Reversed-phase separation of ionic organoborate clusters by high-performance liquid chromatography

Sven Harfst, Detlef Moller, Hartmut Ketz, Jens Rösler, Detlef Gabel*

Department of Chemistry, University of Bremen, P.O. Box 330 440, D-28334 Bremen, Germany

Abstract

Chromatographic separation on reversed-phase materials was carried out for mercaptoundecahydrododecaborate ($B_{12}H_{11}SH^{2-}$) and derivatives with organic residues attached to the sulfur. Solvent and ion-pair systems are described that allow the separation of compounds with greatly different structures. Gradient systems of ion-pair reagents with methanol could be used to separate compounds with greatly different degrees of hydrophobicity. A gradient system was developed in which $B_{12}H_{11}SH^{2-}$-substituted porphyrins and other polar and non-polar porphyrins could be separated.

1. Introduction

The preparation of boron-containing compounds for use in boron neutron capture therapy has received increased interest in recent years [1]. For compounds to be of use in BNCT, they must possess a certain degree of water solubility. This is often achieved through the introduction of solubilizing moieties [2]. Recently, the sulfhydryl-substituted derivative mercaptoundecahydrododecaborate ($B_{12}H_{11}SH^{2-}$) (BSH) of the ionic boron hydride cage $B_{12}H_{12}^{2-}$ has been found to lend itself to substitution chemistry on the sulfur [3]. Although compounds can be prepared readily, analytical separation by chromatography is not trivial, owing to the ionic nature of the boron hydride cage. One of the main problems in this connection is to achieve fast and reproducible reaction control. Simple chromato-

graphic methods such as thin-layer chromatography suffer from bad resolution; further, BSH derivatives are difficult to detect. In contrast, high performance liquid chromatography (HPLC) with ion-pairing reagents allows analysis with good resolution and short analysis times. UV absorption monitoring allows sensitive detection for boron cage derivatives. In addition, HPLC offers easy and practicable purity assays for compounds used in clinical studies (e.g., BSH).

Heteroborane anions have been separated by reversed-phase (RP) ion-pair HPLC on C_1-bonded columns with n-alkylamines as ion-pair reagents [4]. More recently, chromatography of inorganic $B_{12}H_{12}^{2-}$ derivatives on hydroxyethylmethacrylate gels has been described [5]. For preparative chromatography of halogenated boron hydride cages, ion-exchange chromatography has been used [6]. For BSH and its oxidation products, RP-HPLC in the presence of

* Corresponding author.

tetrabutylammonium as ion-pair reagent has been used [7].

We report here that analytical separations of organic derivatives of BSH can be carried out using HPLC in the presence of ion-pair reagents. We have investigated systematically the chromatographic behaviour of many different sulfur-substituted derivatives of $B_{12}H_{11}SH^{2-}$, in order to establish the separation conditions for reaction control and purity assay with maximum resolution and minimum elution times.

2. Experimental

RP chromatography was carried out on a Merck–Hitachi system consisting of an L-6200 pump, a L-4200 UV–Vis detector and a D-2500 chromatointegrator. A Merck LiChrospher RP-18 (5 μm) column (125 × 4 mm I.D.) was used, with a precolumn containing the same material (4 × 4 mm I.D.). The injection loop held 20 μl. The flow-rate was 1 ml/min, unless indicated otherwise. Changes in the flow-rate of the gradient systems lead to shorter run times without loss of resolution. Retention times are given as

k' values. UV detection was carried out at 220 nm [BSH derivatives and BSH possess an absorption maximum at 220 nm ($\varepsilon = 2400$)] or 400 nm (porphyrin derivatives). Methanol and tetrabutylammonium hydrogensulfate (TBAS) used for the mobile phase were of analytical-reagent grade. Water was desalted and doubly distilled. Triethylammonium formate (TEAF) was prepared from triethylamine [purified with aluminium oxide (basic, super 1, ICN)] and formic acid, both of analytical-reagent grade. The solvent systems listed in Table 1 were used. The pH was adjusted to 6.5 with sodium hydroxide.
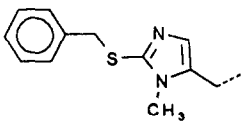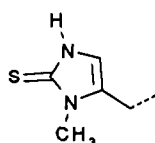
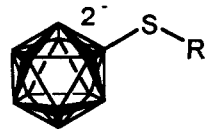The boron compounds used are shown in Fig. 1.

