Journal of Chromatography, 174 (1979) 483–487

CHROM. 11,817

Note

Analytical utility of 2-halopyridinium salts

I. Paper electrophoretic characterization of thiols as 2-alkyl(aryl)thio-1-methylpyridinium *p*-toluenesulphonates

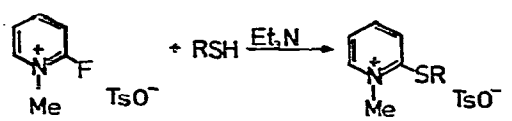
EDWARD BALD

Department of Chemical Technology, Lodz University, 18 Nowotki Street, 91-416 Lodz (Poland)

(Received March 1st, 1979)

In recent years, a new type of dehydration condensation reaction utilizing 2-halopyridinium salts has been developed by Mukaiyama's group (*e.g.*, refs. 1–3). This reaction has a wide range of applications; it is suitable, for example, for the synthesis of esters[1], amides[2] and thiol esters[3]. It appeared to the author that 2-fluoro-1-methylpyridinium *p*-toluenesulphonate could be a promising reagent for the derivatization of thiols subjected to electrophoretic separation, and results are reported here that support this suggestion.

2-Alkyl(aryl)thio-1-methylpyridinium *p*-toluenesulphonates (3) (Fig. 1) were produced easily and rapidly by a nucleophilic attack of thiols (2) on 2-fluoro-1-methylpyridinium *p*-toluenesulphate (1) in the presence of triethylamine. The reaction was carried out in various solvents such as acetonitrile, chloroform, benzene, ethanol and water and no substantial solvent effect was observed. Based on the above considerations, several reactions of thiols and pyridinium salt (1) were carried out, and corresponding derivatives (3) were obtained. The derivatives (3) were separated by high-voltage paper electrophoresis followed by detection with iodine vapour.



1 2 3

Fig. 1. General reaction of thiols and 2-fluoro-1-methylpyridinium *p*-toluenesulphonate.

Few electrophoretic separations of thiols have been described, apart from mostly thiols that have other ionizable functions in the same molecule. Schrauwen[4] referred to the separation of thiols by paper electrophoresis at pH 6, using acetate buffer, at 220 V. His work was concerned primarily with the detection procedure, which he performed with 1-(4-acetoxymercuriphenylazo)-2-naphthol. Wroński[5] described the high-voltage paper electrophoretic separation of thiols, disulphides and

other sulphur-containing compounds. He used *o*-hydroxymercuribenzoic acid and dithiofluorescein as detection reagents.

The electrophoretic separation of simple thiols does not appear to have been described previously. The procedure reported here provides a useful complementary method to chromatographic techniques (for a comprehensive survey, see ref. 6), which normally require some preliminary separation. The derivatization procedure is simple and the equipment involved is simple and inexpensive.

EXPERIMENTAL

Chemicals

2-Fluoro-1-methylpyridinium *p*-toluenesulphonate was prepared in this laboratory. To 2-fluoropyridine (4.85 g, 50 mmole) was added methyl *p*-toluenesulphonate (9.4 g, 50 mmole) and the mixture was stirred for 3 h followed by heating (oil-bath, 80°) for a further 3 h. A precipitate appeared, which was filtered off and washed with dry diethyl ether (25 ml). After drying under reduced pressure, 2-fluoro-1-methylpyridinium *p*-toluenesulphonate (13.9 g, 92%) was obtained and used for the derivatization of thiols without recrystallization. The 2-fluoropyridine and methyl *p*-toluenesulphonate used (Fluka, Buchs, Switzerland) were of synthetic grade.

n-Hexanethiol, *n*-nonanethiol and benzylmercaptan were synthesized in this laboratory from the corresponding iodides according to the literature[7] and purified by double distillation. All other thiols were of analytical-reagent grade from Fluka, Merck (Darmstadt, G.F.R.) or Aldrich Europe (Beerse, Belgium).

Triethylamine (Loba Chemie, Vienna, Austria) was redistilled before use.

