, 1973.
11 H. G. Shertzer and W. M. Tabor, *J. Environ. Sci. Health*, A20 (1985) 845.
12 M. Verzele and C. DeWaele, *Preparative High Performance Liquid Chromatography*, Deinze, Gent, 1986, pp. 90–91.
13 B. C. Gilbert and A. J. Dobbs, *Organic Peroxides*, Vol. III, Interscience, New York, 1972, pp. 271–339.
14 D. Swern (Editor), *Organic Peroxides*, Vol. II, Wiley-Interscience, New York, 1971, pp. 17–19, 23–25, 31–59, 68–69, 94–127, 178–183 and 223–224.
15 S. Patai (Editor), *The Chemistry of Peroxides*, Wiley, Chichester, 1983, pp. 142–143, 501, 503–506, 652, 721–722, 755 and 760–761.
16 P. Bilski and J. Szychlinski, *J. Photochem.*, 38 (1987) 117.
17 F. Feigl, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1966, p. 14.
18 L. G. Wade, Jr., *Organic Chemistry*, Prentice-Hall, Englewood Cliffs, NJ, 1987, pp. 509–10.
19 A. M. Trzeciak and J. J. Ziolkowski, *J. Mol. Catal.*, 39 (1987) 85.
20 V. K. Mohan, K. R. Becker and J. E. Hay, *J. Hazardous Mater.*, 5 (1982) 197.