BSH derivatives (Fig. 1a and b) were normally prepared by the following procedure [3]. The tetramethylammonium salt of BSH was converted into the sodium thiolate by titration with an equimolar amount of NaOH in water and recovered by lyophilization. This salt (1 g, 2.9 mmol) was suspending in 250 ml of acetonitrile. A solution of 15 mmol of alkyl bromide in 40 ml of acetonitrile was added through a dropping funnel at room temperature over 10 min. After 24 h the solvent was removed under vacuum.

Table 1
Isocratic and gradient solvent systems used

Isocratic systems

Solvent	Methanol (%)	Water (%)	TBAS (mM)	TEAF (mM)
A	57	43	20	—
B	30	70	—	30

Gradient systems

Gradient	Time interval (min)	Methanol (%)	Water (%)	TEAF (mM)	Flow-rate (ml/min)
1	0 → 1.1	20	80	30	1
	1.1 → 5.1	20 → 60	80 → 40	30 → 15	1
	5.1 → 10.1	60 → 100	40 → 0	15 → 0	2
	10.1 → 15.0	100	0	0	2
2	0 → 5.1	20 → 64	80 → 36	30 → 13.5	2
	5.1 → 30.1	64 → 100	36 → 0	13.5 → 0	1
	30.1 → 40.0	100	0	0	3

a

$B_{12}H_{11}S^{2-}$-R	
R	Designation
H	BSH
Cyanomethyl	S-1
Cyanoethyl	S-2
Allyl	S-3
Benzylmethimazole	S-4
Methimazole	S-5
Acetyl	S-6
Pentenoyl	S-7
Benzoyl	S-8

b

$B_{12}H_{11}S$-R_2	
R	Designation
Cyanomethyl	D-1
Cyanoethyl	D-2
Allyl	D-3
Butenyl	D-4
Pentenyl	D-5
(1,3-Dioxolanyl)-2-propyl	D-6
Cyanopropyl	D-7
3,3-Diethyloxypropyl	D-8
p-Cyanobenzyl	D-9
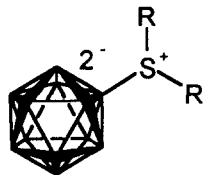
Fig. 1.

C

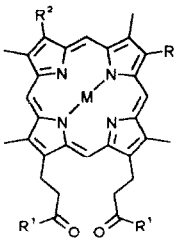| Porphyrins			
R^1 (propionic acid)	R^2	M	Des.
OH	H (deuteroporphyrin IX)	2H	P-1
OMe	H	2H	P-2
B$_{12}$H$_{11}$$^{2-}$-S	H	2H	P-3
OMe	t-butyl-acrylate	Zn	P-4
OMe	acrylate	2H	P-5
OMe	B$_{12}$H$_{11}$$^{2-}$-S-acrylate	2H	P-6
OMe	t-butyl-pentenoate	Zn	P-7
OMe	pentenoate	2H	P-8
OMe	CH=CH-(CH$_2$)$_2$-SB$_{12}$H$_{11}$$^{2-}$	2H	P-9

Fig. 1. Compounds studied.

The residue was suspended in acetonitrile and filtered to remove NaBr. On addition of diethyl ether, the product precipitated.

Boronated porphyrin derivatives (Fig. 1c) were normally prepared by the following procedure. The porphyrin (1 mmol) with free acid side-chains was dissolved in dichloromethane (50 ml). Oxalyl chloride (10 ml) was added and the mixture was refluxed for 45 min. The solvent was evaporated under vacuum. The residue was dissolved in acetonitrile (50 ml) and mixed with dry pyridine (1 ml) and dry tetramethylammonium BSH (1 g, 3.1 mmol, dried at 125°C for 2 h). The mixture was stirred overnight. The solvent was then removed, the residue was dissolved in acetonitrile–water and the counter ion was exchanged against sodium with an ion exchanger (Amberlite IR-120, Na$^+$ form). The solvent was evaporated, the residue was dissolved in a small amount of acetonitrile, filtered off and the solvent removed. The porphyrin was purified by two-step reversed-phase column chromatography with ion-pair reagent (methanol–water between 20:80 and 65:35, triethylammonium formate between 10 and 25 mM) in a flash column (details of preparation and purification will be reported elsewhere). The ion-pair reagent was removed by repeated lyophilization.

