ELSEVIER

JOURNAL OF CHROMATOGRAPHY A

Journal of Chromatography A, 685 (1994) 360–364

Short communication

Resolution of amino acid racemates on borate-gelled guaran-impregnated silica gel thin-layer chromatographic plates

V. Mathur, N. Kanoongo, R. Mathur, C.K. Narang, N.K. Mathur*

Department of Chemistry, JNV University, Jodhpur-342003, India

First received 17 November 1992; revised manuscript received 28 June 1994

Abstract

Borate-gelled guaran-impregnated silica gel TLC plates act as a very good chiral chromatographic media. Tetracoordinated boron is complexed to the *cis*-OH pairs in guaran polysaccharide, creating a chiral environment around boron. The complexed boron can undergo a ligand-exchange reaction or stereoselective dual H-bonding to a bis-oxygen or N,O-ligand, such as conformationally favourable glycol, α-hydroxy acids and α-amino acids. These reactions, due to chiral selectivity, result in a difference in the migration rates of the enantiomers of a racemic mixture on a TLC plate. This provided a simple, rapid and effective method for the resolution of α-amino acid racemates.

1. Introduction

A chiral chromatographic sorbent can recognize molecular chirality of a low-molecular-mass solute owing to the interactions such as host–guest interactions [1], chiral metal complex–ligand exchange [2,3], charge transfer [4] and even weak, but sterically selective, hydrogen bonding association [5]. We have observed certain types of chiral discriminating properties in galactomannan (guaran) polysaccharide [6] which do not occur with anhydroglucose polymers, e.g., starch and cellulose. This has been attributed to the sterically selective dual bonding (or a two-point contact), due to the presence of a pair of *cis*-OH groups in a locked-in conformation of the pyran ring of the hexoses, i.e., galactose and mannose residues of the polysaccharide. The chiral selectivity of galactomannan is further enhanced by introducing an additional chiral stationary selector on the guaran polymer matrix or by carrying out a ligand-exchange reaction of a polymer-bound tetracoordinated borate group with ligands such as chiral 1,2-diol or α-hydroxy acid [7].

1,2-Dihydroxy ligands, α-amino acids and α-amino alcohols can also form borate complexes. We have considered the possibility of developing a rapid TLC method for the separation of amino acid racemates, based on the interaction of amino acids with tetracoordinated borate immobilized on guaran gel. In this paper, we report the effective separation of several amino acid racemates on silica gel impregnated with borate-gelled guaran.

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00731-4

2. Experimental

Guaran (food grade, with a viscosity of 3800 cP at 1.0% concentration as measured with a Brookfield LVT viscometer spindle No. 3 at 20 rpm) was purified as described earlier [8]. All other reagents were high-purity commercial products and were used as received.

2.1. Preparation of borated guaran-impregnated silica gel TLC plates

A 1.0-g amount of guaran was dissolved in 100 ml of distilled water and 50 g of TLC-grade silica gel was slurried into it using a Waring blender at 3500 rpm for 2 min. This was followed by addition of 0.2 g of sodium tetraborate dissolved in 100 ml of water and the mixture was again blended for 2 min at 3500 rpm. The resulting slurry was coated (0.5 mm) on TLC glass plates (20 × 5 cm) using a Stahl-type applicator. The plates were air dried and stored. For resolution studies, a solution of amino acid racemate (0.001 M, 25 μl) was applied on the TLC plate with a Hamilton syringe along with an equal amount of individual isomers of the same amino acid. For good resolution, the spot diameter was kept to about 2 mm.

The chromatographs were developed in glass jars of suitable size containing 30–40 ml of solvent and with the chamber saturated at 25°C.

As we employed laboratory-made TLC plates, the nature of TLC-grade silica gel and guaran (natural product; the commercially available form can have different molecular masses and viscosities) employed are likely to affect the resolution. Similarly, the reversible borate complexing of guaran and amino acid is pH dependent (optimum pH range 7–8) and at more alkaline pH (ca. >9) the exchange reaction becomes sluggish.

