Journal of Chromatography, 396 (1987) 273–279
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 481

QUANTITATION OF SODIUM MERCAPTOUNDECAHYDRODODECABORATE BY CAPILLARY ISOTACHOPHORESIS

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(Received January 19th, 1987)

SUMMARY

A simple, specific and accurate method for the quantitation of sodium mercaptoundecahydrododecaborate, which is a very hygroscopic agent for boron neutron-capture therapy, was developed by using capillary isotachophoresis with sodium *p*-toluenesulphonate as an internal standard. Compound 1a was separated and identified using 0.01 *M* hydrochloric acid and *β*-alanine as the leading electrolyte and 0.01 *M* *n*-caproic acid as the terminating electrolyte. It could be resolved well from the oxidation products, sodium sulphidobis (undecahydrododecaborate) and sodium sulphidobis (undecahydrododecaborate) S-oxide, and the detection limit was 0.7 μmol/ml.

INTRODUCTION

The boron-10 enriched compoound, sodium mercaptoundecahydrododecaborate (1a), $Na_2{}^{10}B_{12}H_{11}SH$, is one of the most useful agents for boron neutron-capture therapy, which is based on the destruction of tumour cells by short-range α particles produced in nuclear reactions of boron-10 selectively incorporated into malignant tissue. Boron neutron-capture therapy of malignant brain tumour using compound 1a has been carried out successfully by Hatanaka[1–3]. In order to evaluate the distribution of boron-10 around the tumour, we developed a colorimetric method with curcumine for determining boron levels in biological samples[4].

Since compound 1a is very hygroscopic and prone to oxidation in air, its quality has been monitored by detecting the disulphide, the main contaminant, by thin-layer chromatography[5]. However, the resolution of this method is poor.

In this report, we describe a simple assay procedure for measuring compound 1a as an anion by capillary isotachophoresis using sodium *p*-toluenesulphonate as an internal standard. This method is accurate, rapid and sensitive for the separation and determination of compound 1a.

0021-9673/87/$03.50

EXPERIMENTAL

Apparatus and conditions

Isotachophoretic analysis was performed with a Shimadzu IP-1B isotachophoretic analyzer equipped with a PG-1 potential gradient detector. The separation was performed in a PTFE capillary tube (20 cm × 0.5 mm I.D.) maintained at 20°C. The leading electrolyte was 0.01 *M* hydrochloric acid adjusted to pH 3.70 by adding *β*-alanine; 1.5% Triton X-100 was also added to suppress electro-osmosis. The terminating electrolyte was 0.01 *M* *n*-caproic acid.

Chemicals

Caesium mercaptoundecahydrododecaborate monohydrate (1b), $Cs_2{}^{10}B_{12}H_{11}SH \cdot H_2O$, was obtained as non-hygroscopic crystal (Fig. 1) and therefore was used instead of the very hygroscopic compound 1a. Also used were caesium sulphidobis (undecahydrododecaborate) (2b), caesium sulphidobis(undecahydrododecaborate) S-oxide (3b) as an oxidation product and caesium undecahydrododecaborate (4b) as an intermediate of compound 1b. These compounds 1b–4b were synthesized in our laboratories from boric acid with boron-10 as the starting material. Commercially available sodium *p*-toluenesulphonate was recrystallized from 95% (v/v) ethanol and used as an internal standard. Other chemicals were of analytical grade.

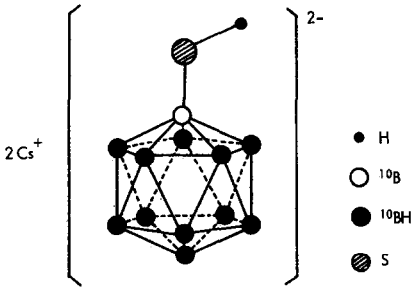

Fig. 1. Structural formula of compound 1b (ref. 6).