Subsequently, the counter ion was exchanged against sodium. The product was dissolved in acetonitrile, filtered and the solvent was evaporated. Reaction control was carried out using the HPLC gradient system 2, taking 10 μl from the reaction mixture dissolved in 100 μl of solvent. Purity assays were carried out by dissolving 1 mg of the pure compound in 250 μl of solvent. A volume of 50 μl of this solution was injected.

For the investigation in Table 2, BSH itself, a sulfonium salt with k' smaller than that of BSH (D-9) and one with k' larger than that of BSH (D-8) were chosen. The solutions of compounds used in Table 2 were prepared from the pure compounds.

Loading experiments were carried out with porphyrin P-3 with five different concentrations (55, 27.5, 14, 10.5 and 7 μg per 500 μl solvent). The retention times at all concentrations varied by only about ±5 s.

3. Results and discussion

A systematic search for optimum separation conditions was carried out. The ratio of methanol to water and the concentrations of the ion-pair reagents TBAS and TEAF were varied and

Table 2
Separation of selected compounds by buffers with various concentrations of methanol and TBAS at pH 7.0

TBAS (mM)	MeOH (%)	k' for compound			Separation of ternary mixture
		BSH	D-8	D-9	
20	60	4.03	5.88	3.37	Yes
	55	7.41	13.17	6.11	Yes
	50	15.24	32.33	12.16	Yes
10	60	2.84	4.54	2.30	Yes
	55	4.46	8.35	3.55	Yes
5	60	2.02	3.31	1.87	No
	55	2.80	5.96	2.65	No

the change in k' for a set of different compounds was recorded.

The values for k' for the three compounds BSH, D-9 and D-8 were greatly influenced by the methanol concentration. Fig. 2 shows that an increase in methanol concentration from 50% to 60% leads to a considerable decrease in the
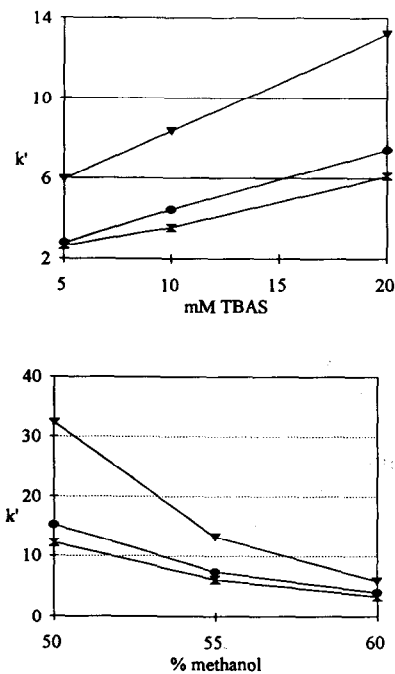

Fig. 2. Dependence of k' values on TBAS concentration (methanol-to-water ratio = 55:45) and methanol-to-water ratio (TBAS concentration = 20 mM). ● = k'(BSH); ▼ = k'(D-8); ✗ = k'(D-9)].

retention times of all compounds. The retention times were also influenced by the concentration of the ion-pair reagent. For TBAS, a nearly linear increase in k' was found between 5 and 20 mM concentrations (Fig. 2). Fig. 3 shows the interdependence of k' values for BSH on methanol and TBAS concentrations. The effects of methanol and TBAS concentrations appear to be additive. No reversal of elution times was found for any of the compounds investigated for any of the concentration combinations of methanol and TBAS. With low concentrations of TBAS, the difference between the k' values decreased. Table 2 summarizes these data. Fig. 4 shows a chromatogram of the reaction mixture of compound D-3.

