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Note

Self-amplification of optical activity by chromatography on an achiral adsorbent

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Recently[1], it was reported that ^{14}C-labelled nicotine was resolved into its enantiomers by high-performance liquid chromatography on Partisil ODS and SCX, when coinjected with optically active nicotine. Independently of this report, we have studied the self-amplification of optical isomers on achiral adsorbents, and wish to communicate herewith some of our results.

On isolation on silica gel of an optically enriched N-lauroyl-valine-*tert.*-butylamide [(−)-I] from by-products of its synthesis, it was observed that fractions which were obviously chemically pure and identical, differed in their melting points[2]. This finding led us to examine whether amplification of optical purity (o.p.) could occur by chromatography on an achiral adsorbent without addition of a chiral reagent.

In the chromatographic experiments a column (80 × 1.5 cm I.D.) was packed with Kieselgur 60 (70–230 mesh, 40 g/g sample), deactivated with 6% of water. The optically active substrate was either a diamide [(−)-I][3], a N-trifluoroacetyl-valine-valine-cyclohexyl ester[4] [(+)-II or (−)-II] or a N-trifluoroacetyl-alanine-glycine isopropyl ester [(−)-III], enriched to 67–74% o.p., containing no chemical impurities. Samples of (−)-I of varying enantiomeric purity were available in our laboratory[3,5], whereas optically enriched (−)-II and (+)-II were prepared by mixing (−)-II and (+)-II of 99.8–100% o.p.[6]. The (−)- and (±)-alanylglycine were commercial samples (Sigma) and were derivatized in our laboratory. The o.p. of all materials submitted to chromatography was determined by gas chromatography (GC), as described below. The mixtures of enantiomers were introduced into the column, adsorbed on 2–3 g Chromosorb W AW (30 mesh). The eluent was first pure *n*-hexane, then *n*-hexane–ethyl acetate (95:5), and finally, where necessary, *n*-hexane–ethyl acetate (92.5:7.5). The amino acid derivatives were eluted from the column only after addition of ethyl acetate to the alkane.

The substrate in each fraction was weighed and a small portion hydrolyzed with 6 *N* hydrochloric acid–1.25 *N* hydrogen chloride in isopropanol (1:1)[5] to form valine isopropyl esters of I and II. The enantiomeric composition of the ester was determined by conversion to its N-trifluoroacetyl derivative[5], followed by GC on a capillary column (50 m × 0.5 mm I.D.) coated with either the *tert.*-butylamide of N-lauroyl-D-valine[3] or of N-docosanoyl-L-valine[7]. Compound III was directly chromatographed (without hydrolysis or derivatization) on a capillary column coated with N-docosanoyl-L-leucine-*tert.*-octylamide[8].

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The o.p. of the substrate in the consecutively eluted fractions is plotted in Figs. 1, 2 and 3 for (−)-I, (−)-II and (−)-III, respectively.

The results clearly establish the occurrence of optical enrichment during the chromatographic experiments. The diamide (−)-I (74% o.p., 87% of L-isomer) initially gave an eluate (*ca.* 30% of the sample) of lower o.p. than the starting material (46% o.p., 73% L-isomer), whereas the latter fractions contained diamide with an average o.p. of 90%, with a maximum enrichment of up to 97% o.p. (see Fig. 1). Chromatography of (−)-I of 70% o.p., with a final eluent composition of *n*-hexane–ethyl acetate (90:10), gave an 8% fraction in which no D-isomer could be detected (>99.8% o.p.).

Similar results were obtained with the dipeptide (−)-II initial 67% o.p. (83%, L,L-isomer), giving fractions of 88% highest and 12.5% lowest o.p. (Fig. 2). However, in contrast to (−)-I, for (−)-II, the first eluted fraction (47.5% of the sample) was the one enriched in the L,L-isomer. Correspondingly, chromatography of (+)-II (62% o.p., 81% D,D-isomer) showed a gain in the D,D-antipode in the first eluted fraction, followed by its depletion in the later emerging fraction. Finally, chromatography of (−)-III (70.7% o.p., 85.35% L-isomer) showed an enrichment in the L-isomer in the first eluted fraction (45% of the sample), as can be seen in Fig. 3. One fraction of 10% gave the highest o.p. of 86.5% (92.25% L), whereas the lowest o.p. observed was 25%.

By inspecting Figs. 1–3, it is seen that the overall depletion of one antipode in part of the eluates is equivalent, within experimental errors, to its enrichment in the others, as required by the mass balance (see shaded areas below and above the line of initial sample composition).

For the resolution of an enantiomerically enriched mixture, in contrast to that

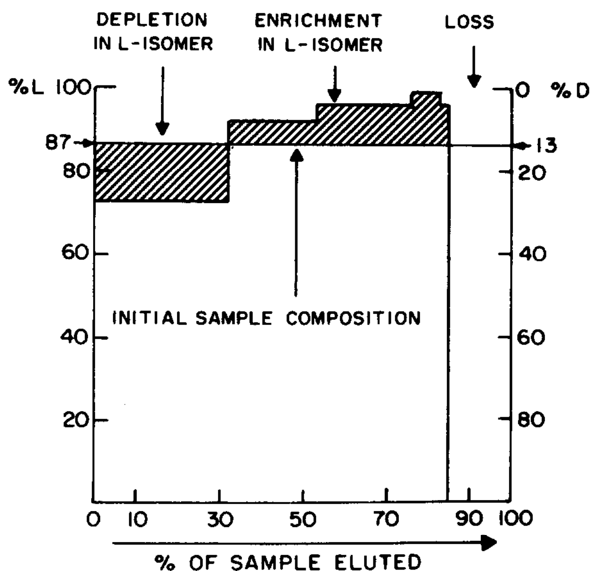

Fig. 1. Chromatographic self-amplification of the optical purity of N-lauroyl-valine-*tert.*-butylamide. *Ca.* 70% of the sample (500 mg) was eluted by *n*-hexane–ethyl acetate (95:5) and the remainder with *n*-hexane–ethyl acetate (92.5:7.5).