AnalaR-grade solvents were used without drying.

Solutions

Buffer solution of pH 7.6 consisted of an aqueous solution of 0.5 mole/l boric acid 0.1 mole/l diethanolamine and 0.025 mole/l disodium salt of ethylene-diamine-tetraacetic acid (EDTA).

Other solutions were a 0.1 *M* solution of 2-fluoro-1-methylpyridinium *p*-toluenesulphonate in acetonitrile and a 0.1 *M* solution of triethylamine in acetonitrile.

Sample derivatization procedure

A 0.1-ml volume of sample solution in the appropriate solvent (or neat) containing not less then 50 nmole of the thiol was pipetted into a small glass test-tube, then 0.1 ml of 2-fluoro-1-methylpyridinium *p*-toluenesulphonate solution and 0.1 ml of triethylamine solution were added*. After shaking the tube and diluting with buffer, the sample was ready for electrophoretic separation.

Electrophoresis procedure

The apparatus for high-voltage electrophoresis (type AEA; WSR-Olsztyn, Poland) was set up in accordance with the manufacturer's instruction. Whatman No. 1 chromatography paper (12 × 37 cm) was impregnated with buffer solution by immersion

* If the sensitivity limit was to be determined, 5 μl of each solution were applied with a Hamilton microdispenser.

in a trough containing the boric acid–diethanolamine–EDTA buffer and excess of buffer solution was removed by light blotting with filter-paper. Sample solutions were applied across the moist paper, 5 cm from the edge, as 5-μl spots using a microsyringe. The safety case of the electrophoresis unit was then closed and a potential of 3000 V applied for 30 min. The paper was removed and the compounds were made visible with iodine vapour as violet-brown compact spots on a white background. All spots were 0.9 $\pm$ 0.1 cm in diameter.

RESULTS

The results show that is possible to correlate migration distance with the carbon number of the *n*-alkyl group for series of C_1–C_{12} thiols.

Fig. 2 shows the electrophoretic migration pattern of the 2-alkylthio-1-methylpyridinium cations obtained for the series of *n*-alkylthiols when examined under the standard conditions described above. Standard mixtures of the thiol derivatives were applied on either side of the paper in order to show that uniform migration occurred across the paper.

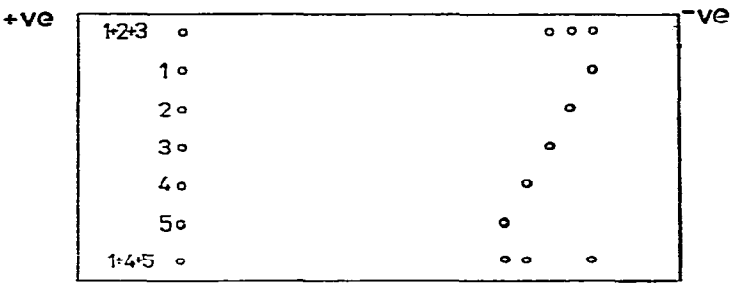


Fig. 2. Migration patterns of thiols separated as 2-alkylthio-1-methylpyridinium *p*-toluenesulphonates. 1 = Methyl; 2 = ethyl; 3 = *n*-propyl; 4 = *n*-butyl; 5 = *n*-pentyl.

Fig. 3 shows the linear relationship between migration distance and carbon number of the *n*-alkyl group for the series of C_1–C_{12} thiols. The aryl series also shows a linear relationship although the migration distances of the cations were smaller.

The relative mobilities and the sensitivity limits of 2-alkyl (aryl)thio-1-methylpyridinium cations are listed in Table I. The relative mobility data are average values of duplicate measurements, and in no instance was the variation between duplicates greater than 4.

Stability of thiol derivatives

The stability of 2-alkyl(aryl)thio-1-methylpyridinium *p*-toluenesulphonates diluted with buffer was followed for 2 weeks and no changes were noticed.

