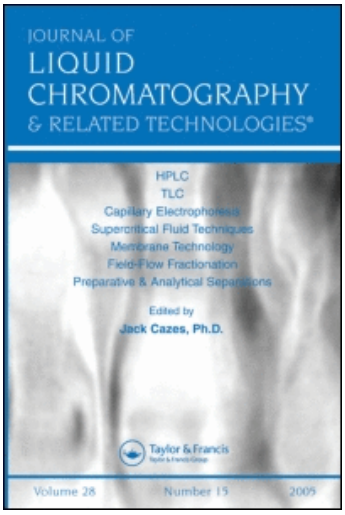

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Guaran: A Novel Polysaccharide for Racemate Resolution

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GUARAN: A NOVEL POLYSACCHARIDE FOR RACEMATE RESOLUTION

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ABSTRACT

The polysaccharide, guaran, as a chromatographic media has been shown to exhibit weak chiral discrimination for the enantiomers in a racemate. On covalent binding of quinine to the polysaccharide, the chiral selectivity of guaran is further enhanced. This paper describes the method for covalent binding of quinine as a chiral selector on guaran back bone. Successful application of this new chiral media has been made for effective resolution of racemic mandelic acid and cotton seed pigment, gossypol. Probable mechanism for the chiral selectivity of underivatized and derivatized guaran, has been suggested.

INTRODUCTION

Chromatographic separation of enantiomers can be achieved either (i) by pre-column derivatization of a racemate with a chiral derivatization reagent, followed by separation of the covalent diastereoisomers thus formed on achiral adsorbents, or (ii) by direct separation of the enantiomers on a chiral adsorbent [1]. The later method has many advantages, but a universal chiral stationary phase (CSP) capable of resolution of a large variety of structurally different racemates is still elusive. Some chiral adsorbent pre-packed HPLC columns are now commercially available, which are claimed to have chiral discrimination behaviour towards a large variety of racemates [2]. Some chiral adsorbents have been used in preparative liquid chromatography. These are based on either a natural chiral polymer, e.g., a polysaccharide such as cellulose [3], or on a purely synthetic polymer prepared from a chiral monomer [4], or by binding a chiral selector to a preformed achiral polymer [5].

Among the chiral polymers, microcrystalline cellulose (MCC) has recently gained favour due to its broad discriminating power for a variety of enantiomers. Cellulose, because of its β-(1 → 4) linkage between anhydroglucose repeat units, and all

equatorial - OH groups, has some crystalline regions, the amount of which is further increased by controlled hydrolysis resulting into MCC. Chiral discrimination of MCC has been attributed to the presence of chiral cavities (lined with chiral groups) in its crystalline regions [6]. Amylose, with its helical structure, also shows some chiral discrimination by preferential inclusion into the helices of one of the isomers in a racemate, but overall it has not found much favour as a chromatographic adsorbent.

In this communication, we report the first ever application of a galactomannan (guaran or guar gum, a polysaccharide from the legume seed, Cyamopsis tetragonolobus) as an adsorbent for enantioseparation.

We have earlier reported the use of cross-linked guaran as gel-filtration media for biopolymers and also for the synthesis and application of gel type ion-exchangers-based guaran [7].

EXPERIMENTAL

Materials and chemicals: Guar gum (200 mesh) was procured from a local manufacturer, and was purified by thorough washing with methanol. All other chemicals

used were of high purity analytical grade and were used as such.

Guaran was derivatised to contain quinine in the form of its quaternary salt. Derivatization was achieved in two steps. The first step involved quaternisation of quinuclidine nitrogen of quinine, using a bifunctional reagent, epichlorohydrin. The quaternised quinine in the second step was immobilised on to the guaran matrix.

Quaternisation of quinine: 19.5 g (0.025 mole) of quinine sulphate was converted into its more soluble salt, quinine hydrochloride by treatment with an equivalent of barium chloride dissolved in 300 ml of water. The solution of quinine hydrochloride was filtered, to which 4.5 g (0.05 mole) of epichlorohydrin was added. The reaction mixture was stirred on a magnetic stirrer at 35^{o} for 4 h when the chlorohydrin (I) was formed. This was followed by addition of 2.0 g (0.05 mole) sodium hydroxide as its 50% aqueous solution. This resulted in the formation of an insoluble quininium salt with epoxide ring (II).