Also for TEAF there is a strong correlation between methanol concentration and elution time. Again, an increase in methanol concentration reduced the elution times. However, there was a much weaker dependence of the
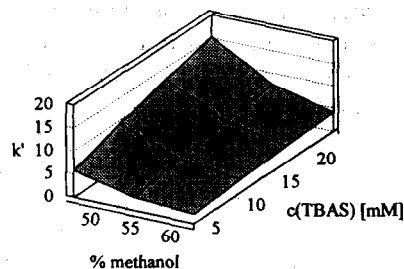

Fig. 3. Dependence of k' value of $B_{12}H_{11}SH^{2-}$ on methanol and TBAS concentrations.
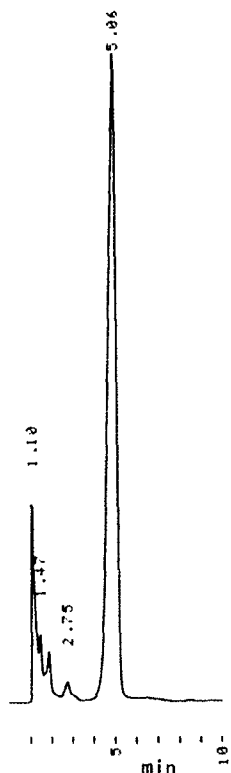
Fig. 4. Chromatogram of the reaction mixture of bisallyl-BSH (D-3). Peaks [retention times (min)]: 1.10 = aceto-nitrile; 1.47 and 1.86 = by-products; 2.75 = BSH; 5.06 = D-3.

Fig. 5. Dependence on k' on methanol-to-water ratio (TEAF concentration = 20 mM) and TEAF concentration (methanol-to-water ratio = 60:40). ● = k'(BSH); ▼ = k'(D-8); ✕ = k'(D-9).

elution time on TEAF concentration between 20 and 60 mM (Fig. 5). For TEAF, much lower k' values were found for all compounds investigated, compared with TBAS as ion-pair reagent. Exceptions are the cyanomethyl and cyanoethyl substituents, where both sulfonium salts showed longer retention times with TEAF than with TBAS.

For a greater number of compounds with similar chemical structures but different carbon side-chain lengths, increased chain length generally led to an increase in k', as shown in Table 3. Despite the fact that sulfonium salts have one more carbon chain than the corresponding thioethers, their k' values did not differ greatly from those of the thioethers (compare, e.g., D-1 with S-1, D-2 with S-2 and D-3 with S-3). This might be because sulfonium salts require only one counter ion for ion-pair formation.
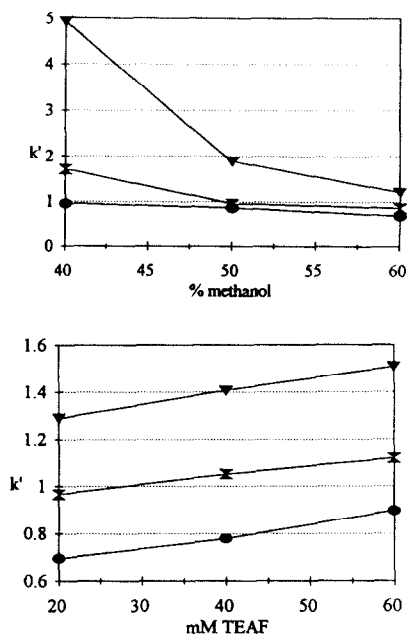
Most of the BSH derivatives could be purified by recrystallization. Only some of the compounds were difficult to purify without chromatographic methods (e.g. S-4 and porphyrins). These compound mixtures contained components that varied greatly in the degree of hydrophobicity. In these cases isocratic elution led to unacceptably long elution times. Therefore, two gradient systems were developed for these separation problems. TEAF was used as ion-pair reagent because it can be removed by lyophilization. Thus, later use of this solvent in preparative chromatography is possible.

