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Note

Chiral resolution of a carboxylic acid using droplet counter-current chromatography

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Liquid chromatographic chiral resolutions of racemic mixtures have typically employed adsorption chromatography utilizing a chiral stationary phase or a chiral mobile phase.[1] Diastereotopic interactions between the chiral element of the stationary or mobile phase and the enantiomeric components of the racemic mixture produce different adsorption behaviours for these components and subsequently different elution times. While such applications have successfully achieved baseline resolution of the enantiomers, these methods are not particularly amenable to large scale resolutions.

Two recent reports have appeared describing the use of liquid–liquid partition chromatography for the chiral resolution of norephedrine (200 mg)[2], and isoleucine[3]. The former procedure utilized the newly described rotation locular counter-current chromatography (RLCC)[4–6] with (*R,R*)-di-5-nonyltartrate as the chiral resolving agent dissolved in the mobile phase. The diastereomeric complexes formed upon solvation of the norephedrine enantiomers by the chiral tartrate ester had sufficiently different partitioning behaviour, different formation equilibria, or both to allow for resolution, though baseline resolution was not achieved.

The resolution of racemic isoleucine employed the more familiar droplet counter-current chromatography (DCCC)[7,8]. Diastereomeric mixed copper(II) complexes of the isoleucine enantiomers and N-dodecyl-L-proline were readily separated, though the quantity of isoleucine used was only 2.6 mg. The authors did state, however, that larger amounts of racemate could also be resolved. Separation of the copper(II) complexes is dependent not only upon their differing partitioning behaviours, but also upon the different ligand exchange equilibria between the N-dodecyl-L-proline–Cu(II) complexes and the two enantiomers of isoleucine.

We were recently faced with the necessity of obtaining various substituted optically pure bicyclo[2.2.1]hept-5-ene-2-carboxylic acids, such as compound 1, routinely synthesized via Diels-Alder cycloadditions (Fig. 1). Initial resolution of the racemates was attempted using more traditional methods. Fractional recrystallization of the brucine salts of the parent carboxylic acid, compound 1, and of the carboxylic acid, compound 3, formed by esterification of alcohol, compound 2, [lithium aluminium hydride (LAH) reduction of compound 1] with phthallic anhydride,[9] both failed to yield satisfactory resolution.

Esterification of compound 1 or 2 with optically pure O-acetylmandelic acid,

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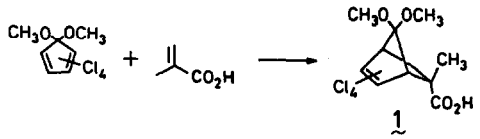

Fig. 1. Synthesis of carboxylic acid 1, via Diels-Alder cycloaddition.

compound 4, or methylmandelate, compound 5, produced diastereomeric esters, compound 6 or 7, respectively, which could be separated via high-performance liquid chromatography (HPLC)[10]. Only diastereomeric esters 6 were routinely resolvable using flash chromatography, and both esterification procedures require chemical modifications which we wished to avoid. The attempts for chemical resolution of carboxylic acid 1 are summarized in Fig. 2.

Subsequent to these efforts, we turned to liquid–liquid partition chromatography to resolve compound 1 using DCCC. We now report the successful use of DCCC in achieving this resolution utilizing (−)-(*R*)-2-aminobutanol as the chiral resolving agent.

EXPERIMENTAL

The DCCC instrument (Model DCC-300 Eyela, Tokyo Rikakikai, Tokyo, Japan) has been previously described.[5] The DCCC employed in this work utilized 225 glass columns, (40 cm × 1.9 mm I.D., 9 racks of columns with 25 columns per rack), connected in series by PTFE tubing, (0.5 mm I.D.). The flow-rate and mode of operation (ascending or descending) were optimized for each experiment; all experiments were run at ambient temperature.