CONCLUSIONS

The method described appears to be a general procedure for the rapid separation of thiols. The method has several advantages: (a) it can be used for

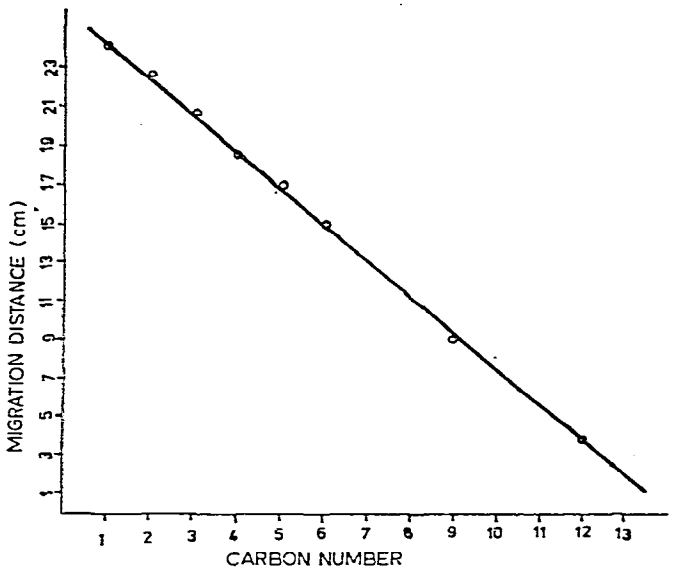


Fig. 3. Relationship between migration distance and carbon number.

TABLE I

CHARACTERIZATION OF THIOLS AS 2-ALKYL(ARYL)THIO-1-METHYLPYRIDINIUM *p*-TOLUENESULPHONATES

Run	*R in thiol*	*Derived cation (Mm × 100)* *	*Sensitivity limit (pmole)* **
1	Methyl	100	1000
2	Ethyl	93	750
3	*n*-Propyl	87	500
4	Isopropyl	88	500
5	*n*-Butyl	76	500
6	Isobutyl	76	500
7	*tert.*-Butyl	79	500
8	*n*-Amyl	71	500
9	Isoamyl	70	500
10	*n*-Hexyl	62	500
11	Cyclohexyl	94	1000
12	*n*-Nonyl	37	500
13	*n*-Dodecyl	16	500
14	Benzyl	64	250
15	Phenyl	71	500
16	2-Pyridyl	75	1000
17	*p*-Cresyl	63	750
18	2-Naphthyl	45	750
19	Allyl	87	250
20	Diethyl dithiophosphate	19	500

* *Mm* values express mobilities relative to the 2-methylthio-1-methylpyridinium ion, which moved approximately 23 cm.

** Sensitivity limit data are, in fact, the combined effect of two procedures, formation of the cation and detection.

the rapid characterization of the thiol compositions in wide range of solvents; (b) it works also with moist samples; (c) it involves readily available reagents and simple and inexpensive equipment; (d) it has a high sensitivity.

Electrophoresis, however, possesses certain disadvantages: (a) it is possible for mixtures containing alkyl, aryl and other thiols to have similar electrophoretic mobilities and thus be indistinguishable; (b) difficulty is experienced with mixtures of isomeric alkyl derivatives, except *tert.*-butyl (see Table I).

Further analytically useful applications of 2-halopyridinium salts are currently being explored.

REFERENCES

1 T. Mukaiyama, M. Usui, E. Shimada and K. Saigo, *Chem. Lett.*, (1975) 1045.
2 E. Bald, K. Saigo and T. Mukaiyama, *Chem. Lett.*, (1975) 1163.
3 Y. Watanabe, S. Shoda and T. Mukaiyama, *Chem. Lett.*, (1976) 741.
4 J. A. M. Schrauwen, *J. Chromatogr.*, 10 (1963) 113.
5 M. Wroński, *Z. Anal. Chem.*, 264 (1973) 406.
6 M. R. F. Ashworth, *The Determination of Sylphur-Containing Groups, Vol. II, Analytical Methods for Thiol Groups*, Academic Press, London, 1976.
7 A. I. Vogel, *A Text-Book of Practical Organic Chemistry*, Longmans, London, 1961.