Gradient 1 is capable of resolving the highly polar S-5 (k' = 2.56) from the S-benzylated derivative S-4 (k' = 11.71) and toluene (k' = 20.86). In this system, BSH has a k' value of 1.03, close to the void volume. Gradient 2 allows the separation of porphyrins. With the high water concentrations necessary when using TEAF, some poorly water-soluble salts, such as the tetramethylammonoum salts of boronated porphyrins, may present problems. In such

Table 3
Retention times of different compounds in (A) MeOH–water (57:43), 10 mM in TBAS (pH 6.5) and (B) MeOH–water (30:70), 30 mM in TEAF (pH 6.5)

Substituent	Number of substituents	Designation	k'	
			Solvent A	Solvent B
H	1	BSH	2.51	0.80
Cyanomethyl	1	S-1	2.83	2.25
Cyanomethyl	2	D-1	2.05	3.20
Cyanoethyl	1	S-2	3.12	1.42
Cyanoethyl	2	D-2	2.12	3.44
Cyanopropyl	2	D-7	2.31	n.d.[a]
Allyl	1	S-3	12.44	n.d.
Allyl	2	D-3	10.80	n.d.
Pentenyl	2	D-5	34.19	n.d.
Butenyl	2	D-4	19.46	n.d.
(1,3-Dioxolanyl)-2-propyl	2	D-6	4.07	n.d.
Acetyl	1	S-6	3.12	1.24
Pentenoyl	1	S-7	7.92	n.d.
Benzoyl	1	S-8	9.92	7.34

[a] n.d. = Not determined.

cases, exchanging the counter ion to Na^+ and thereby enhancing the water solubility proved to be helpful. As can be seen in Table 4, porphyrins with greatly different substituents could be separated successfully. Fig. 6 shows the analytical separation of porphyrin P-6 from its monoboronated derivatives in the reaction mixture. Loading experiments for porphyrin P-3 showed no dependence of k' on sample concentration. The boronated porphyrins P-3 and P-6 must be considered very hydrophilic, based on their short retention times.

HPLC in the presence of ion-pair reagents is capable of separating ionic borates with greatly different degrees of hydrophobicity. The best separations for most of the BSH derivatives are achieved with methanol–water mixtures around 57:43 and tetrabutylammonium concentrations

Table 4
Separation of 3,8-substituted porphyrins using gradient system 2

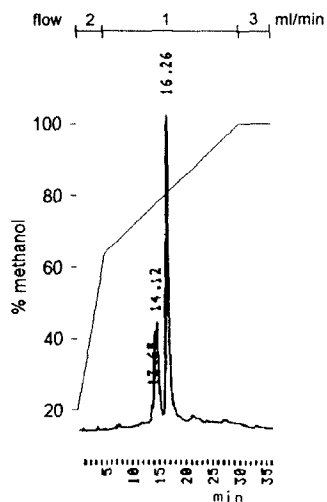| Designation	R at 3- and 8-positions	M	R at propionic acid	k'
P-1	H	2H	OH	35.96
P-2	H	2H	OMe	57.66
P-2	H	Zn	OMe	50.37
P-3	H	2H	$SB_{12}H_{11}^{2-}$	28.84
P-4	CH = CHCOOBu^t	Zn	OMe	62.35
P-5	CH = CHCOOH	2H	OMe	32.23
P-6	CH = CHCOSB$_{12}$H$_{11}^{2-}$	2H	OMe	25.62
P-7	CH = CH(CH$_2$)$_2$COOBu^t	Zn	OMe	57.66/59.68[a]
P-8	CH = CH(CH$_2$)$_2$COOH	2H	OMe	42.40/43.76[a]
P-9	CH = CH(CH$_2$)$_2$SB$_{12}$H$_{11}^{2-}$	2H	OMe	35.96/39.01[a]

[a] cis–trans Isomers.

Fig. 6. Chromatogram of the reaction mixture of porphyrin P-6. Peaks [retention times (min)]: 13.65 and 14.12 = mono boronated porphyrins; 16.26 = P-6.

around 10 mM. The elution times depend very critically on the methanol-to-water ratio, as reported previously for C_1 phases and laurylamine as ion-pair reagent [4]. For the TBAS system described here, a decrease in methanol concentration of 2–3% led to a doubling of the retention time. For TEAF, an increase of the methanol concentration from 30 to 40% led to about 50% shorter retention times. Whereas laurylamine was required for the C_1 phase at concentrations around 2 mM [4], on the C_{18} phase used here higher concentrations of 10 mM TBAS were found to be necessary for adequate separation.

The separation of ionic borates has been achieved previously by ion-exchange chromatography [6]. Recovery of the compounds was achieved by eluting slices of the extruding gel. In contrast, HPLC can be carried out also under analytical conditions, and with greatly enhanced speed.

Acknowledgements

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