CHROM. 3564

Thin layer chromatography of N,N,-disubstituted hydroxylamines and stable nitroxides

Thin layer chromatography has been found useful in studies on the chemistry and separation of hydroxylamines and stable nitroxides. These compounds were needed in an investigation of their properties as lipid antioxidants[1].

Experimental

Hydroxylamines (except diethylhydroxylamine (DEHA)) were synthesized by the method described by SCHÖPF *et al.*[2] N-Hydroxypyrrolidine and N-hydroxymorpholine were synthesized also by the methods of BLOUT *et al.*[3] and of HENRY AND DEHM[4] respectively. DEHA was obtained from Pennsalt Chemicals (Philadelphia, Pa.). The stable nitroxides were synthesized by the method of BRIERE *et al.*[5].

The hydroxylamines were purified by distillation followed by crystallization as the oxalate salt. After recrystallization to constant melting point, the hydroxylamine was freed from the oxalate salt with liquid ammonia and distilled under reduced pressure. Purity was determined by boiling point, and I.R., U.V. and E.S.R. spectra. The hydroxylamines and their parent amines were spotted from chloroform or methanol (50 μg/ml); solutions of the stable nitroxides and their parent amines at the above concentrations were prepared in chloroform, petroleum ether, benzene or methanol (TM-4-OHP* was soluble only in methanol).

Preparation of silica gel plates

A slurry containing 25 g Silica Gel H (E. Merck, Darmstadt, Germany) in 60–65 ml distilled water was applied to glass plates (20 × 20 cm) at a thickness of 250 μ (Kensington Scientific Corp., Oakland, Calif., multi-thickness applicator). The plates were air dried, activated for 20 min at 110°, and stored over calcium chloride until used.

Development of plates

The following three solvent systems were used: (A) methanol, (B) acetone–methanol 60:40 (v:v), and (C) diethyl ether. All solvents were redistilled through an Oldershaw-15 plate fractionation column. The developing tanks were pre-equilibrated with the solvent by wetting Whatman No. 3 MM chromatographic paper on the inside perimeter of the tank. The plates were allowed to develop until the solvent front was near the top of the plate (25–35 min).

Detection

The developed plates were air dried and spots were detected with the following reagents: (a) 1% potassium permanganate in distilled water; hydroxylamines appeared white immediately on a reddish-pink background, nitroxides turned yellow

* Abbreviations: TMP = 2,2,6,6-tetramethylpiperidine; TM-4-C=OP = 2,2,6,6-tetramethyl-4-piperidone; TM-4-OHP = 2,2,6,6-tetramethyl-4-piperidinol; TMPNO = 2,2,6,6-tetramethylpiperidine-1-nitroxide; TM-4-C=OPNO = 2,2,6,6-tetramethyl-4-piperidone-1-nitroxide; TM-4-OH PNO = 2,2,6,6-tetramethyl-4-piperidinol-1-nitroxide.

immediately, other compounds turned yellow more slowly; and (b) iodine vapor; hydroxylamines turned white immediately, other spots were yellow to brown. The tetrazolium technique used by SNOW[6] to detect hydroxylamines was found less useful than permanganate or iodine despite its greater specificity. Two sprays are required (tetrazolium followed by alkali) and the pink spots were very faint.

TABLE I

R_F VALUES OF SOME AMINES, HYDROXYLAMINES AND STABLE NITROXIDES USING THREE SOLVENT SYSTEMS

Compound	*R_F in solvents*		
	A	*B*	*C*
Amines and hydroxylamines			
DEHA	0.63	0.61–0.63	0.36
Pyrrolidine	0.0	0.0	—
N-Hydroxypyrrolidine	—	0.48–0.51	0.10
Piperidine	0.0	0.0	—
N-Hydroxypiperidine	0.58	0.56–0.58	0.20
Morpholine	0.0	0.05	—
N-Hydroxymorpholine	0.58–0.62	0.57–0.62	0.18
Stable nitroxides			
TMP	0.08	0.15	—
TMPNO	0.63	0.70–0.73	0.70
TM-4-C=OP	0.57	—	—
TM-4-C=OPNO	0.68	0.68–0.72	0.51
TM-4-OHP	0.17	—	—
TM-4-OH PNO	0.61	0.67–0.69	0.30