Standard solutions

The standard solutions of compounds 1b and 2b were prepared individually by dissolving about 36 mg in 10 ml of distilled water. The internal standard solution was obtained by dissolving about 29 mg of sodium *p*-toluenesulphonate in 10 ml of distilled water. The working standard solutions for calibration curves were prepared by mixing standard solutions of compound 1b or 2b and sodium *p*-toluenesulphonate. The final concentrations were 0.8–4.8 μmol/ml for compound 1b, 0.4–2 μmol/ml for compound 2b and 3 μmol/ml for sodium *p*-toluenesulphonate.

Procedure

A 10-μl portion of the sample solution with 3 μmol/ml of sodium *p*-toluenesulphonate was introduced into the apparatus. The analysis was performed at 100

μA and the isotachopherogram was recorded at a chart speed of 20 mm/min. The lengths of the separated zones corresponding to compounds 1b, 2b and sodium *p*-toluenesulphonate were noted, and the contents of compounds 1b and 2b in the sample were found from the calibration curves.

RESULTS AND DISCUSSION

Identification

To detect compound 1b as an anion, 0.01 *M* hydrochloric acid–β-alanine (pH 3.70) was used as the leading electrolyte and 0.01 *M n*-caproic acid as the terminating electrolyte as reported previously[7]. Under the analytical conditions, the effective mobilities of the anions of compounds 1b–4b were evaluated according to the method described by Hirokawa *et al.*[8], and are summarized in Table I. Fig. 2 shows an isotachopherogram displaying clear separation of compounds 1b, 2b and the internal standard sodium *p*-toluenesulphonate. Compound 1b could also be separated well from compounds 3b and 4b. The separation of compounds 2b–4b was insufficient because there was little difference in the effective mobilities under the analytical conditions. These results showed that compound 1b could be clearly separated from the related compounds 2b–4b under the analytical conditions used.

TABLE I

EFFECTIVE MOBILITIES OF THE ANIONS OF COMPOUNDS 1b–4b UNDER THE ANALYTICAL CONDITIONS

The values were calculated using the effective mobility of chloride ion ($74.52 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}) and the R_E value of each compound based on acetate anion ($R_E = 5.616$). R_E is the ratio of potential gradient of the sample zone to that of the leading zone in an isotachophoretically steady state[8].

Compound	*Anionic species*	*Effective mobility* ($cm^2\ V^{-1}\ s^{-1} \cdot 10^5$)
1b	$^{10}B_{12}H_{11}SH^{2-}$	45.19
2b	$^{10}H_{12}H_{11}SS^{10}B_{12}H_{11}{}^{4-}$	50.25
3b	$^{10}B_{12}H_{11}S(O)S^{10}B_{12}H_{11}{}^{4-}$	49.90
4b	$^{10}B_{12}H_{12}{}^{2-}$	53.50

Stability

The stability of compounds 1b and 2b in aqueous solution was examined over 9 days at room temperature. Sample solutions of 1.12 and 5.47 μmol/ml were used for compound 1b and of 0.83 and 3.73 μmol/ml for compound 2b. Portions (5–10 μl) were analyzed under the conditions described above at appropriate time intervals after dissolution. As summarized in Table II, both compounds 1b and 2b were readily oxidized by the oxygen dissolved in the solution, and their percentages decreased significantly, depending on the initial concentrations.

Let us consider the oxidation course of compound 1b at 1.12 μmol/ml. Oxidation was slight for the first 3 h then became considerable, as is seen from curve 1 in Fig. 3. As compound 1b decreased, the disulphide (2b), produced in the solution, increased and could be clearly detected after 24 h. Since the effective mobilities of