The biphasic solvent systems used were equilibrated overnight prior to separation of the two phases. Partition coefficients (K') were measured by weight of sample in each phase. Values of K' reported were determined using the same ratio of the

TABLE I

EFFECT OF (−)-(*R*)-2-AMINOBUTANOL ON THE PARTITION COEFFICIENTS, K', OF CARBOXYLIC ACID 1 IN CHLOROFORM–METHANOL–WATER (pH 7, 0.01 *M* PHOSPHATE BUFFER)

Partition coefficients, K', were determined by dissolving 112 mg of compound 1 in 80 ml of the aqueous component, adding 130 ml of methanol and 70 ml of chloroform and equilibrating. The concentration of compound 1 in each phase was subsequently determined by evaporation of the solvent from the separated phases, redissolving the solute in 1 *M* hydrochloric acid and extracting with dichloromethane. Under these conditions, compound 1 is extracted entirely into the dichloromethane phase uncontaminated by the (−)-(*R*)-2-aminobutanol and the phosphate buffer, which remain in the aqueous phase. The dichloromethane was removed under reduced pressure and the weight of recovered compound 1 determined.

Solvating agent	*Concentration (mM)*	K'*
None	–	1.08
(−)-(*R*)-2-aminobutanol	20	1.44
(−)-(*R*)-2-aminobutanol	100	2.13

* K' = wt. of compound 1 in upper phase/wt. of compound 1 in lower phase.

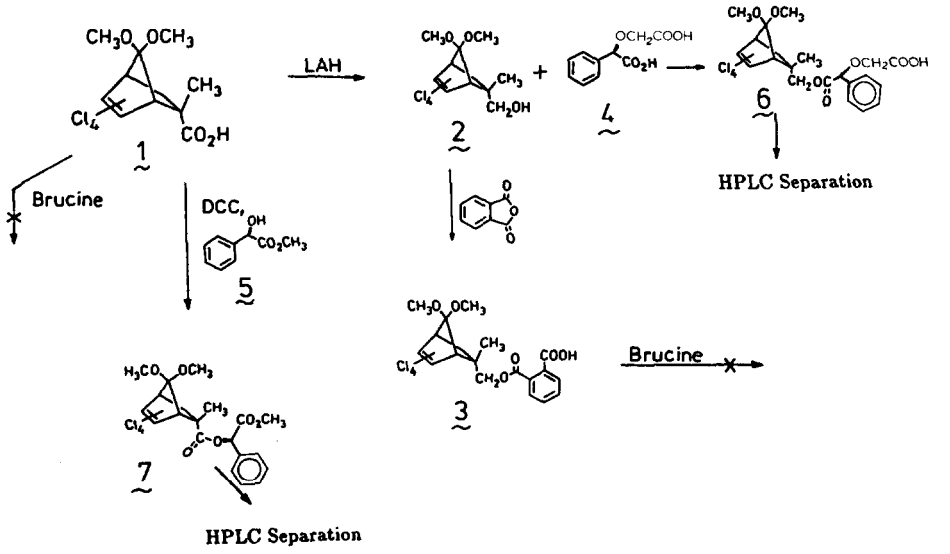

Fig. 2. Attempts at chemical resolution of carboxylic acid 1.

two solvent phases that results from the overnight equilibration rather than equal volumes of the two phases (see Table I). The samples were applied to the instruments by dissolution in a 1:1 mixture of the two phases.

All solvents were distilled prior to use. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The HPLC separations employed a Rainin Microsorb silica column (25 cm × 10 mm I.D.).

RESULTS AND DISCUSSION

Selection of the solvent system

The solvent system employed for the resolution of racemic compound 1 was selected by measuring its partition coefficient in various biphasic systems, both in the presence and the absence of the chiral resolving agent. We initially wanted a system which gave a partition coefficient of approximately unity in the absence of the chiral resolving agent. Such an appropriate system proved to be chloroform–methanol–water (pH = 7, 0.01 *M* phosphate buffer) (7:13:8) with a partition coefficient (upper phase/lower phase) of 1.08 for carboxylic acid 1. The relative volumes of the two phases which results from this solvent system is 5/2 (upper/lower).