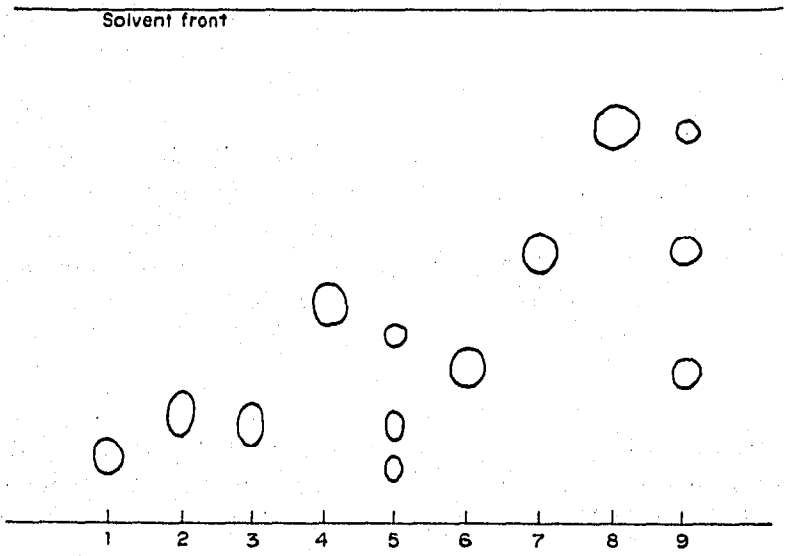

Fig. 1. Thin layer chromatographic separation of N,N-disubstituted hydroxylamines and stable nitroxides. 1 = N-hydroxypyrrolidine; 2 = N-hydroxypiperidine; 3 = N-hydroxymorpholine; 4 = diethylhydroxylamine; 5 = mixture of 1–4; 6 = TM-4-OH PNO; 7 = TM-4-C=OPNO; 8 = TMPNO; 9 = mixture of 6–8.

Results and discussion

The R_F values of the parent amines, the hydroxylamines, and the stable nitroxides are given in Table I. Good separations were achieved. Oxidation products of hydroxylamines were best separated with solvent system (B), but (A) gave similar results. Mixtures of hydroxylamines or of nitroxides were separated best by (C) (see Fig. 1). The systems outlined were quick (25–35 min), convenient methods for separating hydroxylamines and nitroxides, and for determining the purities of the various preparations. Preparative thin layer chromatography was satisfactory for obtaining larger amounts of these products. Up to 10 mg could be streaked on each plate.

Acknowledgements

The author would like to acknowledge the help of Dr. BARBARA BECK in purifying the hydroxylamines. This work was supported in part by grant No. UI 00238-01 from the National Institute of Health.

Institute of Marine Resources, Department of Nutritional Sciences, University of California, Berkeley, Calif. (U.S.A.) JAIR T. WEIL

1 J. T. WEIL, *Ph. D. Thesis*, University of California, Berkeley, Calif., 1968.
2 C. SCHÖPF, A. KOMZAK, F. BROWN AND E. JACOBI, *Ann. Chem.*, 559 (1948) 40.
3 E. R. BLOUT, S. G. COHEN AND M. GREEN, *U.S. Patent*, 2,843,481 (1958).
4 R. A. HENRY AND W. M. DEHM, *J. Am. Chem. Soc.*, 72 (1950) 2280.
5 R. BRIERE, H. LEMAIRE AND A. RASSAT, *Bull. Soc. Chim. France*, (1965) 3273.
6 G. A. SNOW, *J. Chem. Soc.*, (1954) 2588.

Received April 19th, 1968

CHROM. 3553

Two-dimensional thin-layer chromatography of polyphenols from *Dryopteris* species

The medicinally-important *Dryopteris* ferns contain a large number of interrelated phloroglucinol derivatives and the chromatographic separation of these is important both in taxonomic studies of this complex genus and in the evaluation of Male Fern and related drugs. Previous chromatographic studies have been reported[2–5], including two-dimensional paper[6] and thin-layer[7] methods. In these latter, however, the same solvent was used in both directions.

We now present a two-dimensional, thin-layer method which provides differential separation in the two directions of development and in which decomposition of the labile polyphenols is prevented by incorporation of an anti-oxidant in the chromatoplate layer. A further advantage is that visualisation of the compounds is possible, without recourse to spraying, by examination under ultraviolet light.