

Note

Chromatography of carborane monoanions on silica

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The application of chromatographic methods to the separation and purification of boron-containing compounds is not without precedent. Particularly relevant are reports of complementary thin-layer chromatographic (TLC) separations of polyhedral borane anions by Wellum *et al.*¹ and of high-pressure liquid chromatography (HPLC) of metallocarboranes by Evans and Hawthorne². Our recent efforts in the synthesis of new carborane monoanions capable of conjugation to monoclonal antibody for animal and patient studies in one approach to a neutron-capture therapy of cancer³ has prompted our investigation into their purification. Until now, the purification of simple carborane monoanions by recrystallization has sufficed⁴. Chemical modification of side chain functionalities on these monoanions can result, however, in the formation of impurities which co-crystallize with the desired compound, and/or the formation of impure and syrupy monoanions which crystallize only with difficulty, if at all. Moreover, when repeated recrystallizations are necessary, up to 10% of the monoanion is sacrificed with each recrystallization. Herein we wish to describe our initial application of TLC and medium-pressure liquid chromatography (MPLC) on silica to the separation and purification of carborane monoanions⁵.

EXPERIMENTAL

Analytical TLC was performed on glass plates which were precoated with silica gel 60 F-254 (0.25 mm thickness, 20 × 20 cm) as obtained from Merck and cut to 2.0 × 6.6 cm. R_f values represent a mobility relative to the solvent phase and were measured on analytical plates with an average standard deviation of ± 0.01 . TLC spots were visualized by dipping the plates, from which the solvent had been evaporated, in an aqueous solution of silver nitrate followed by towel drying. Gentle heating of the plate facilitated visualization of the spot in some cases but was usually unnecessary. All column chromatography was performed on Merck Kieselgel 60 (40–63 μm) which was vibrated dry into Altex glass columns with either isocratic or gradient elution of the solvent using Altex Model 110A pumps interfaced with an Altex Model 420 programmer at 50–150 p.s.i. Peak detection was made possible by the use of a Hitachi Model 100-40 variable-wavelength UV-VIS spectrophotometer which is equipped with an Altex Model 155-00 flow cell. Sample fractionation was

aided by the use of an LKB-7000 UltraRac fraction collector. In general, 8-ml fractions were collected in 100×13 mm disposable tubes. Ethyl acetate, acetonitrile, and methanol were ChromAr grade as supplied by Mallinckrodt.

RESULTS AND DISCUSSION

As relatively polar molecules, carborane monoanions require the use of relatively polar solvent systems to move on silica. For several reasons we wanted to avoid the use of aqueous ionic solvent systems and have found that mixtures of methanol (1–20%) or acetonitrile (1–5%) in ethyl acetate will provide excellent and reproducible separations of carborane monoanions on silica. Our preliminary observations indicate, however, that the binding capacity of silica for these compounds is significantly less than for the corresponding undegraded cages, resulting in streaking or tailing on the plate or column of silica if too much monoanion is applied. Up to 3 mg of monoanion per gram of silica has been applied, however, to our columns for preparative separations.

In the case of the pyranosyl monoanion (entry 13), not only was separation from the faster-moving borate impurity possible, but anomeric purification in the sugar residue was achieved with the resulting isolation of pure α -anomer. The corresponding β -anomer, originally formed as the minor component of a 9:1 anomeric mixture in a $\text{BF}_3 \cdot \text{OEt}_2$ -catalyzed O-glycosylation⁶, was identified by ^1H NMR in the later fractions of the peak recorded at 254 nm (Fig. 1). Furthermore, the tetramethylammonium (TMA) counterion was not lost or exchanged in the chromatographic purification as evidenced by both ^1H NMR and combustion analysis, suggesting that surface interactions on silica do not perturb the nature of this tight-ion pair.

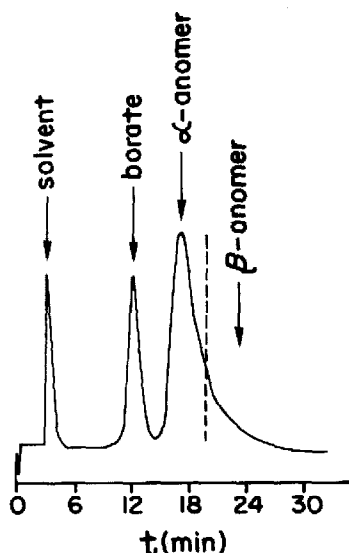


Fig. 1. Chromatographic purification of 1-(2,3-dideoxy- α -D-erythro-hex-2-enopyranosyl)-methyl-1,2-dicarbadoecahydroundecaborate (1-) ion, tetramethylammonium salt. Solvent: methanol-ethylacetate (5:95). Chart speed: 10 cm/h. Flow-rate: 7 ml/min. Detector: UV (254 nm). Column: 250×15 mm.