Ideally, we desired a chiral solvating agent which would remain exclusively in the stationary phase in the absence of such solvation complex formation with compound 1. Under such circumstances, the amount of chiral resolving agent required would be reduced as more mobile phase is usually consumed in DCCC separations than stationary phase. Furthermore, possible difficulties in removing the resolving agent from the resolved carboxylic acids would be minimized as the concentration of the resolving agent in the mobile phase would be limited to that which partitions

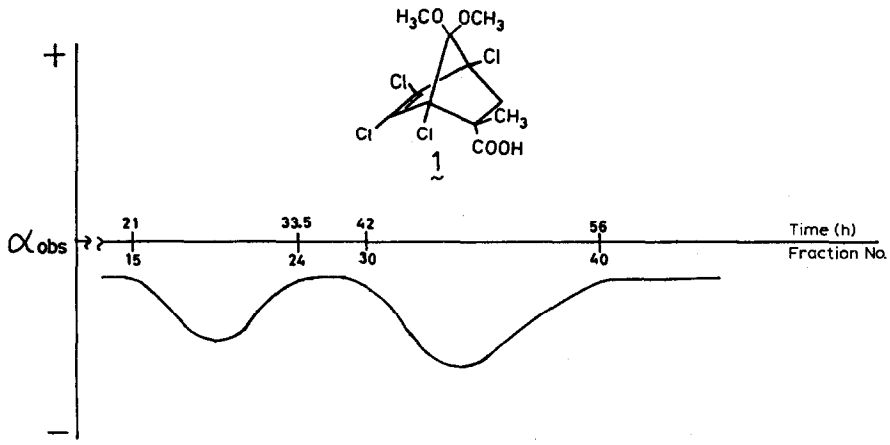

Fig. 3. DCCC chiral resolution of 100 mg of carboxylic acid 1, using chloroform–methanol–water (7:13:8) (pH 7, 0.01 *M* phosphate buffer), descending mode. 100 m*M* (−)-(*R*)-2-amnobutanol, flow-rate = 10 ml/h.

as the solvation complex with compound 1. The concentration of the resolving agent in the stationary phase, however, would not remain constant but would gradually be reduced due to loss via partitioning in the solvation complex form into the mobile phase.

Numerous potential chiral resolving agents, including sugars, amino acids, tartaric acid, and aminoalcohols, were then screened for their ability to alter this partition coefficient of compound 1. We had rationalized that variation in the partition coefficient of compound 1 could be due to the formation of a solvation complex between compound 1 and the resolving agent. Since the resolving agent employed is optically pure, the two solvation complexes formed from the two enantiomers of

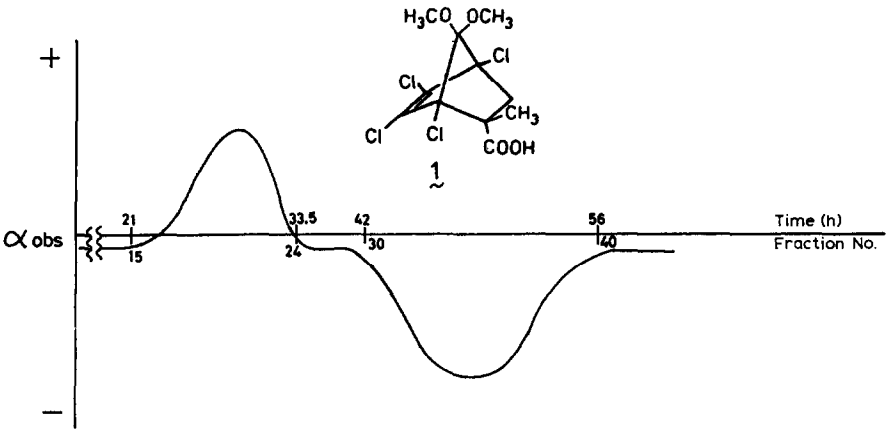

Fig. 4. Observed optical rotation of DCC fractions from chiral resolution of carboxylic acid 1, after removal of (−)-(*R*)-2-aminobutanol. For conditions of chromatography see Fig. 3.

compound 1 and the resolving agent are diastereotopic. Chiral resolution can therefore be achieved either through the different partitioning behaviour of the diastereotopic complexes, the different formation equilibria of the complexes, or a combination of these two effects. The phase which contained the lesser amount of compound 1 after equilibration was then chosen to be the mobile phase.