The chromatograms were developed using suitable solvent systems (Table 1) and, after drying, the chromatograms were developed by spraying with a 0.1% ethanolic ninhydrin solution followed by heating for 5 minutes at 90°C. In most instances two distinct and sharp spots corresponding to the D- and L-isomers were clearly visible on the TLC plate. These were identified by measuring and comparing the R_F values with those of co-chromatographed D- and L-isomers (Figure 1).

3. Results and discussion

Two extreme views have been expressed about the mechanism of gelling of galactomannans by

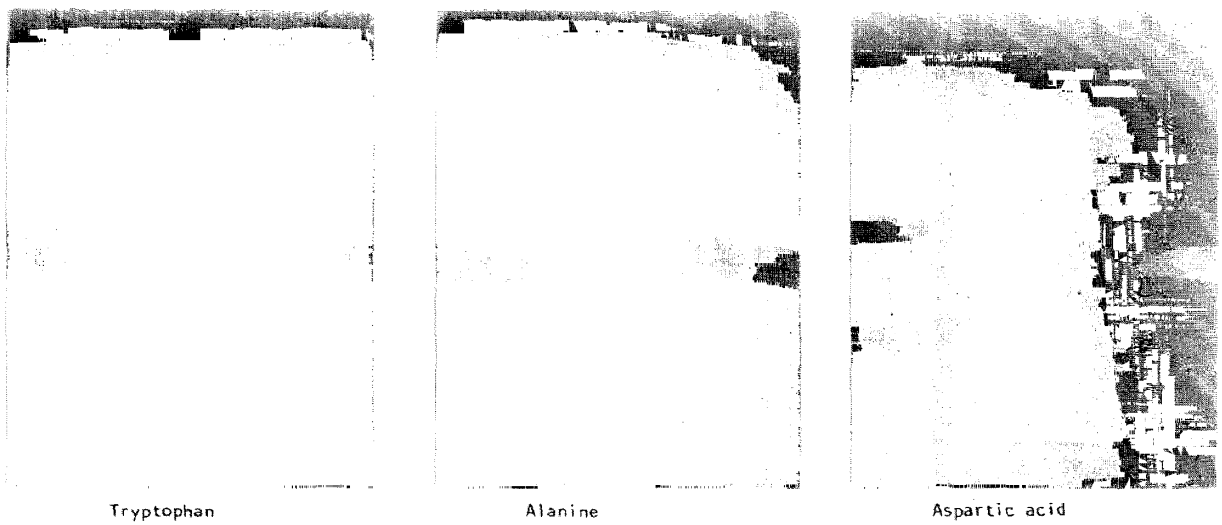

Fig. 1. Typical thin-layer chromatograms showing resolution of amino acid racemates.

borates [9]. According to one of the views, borate forms a bis-diol complex (Fig. 2, Structure 1a) due to the presence of pairs of *cis*-OH groups on the hexose units of the adjacent polymer chains resulting in a pH-dependent cross-linking. According to the other view, the cross-linking results solely due to H-bonding of the tetrahydroxy borate anion with the *cis*-OH groups on the adjacent polymer chains (Fig. 2, Structure 1b) [10]. As the borate gelling of guaran is always accompanied by the liberation of acid, the latter view cannot be considered to be correct. Also, in aqueous medium, there is a predominant possibility of forming a 1:1 diol–borate complex [11,12]. Hence the gelling of galactomannan by borate is best explained as being due to the formation of 1:1 complexes followed by H-bonding to the adjacent polymer chain (Fig. 2, Structure 1c). With this model, we can make a reasonable hypothesis for the chiral discrimination of 1,2-diol and α-amino acid enantiomers by borate-gelled guaran.