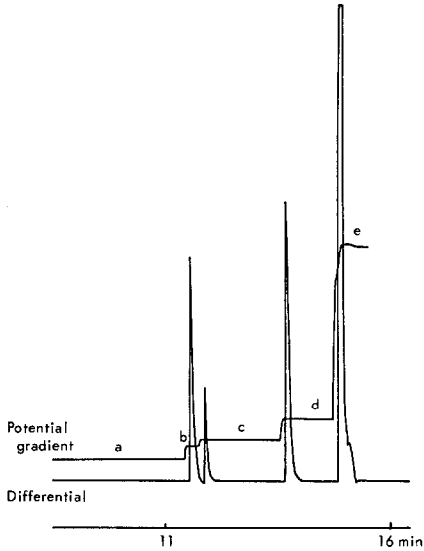

Fig. 2. Isotachophoretic separation of compounds 1b, 2b and sodium *p*-toluene sulphonate. A 10-μl portion of a standard mixture containing compound 1b (3.21 μmol/ml), 2b (0.42 μmol/ml) and sodium *p*-toluenesulphonate (3.05 μmol/ml) was injected. Analytical conditions as in the text. a, Cl^-; b, $(^{10}B_{12}H_{11}S_2)_2^{4-}$ (2b); c, $^{10}B_{12}H_{11}SH^{2-}$ (1b); d, *p*-toluenesulphonate; e, *n*-caproic acid.

compounds 2b and 3b are almost the same, another derivative, the thiosulphinate (3b) is detectable isotachophoretically together with the disulphide if it is produced in the sample solution by subsequent oxidation. The amounts of disulphide produced and possibly of thiosulphinate were used to calculate the amount of compound 1b.

The subsequent oxidation product, the unknown derivative, was found in the sample solution after 48 h; the migration order was chloride, the unknown derivative, the disulphide and thiosulphinate, compound 1b and *n*-caproic acid (Fig. 4). The unknown derivative seems to be very mobile, due to an highly charged ionic structure

TABLE II

STABILITY OF COMPOUNDS 1b AND 2b IN AQUEOUS SOLUTION

Time after dissolution (h)	*Percentage remaining*			
	Compound 1b (μmol/ml)		*Compound 2b (μmol/ml)*	
	1.12	*5.47*	*0.83*	*3.73*
0.1	100.0	100.0	100.0	100.0
6	92.3	98.4	96.1	99.3
24	70.8	95.9	90.4	96.2
48	46.2	93.6	75.7	92.2
72	37.2	91.0	65.0	85.3
120	26.9	86.1	41.6	73.4
226	19.2	77.3	17.4	52.0

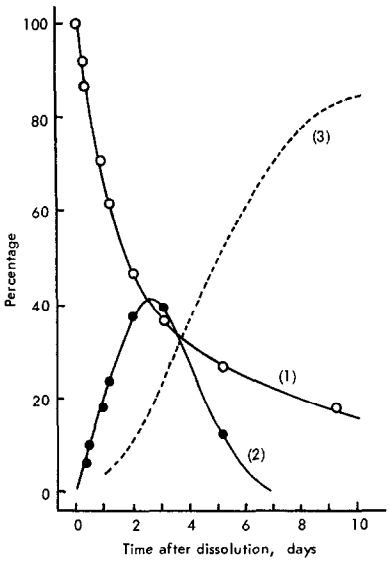

Fig. 3. Time course of the oxidation of compound 1b (1.12 μmol/ml) in aqueous solution. Curve 1 represents the percentage of compound 1b in aqueous solution and curves 2 and 3 those of the disulphide (2b) and possibly the thiosulphinate (3b) derived from compound 1b, and the subsequent oxidation products 5b and 6b, respectively.

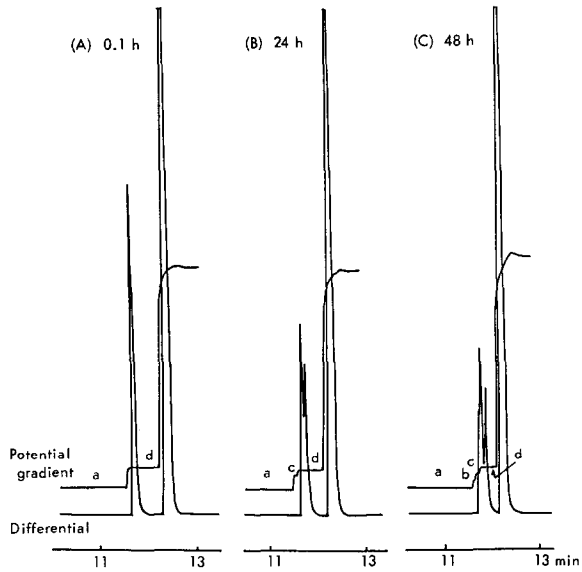

Fig. 4. Isotachophoretic monitoring of the oxidation of compound 1b in aqueous solution. Portions of sample solution (1.12 μmol/ml), each 10 μl were analyzed under the conditions described in the text. a = Cl^-; b = unknown derivative; c = the disulphide (2b) and thiosulphinate (3b); d = compound 1b; e = *n*-caproic acid.