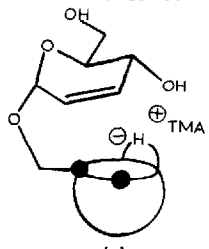
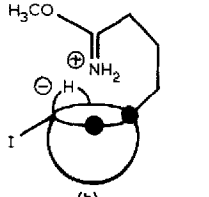
Upon examination of the data presented in Table I, we find that R_F values for a monoanion do not vary to an appreciable extent with variations in the counterion. For $M^+C_2B_9H_{11}Ph^-$, the larger tetraethylammonium (TEA) complex appears to be only slightly slower than both the potassium and TMA complexes. With respect to cage isomers, the R_F values of *ortho*- and *meta*- $C_2B_9H_{12}^-M^+$ suggest that the *meta*-isomer is slightly more polar than the corresponding *ortho*-isomer, a fact which is not inconsistent with theoretical considerations. Additionally, bis-carborane monoanion (entry 11) was found to be slower-moving than mono-carborane monoanion (entry 6) and the R_F value of bis-carborane bisanion (entry 12) was about 20% less than that of bis-carborane monoanion.

In the monoiodination of phenylcarborane monoanion^{3,7}, unreacted starting material was detected as the faster-moving component of a mixture on a 500×6

TABLE I

 R_F VALUES OF CARBORANE ANIONS

Solvent A: acetonitrile-ethyl acetate (5:95), solvent B: methanol-ethyl acetate (5:95).

Entry	Sample	Solvent A	Solvent B
1	$K^+C_2B_9H_{11}Ph^-$	0.33	0.23
2	$TMA^+C_2B_9H_{11}Ph^-$	0.33	0.23
3	$TEA^+C_2B_9H_{11}Ph^-$	0.31	0.21
4	$TMA^+C_2B_9H_{10}IPh^-$	0.30	0.20
5	<i>ortho</i> - $K^+C_2B_9H_{12}^-$	0.36	0.27
6	<i>ortho</i> - $TMA^+C_2B_9H_{12}^-$	0.36	0.27
7	<i>meta</i> - $TMA^+C_2B_9H_{12}^-$	0.33	0.23
8	<i>meta</i> - $Cs^+C_2B_9H_{12}^-$	0.33	0.23
9	$TMA^+C_2B_9H_{11}C_6H_4-p-NH_2^-$	0.28	0.17*
10	$Na^+C_2B_9H_{11}C_6H_4-p-CO_2H^-$	0.62**	0.27***
11	$TMA^+C_2B_9H_{11}C_2B_{10}H_{11}^-$	0.27	0.20
12	$TMA^{2+}C_4B_{18}H_{22}^{2-}$	0.22	0.18
13		0.24	0.15 [§]
14		0.30	0.20

* In its preparation³ impurities were seen with R_F 0.02, 0.22 and 0.30.

** Solvent: methanol-ethyl acetate (20:80).

*** Solvent: methanol-ethyl acetate (10:90).

[§] Impurities were seen with R_F 0.02 and 0.27.

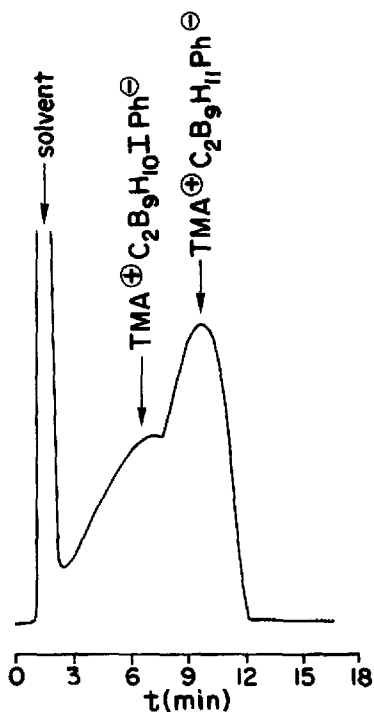


Fig. 2. Chromatographic detection of phenylcarborane monoanion in the presence of iodinated species. Solvent: methanol-ethylacetate (5:95). Chart speed: 20 cm/h. Flow-rate: 5 ml/min. Detector: UV (254 nm). Column: 6 × 500 mm.

mm analytical column (Fig. 2), whereas the corresponding thin-layer chromatogram was inconclusive. In independent runs, the iodinated monoanion had a retention time of 8.9 min as compared to 10.5 min for the parent monoanion. Chromatography of this and other iodinated monoanions (entry 14) indicate that the B-I bond is stable to these chromatographic conditions as evidenced by TLC, ^{11}B NMR, and combustion analysis.

In conclusion we have found that TLC can be very useful in the detection of impurities and/or side products in reactions of carborane monoanions and that MPLC can be an inexpensive and yet valuable aid in the separation and purification of these compounds without the loss or exchange of counterion. The application of HPLC both in normal and reversed-phase will be the subject of future investigations.

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