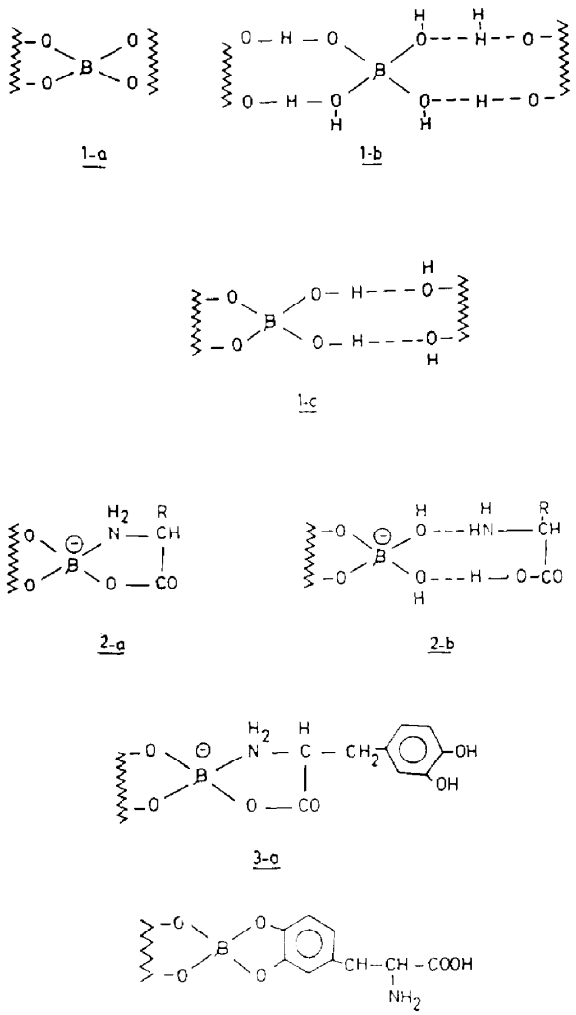

Fig. 2. Modes of interaction of borate and guaran analytes. 1a–1c: Possible modes of complexing of borate with guaran. 2a,2b: Possible interactions of guaran borate complex with amino acids. 3a,3b: Possible interactions of DOPA with guaran–borate complex.

The polymeric borate–guaran diol (1:1) complex interacts steroselectively with one of the amino acid enantiomers to form a mixed bis-complex (Fig. 2, Structure 2a), resulting in the sorption of an amino acid isomer from the mobile phase, the mobility of which is decreased (lower R_F value). Alternatively, the free hydroxyl groups [$B(OH)_2$] in the 1:1 complex interact steroselectively with one of the enantiomers by H-bonding [7] (Fig. 2, Structure b). It is expected that in the former instance, the interaction, which amounts to a ligand-exchange reaction, will have a higher stereoselectivity, but in a counter-current process even the weak stereoselectivity in H-bonding should be sufficient to resolve the enantiomers.

Our contention that bonding due to *cis*-OH groups plays an important role in chiral selectivity is further supported by the fact that even the low-molecular-mass disaccharide lactose (containing a *cis*-OH pair in the galactose half) has been shown to be effective in the separation of certain racemates [13], although no explanation was given.

Table 1 shows representative α-amino acids which have been resolved on borated guaran-impregnated silica gel. The R_F values of the D- and L-isomers are given, along with the solvent systems. It can be observed that the overall difference between the R_F values of D- and L-isomers is sufficient to make a clear distinction between the two enantiomers. However, there is no common solvent system applicable to most of the amino acids. This is to be expected because of the difference in the polarity of various amino acids. There is no common trend for the R_F values of D- and L-isomers, although a decreased

Table 1
Amino acids resolved by TLC on borated guaran-impregnated silica gel

DL-Amino acid	Solvent system[a]	R_F values of isomers[b]	
		L-	D-
Alanine	B	0.60	0.41
Valine	B	0.42	0.61
Leucine	A	0.25	0.44
Isoleucine	A	0.25	0.43
Phenylalanine	C	0.44	0.65
Tyrosine	C	0.41	0.62
DOPA	C	0.38	0.60
Tryptophane	C	0.41	0.61
Serine	C	0.23	0.44
Threonine	C	0.25	0.46
Cystine	A	0.33	0.46
Aspartic acid	C	0.61	0.80
Glutamic acid	C	0.64	0.82
Proline	A	0.62	0.29

[a] Solvent systems: (A) 2-propanol–water (7:3); (B) phenol–water (4:1) [15]; (C) butanol–acetic acid–water (3:1:1). Elution time: 30–45 min.