or small size. It was also detected in the solution of compound 2b under comparable conditions. Consequently, we speculate that this derivative is probably the conjugated form of the disulphide, $Cs_6(^{10}B_{12}H_{11}S^+-S^{10}B_{12}H_{11})_2$ (5b), which exists in equilibrium with the unstable radical derivative, $Cs_3(^{10}B_{12}H_{11}S^{\cdot+}-S^{10}B_{12}H_{11})$ (6b), in aqueous solution[9]. The time course of the derivatives 5b and 6b was estimated from curve 3 in Fig. 3, corresponding to the difference between the initial amount of compound 1b and the measured amounts of residual compound 1b and of disulphide and thiosulphinate.

Calibration curve

The calibration curve for compound 1b was constructed using six standard solutions containing 3 μmol/ml of sodium *p*-toluenesulphonate as the internal standard. As shown in Fig. 5, the relationship between the zone length and the amount of compound 1b injected was linear over the range of 0.8–4.8 μmol/ml. Furthermore, as no oxidized anions such as compounds 2b and 3b were detected, we conclude that oxidation of compound 1b did not occur during analysis.

A linear relationship was also observed for compound 2b in the range of 0.4–2 μmol/ml. The detection limit was 0.7 nmol/10 μl for compound 1b and 0.5 nmol/10 μl for compound 2b.

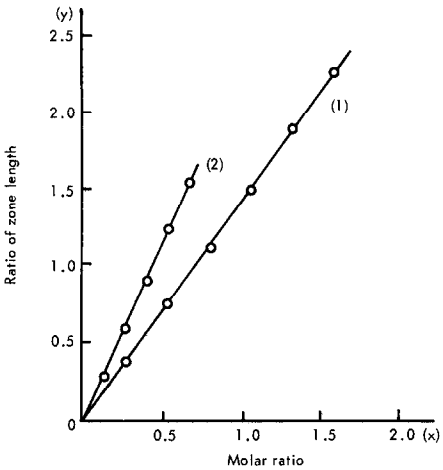

Fig. 5. Calibration curves: (1) compound 1b, regression equation $y = 1.423x - 0.01$, S.D. = 0.02, n = 6; (2) compound 2b, regression equation $y = 2.048x - 0.06$, S.D. = 0.03, $n = 5$. x = Molar ratio of compound 1b or 2b to *p*-toluenesulphonate; y = ratio of zone length of compound 1b of 2b to *p*-toluenesulphonate.

Regression analysis

Regression analysis was used to determine the amount of compound 1b in the concentration range of 0.8–4 μmol/ml using a sample solution in which compound 2b was present in 0.05–2-fold molar excess over compound 1b. The resulting regression equation for compound 1b was $y_1 = 1.013x_1 - 0.14$ (S.D. = 0.21; C.V. =

0.87%; $n = 5$). For compound 2b, which was simultaneously detected, the regression equation was $y_2 = 1.008x_2 - 0.13$ (S.D. = 0.19; C.V. = 2.27%; $n = 5$).

Analytical evaluation

The present method was used to evaluate the purity of the crude product (1b) prepared for clinical study of boron neutron-capture therapy. The amounts of compound 1b in six raw products ranged from 99.2 to 100.2%. Impurities such as compounds 2b, 3b and 4b were not detected.

CONCLUSION

We established a simple and specific method for the determination of compound 1b by using capillary isotachophoresis with sodium *p*-toluenesulphonate as an internal standard. This method is rapid, accurate and suitable for quantitation of the very hygroscopic compound 1a. Use of the method to examine compound 1b showed that it is not oxidizied very rapidly in aqueous solution, which means that compound 1a in such a solution would be available for clinical therapy.

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