Immobilisation of quaternised quinine: In a 500 ml. R.B. flask, 35.5 g of guaran was suspended in 80 ml of dioxan . 1.0 g of sodium hydroxide as 50% aqueous

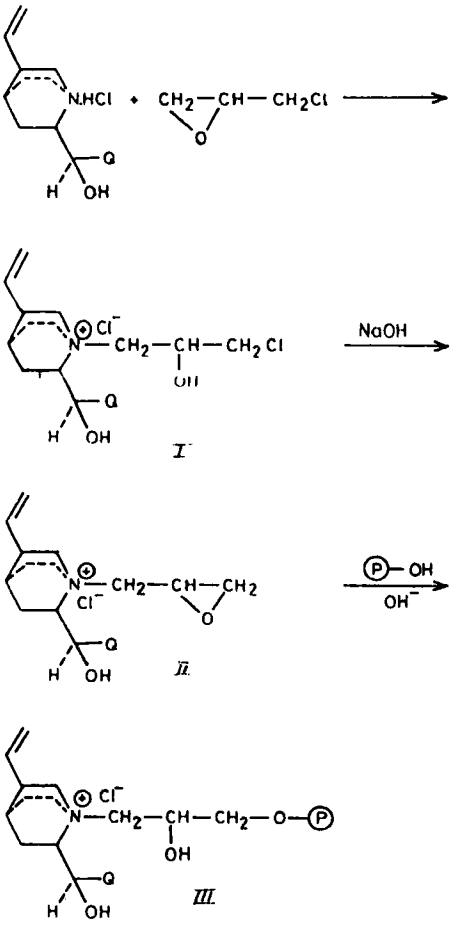

FIG. 1 - COVALENT BINDING OF QUININE TO POLYSACCHARIDE THROUGH QUATERNIZATION

solution, was added followed by addition of 0.3 g of tetrabutylammonium bromide to act as a phase transfer catalyst [8]. The quaternised quinine (II) dissolved in 100 ml of dioxan was then added with constant stirring on a magnetic stirrer, at 60° C, for 6h. The product (III) was filtered under vacuum and washed, first with 2x50 ml of 80% aqueous methanol containing little acetic acid. It was then washed thoroughly with pure methanol to remove unreacted low molecular weight compounds and dried in an oven at 60° C. A light yellow, free flowing powder was obtained.

The product was characterised by ir spectroscopy, and showed absorption at 1620, 1590, 1205 and 1200 cm^{-1}, characteristic of quinine molecule while absorption at 1480 cm^{-1} due to C-N linkage and at 2300 cm^{-1} due to quaternary salt, were also observed. Quinine content of the product was found by ionic chloride determination by Volhard's method. The degree of substitution of quinine in the product was found to be 0.28.

Chromatographic resolution of mandelic acid. 8.0 g of the above quaternary derivatised guaran was allowed to swell for 2 h in 50 ml of solvent mixture containing toluene and methanol (95:5). The slurry was then packed into a glass column (1x55 cm size), and flow

rate was adjusted at 1.5 ml/min. 100 mg of (±) mandelic acid was taken in 5 ml of the eluent and applied to the column. After equilibration of the solute, 2 ml fractions of eluate were collected. Absorbance of the fractions was determined at 220 nm corresponding to λmax of mandelic acid. When all the solute had eluted out, a graph was plotted between absorbance and eluate volume. This showed two identical peaks (Fig. 2 a). Fractions corresponding to the two peaks were pooled separately and used for optical rotation measurement. The solvent was removed on a rotavapour and the residue was redissolved in water. Specific rotation $[\alpha]^{25}$ D of the two enantiomers was: + 123° (Fig. 2 a, A) and -118° (Fig. 2 a, B) (c,0.16, H_2O).

Racemic mandelic acid was similarly attempted for resolution on underivatised guaran (Fig. 2 b).

Resolution of ± gossypol. The cotton seed pigment gossypol is a bisnapthyl derivative and owes its optical activity to restricted rotation on the pivot bond [9]. Because of its activity as a male fertility control agent, several attempts have been made for its resolution, based on its conversion into covalent diastereoisomers [10]. In the present investigation, the resolution has been carried without any pre-column derivatization.