Only the amino alcohols showed significant alteration of the partitioning behaviour of compound 1. Of those amino alcohols tested, (−)-(*R*)-2-aminobutanol had the greatest effect (Table I) and was therefore selected as the resolving agent. Under the solvent conditions employed (pH = 7), this aminoalcohol remained exclusively in the upper layer (designated the "aqueous" phase), which was used as the mobile phase in the resolution.

Resolution of carboxylic acid 1

With the partitioning data presented in Table I in hand, we applied 100 mg of compound 1 to the DCCC system using the decending mode of operation with a flow-rate of 10 ml/h. Baseline resolution of the enantiomers of compound 1 was achieved (Fig. 3) as determined by measuring the optical rotation of each fraction (*ca.* 12 ml per fraction). The negative rotation for both solute bands was thought to be a consequence of the negative optical rotation of the (−)-(*R*)-2-aminobutanol. This was confirmed by removing the aminoalacohol via an acid wash, and remeasuring the optical rotation of each fraction. As illustrated in Fig. 4, after removal of the aminoalcohol, the (+)- and (−)-enantiomers of compound 1 show the expected signs of rotation in their respective peak with baseline resolution confirmed.

Fractions from the latter portion of peak 1 and from the beginning portion of peak 2 were reduced to the corresponding alcohol and esterified with (+)-O-acetylmandelic acid, compound 4, to produce ester 6 (Fig. 2) and subjected to HPLC analysis. The diastereomeric purity of the esters produced from each fraction confirmed baseline resolution of the enantiomers of compound 1 (Fig. 5). We have not yet assigned the absolute stereochemistry of the enantiomers of compound 1.

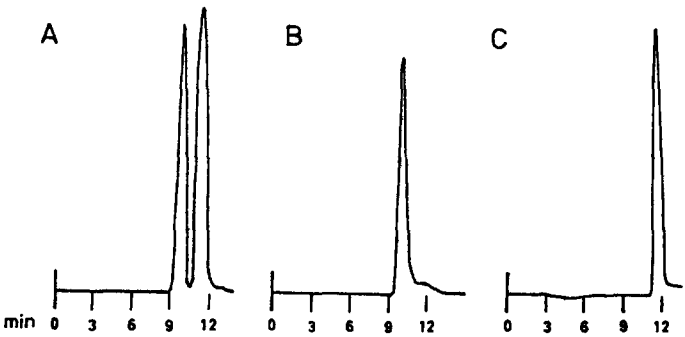

Fig. 5. Confirmation of baseline resolution of enantiomers of compound 1 following conversion to ester 6 using normal phase HPLC, dichloromethane–pet ether (1/2, v/v) flow-rate = 1.0 ml/min, UV detection 254 nm. (a) Chromatogram of diastereomeric esters 6 from racemic compound 1. (b) Chromatogram of diastereomerically pure compound 6 from optically pure (+)-compound 1 in peak 1 (see Fig. 4). (c) Chromatogram of diastereomerically pure compound 6 from optically pure (−)-compound 1 in peak 2 (see Fig. 4).

CONCLUSIONS

Chiral resolution of carboxylic acid 1 using liquid–liquid partition chromatography has been achieved using DCCC. Liquid–liquid partition chromatography using DCCC has the capability of resolving relatively large amounts of sample. We are currently examining other chiral resolving agents in hopes of successfully resolving gram quantities of material.

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