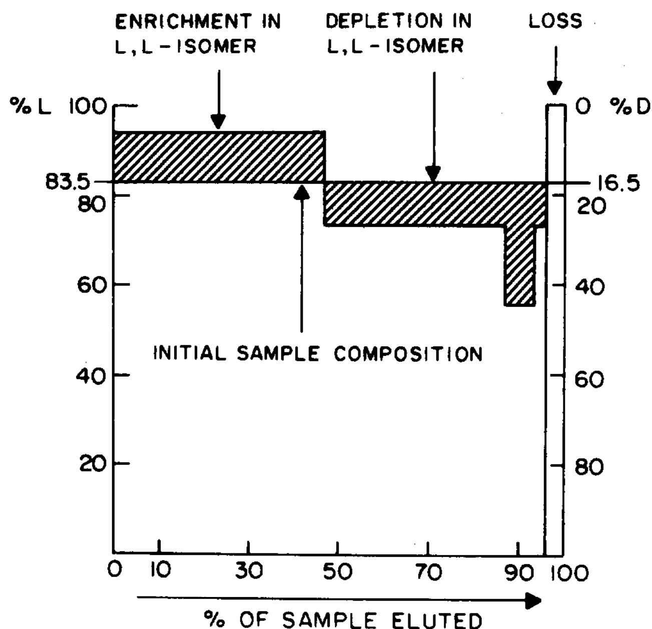

Fig. 2. Chromatographic self-amplification of the optical purity of N-trifluoroacetyl-valine-valine-cyclohexyl ester. The sample (545 mg) was eluted with *n*-hexane–ethyl acetate (95:5).

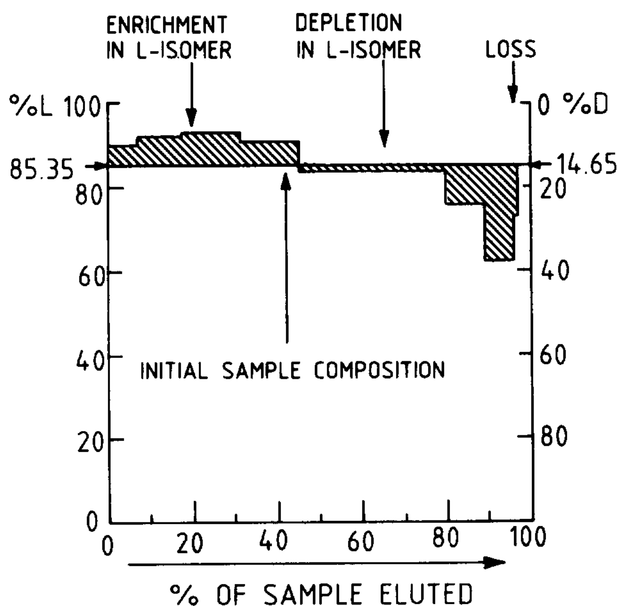

Fig. 3. Chromatographic self-amplification of the optical purity of N-trifluoroacetyl-alanylglycine isopropyl ester. The sample (413.5 mg) was eluted with *n*-hexane–ethyl acetate (90:10).

of a racemic one, resort to an external source of chirality is not a *sine qua non*. For instance, crystallization, using the entrainment method[9], is known to lead to the separation of pure antipodes from initially dissymetric materials. However, the application of chromatography on an achiral adsorbent for this purpose is not normally brought into consideration, because of the prevalent feeling that such an approach does not hold out much promise of success.

The difference in rates of migration, *i.e.* partition coefficients, reported for the enantiomers can be explained by self-association of the solutes. The effect can be understood by the following simplified considerations. Let us assume that self-association occurs only in the adsorbed layer on the silica gel, whereas in the eluent it is negligible due to hydrogen bonding of the solute with the polar component (ethyl acetate). If the L-isomer is in excess in the sample chromatographed, and if, simultaneously, formation of the L,L-hydrogen bonded dimer is preferred over that of the L,D-diastereomeric associates, the adsorbed layer will be enriched in the L-isomer. This means that the partition coefficient of the L-isomer between the stationary and the mobile phase will be larger than that of the D-isomer, and hence, the first fractions will be depleted in the L-antipode. Correspondingly, if under the same set of assumptions, the L,D associate is formed preferentially, the relative rates of migration of the enantiomers will be reversed.

The procedure should, in certain cases, be useful for the preparative-scale isolation of optical isomers, and it would be of great interest to explore what further classes of compounds are amendable to this approach. By extrapolation of the present findings, it should further be possible to resolve racemates by the admixture of a suitable optically active substance of a different structure, followed by chromatography on an achiral adsorbent.

Finally, it has to be noted that the experiments described could lead to a model for the amplification of optical activity under prebiotic conditions. Several workers[10–12] have examined whether adsorbtion of racemic amino acids from aqueous solutions on clay is stereoselective. Some affirmative data reported for ^{3}H-labelled amino acid[10] have, however, been shown to be due to preferentially adsorbed impurities[11], and other investigations have not estasblished conclusive results[12]. In addition, if segregation of enantiomers is claimed in such experiments, highly speculative assumptions have to be made to explain the formation of chiral sites on clay. In contrast, amplification of the optical activity of initially enriched substances involving an achiral adsorbent is not excluded theoretically, and its feasibility is supported by the present investigation. It would be of great interest to find out to what extent the phenomenon reported could occur in systems which can be assumed to exist under prebiotic conditions.

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