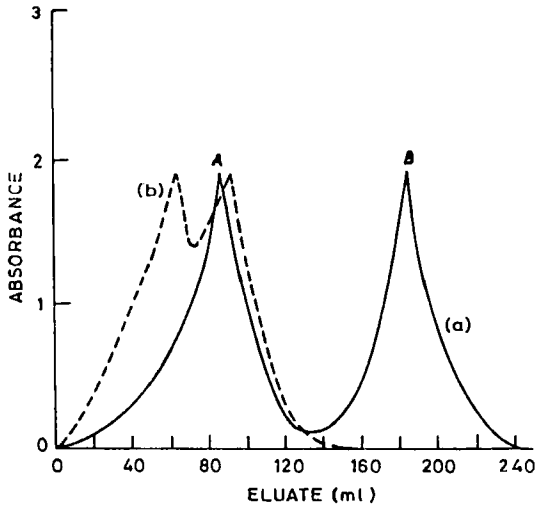

FIG. 2 - CHROMATOGRAPHY OF 100 mg OF (±) MANDELIC ACID ON

(a) 8.0 g QUININIUM LOADED GUARAN

(b) GUARAN

10 mg of (±) gossypol was resolved on guaran containing quaternary quininium salt, using the method similar to one used for mandelic acid. The eluant used was a mixture of toluene: methanol (90:10) and absorbance of the eluate was measured at 370 nm (λmax for gossypol). A graph was plotted between absorbance and eluate volume (Fig. 3). Specific rotation of the

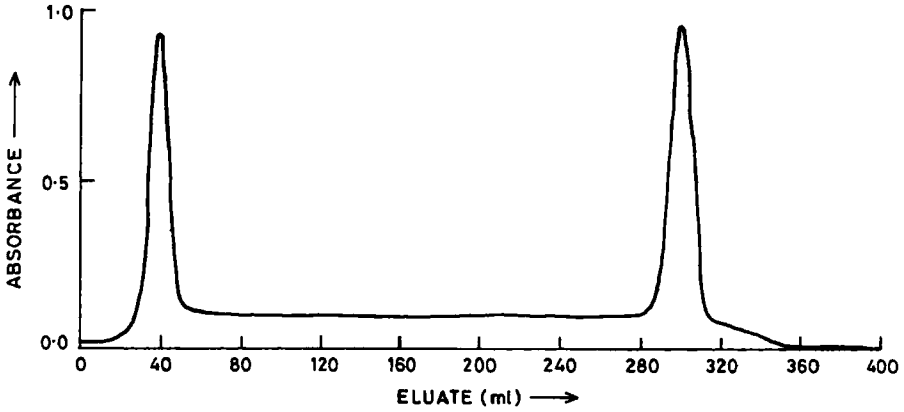

FIG. 3 - CHROMATOGRAPHY OF GOSSYPOL

two enantiomers were found to be ($+373^{\circ}$ and -370° c, 0.15, $CHCl_3$).

RESULTS AND DISCUSSION

Proper understanding of chiral recognition mechanism by polysaccharide adsorbents is still lacking. Polysaccharide guaran consists of a β-(1 → 4) linked mannose back-bone, which like cellulose has a rigid rod like structure, yet it is noncrystalline because of the numerous single galactose grafts preventing strong interchain H-bonding [11]. This leaves large voids in cross-linked guaran gels, and it acts as a gel permeation media [7]. However, both monomeric units in guaran, i.e. mannose and galactose,

differ from glucose polymers in having a pair of cis-OH groups on each anhydrohexose unit. This confers the property of strong, dual H-bonding interaction between guaran and other molecules, including suspended solids having surface hydroxyl groups. This property has beeen extensively used for industrial utilization of galactomannan polymer in mineral beneficiation [12], effluent treatment [11] and controlled release of bioactive molecules [13]. We considered that the cis hydroxyl groups in galactose and mannose units in these polymers can readily form a two point contact, preferentially with one of the enantiomers in a racemate. This results in a weak chiral discrimination. The hypothesis that sterically selective hydrogen bonding is mainly responsible for the resolving power of the proposed chiral chromatographic media, is further supported by the fact that the solvent system used favours strongly the hydrogen bonding, and that the separation of gossypol, a non-acidic, polyphenolic compound, was also carried effectively.

It has been found that introduction of additional chiral selector, i.e., quinine moieties, increases the resolving power of the medium (Fig. 2 a). The separation factor for resolution of mandelic acid on quinine-derivatized guaran was found to be 2.6 against

1.3 for underivatized guaran. The factors likely involved in the enhanced resolving power, may be greater hydrophobicity and π - bonding interactions.

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