[b] The R_F values at 25°C are averages of four determinations, the standard deviation being 0.02; 25 μl of 0.001 M amino acid solution were spotted in each case.

retention (higher R_F value) has been observed for ten out of thirteen D-isomers studied. (Gupta [14] successfully achieved similar resolution, but with different R_F values.)

A unique separation on borate-gelled guaran is that of DL-DOPA enantiomers, which have a pair of catechol-type OH groups, in addition to the α-aminocarboxylic group common to natural amino acids. Here the interaction of the polymer-coordinated boron and DOPA can be either via aminocarboxylic functionalities (Fig. 2, Structure 3a) or via catchol-type 1,2-OH group pairs (Fig. 2, Structure 3b). In the latter instance, the chiral centre (α-C) of the amino acid is somewhat distant from the boron atom, which cause stereoselectivity, in acquiring chirality. Hence the overall "fit" of one of the enantiomers in the coordination sphere of tetrahedral boron must play an important role in stereoselectivity. No resolution was observed when silica gel was impregnated with guaran alone.

As there are a large number of biologically active molecules and synthetic drugs that have oxygen and/or nitrogen functional groups suitably juxtapositioned to interact with the chiral guaran–borate complex, we expect this system to be applicable to the resolution of a variety of racemates, and work on these lines is in progress.

4. Conclusions

Borated guaran-impregnated silica provides a chiral chromatographic medium for the resolution of not only α-amino acids, but also a large number of bis-oxygen or N,O-ligands such as glycols, α-hydroxy acids and α-amino alcohols which can interact with borate ion. As the chiral galactomannan also forms borate complexes, stereoselectivity is observed in either the formation of mixed bis-borate complex by a ligand-exchange reaction, or H-bonding of the solute to coordinated borate, resulting in enantiometer separation.

Acknowledgements

N.K. and V.M. are grateful to the CSIR and UGC India, respectively, for financial assistance.

References

[1] S.G. Allenmark, *Chromatographic Enantioseparation: Methods and Applications*, Ellis Horwood, Chichester, 1988.
[2] R.C. Helgeson, K. Koga, J.M. Timko and D.J. Cram, *J. Am. Chem. Soc.*, 95 (1973) 3021, and subsequent papers.
[3] S.V. Rogozhin and V.A. Davankov, *Chem. Commun.* (1971) 490.
[4] Y.H. Kim, A Tishbee and E. Gil-Av, *J. Am. Chem. Soc.*, 102 (1980) 5915.
[5] R. Charles, V. Beitler, B. Feibuch and E. Gil-Av, *J. Chromatogr.*, 112 (1975) 121.
[6] R. Mathur, S. Bohra, C.K. Narang and N.K. Mathur, *J. Liq. Chromatogr.* 15 (1992) 573.
[7] R. Mathur, S. Bohra, V. Mathur, C.K. Narang and N.K. Mathur, *Chromatographia*, 33 (1992) 336.
[8] K.C. Gupta, M.K. Sahani, B.S. Rathore, C.K. Narang and N.K. Mathur, *J. Chromatogr.*, 177 (1979) 218.
[9] H. Deuel, H. Nevkom and P. Weber, *Nature*, 161 (1948) 96.

[10] R.J. Chydzikowski, *J. Soc. Cosmet. Chem.*, 22 (1971) 43.
[11] R.F. Nickerson, *J. Inorg. Nucl. Chem.*, 32 (1970) 1401.
[12] K. Kustin and R. Piezer, *J. Am. Chem. Soc.*, 91 (1969) 317.
[13] E. Gil-Av, B. Feiburt and R. Charles-Sigier, *Tetrahedron Lett.*, (1966) 1009.
[14] K.C. Gupta, CSIR Center for Biochemical, Delhi, personal communication.
[15] E. Von Arx and R. Neher, *J. Chromatogr.*, 12 (1963) 329.