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Chromatographic Chiral Separations

edited by

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Preface

Ever since Louis Pasteur meticulously separated the enantiomers of sodium ammonium tartrate under a microscope in 1848, investigators have searched for improved methods to resolve and purify optically active compounds. Traditionally, a combination of chemical and physical separations has prevailed; conversion of enantiomers to diastereomers followed by crystallization has been the dominant approach.

Very little progress was evident until various forms of chromatography were investigated in the 1950s. Isolated experiments with thin-layer, paper and gravity liquid chromatography (GLC) were moderately successful. In 1966, however, the first reliable optically active stationary phase for enantiomeric separation by gas chromatography (GC) was developed. N-Trifluoroacetyl L-isoleucine lauryl ester was synthesized for the resolution of N trifluoroacetyl amino acid esters. In the first chapter of this book one of the codevelopers of this phase delineates the modifications of this original monoamide by dipeptide and diamide phases to achieve superior resolution by GC. He traces the blending of hydrogen bonding, $\pi-\pi$ interaction and polysiloxane chemistry in the development of sophisticated GC chiral phases.

In the late 1960s the experience gained with GLC was gradually transferred to high-performance liquid chromatography (HPLC).

With the advent of new microparticulate packings and sophisticated instrumentation for analytical and preparative work, HPLC has clearly become the method of choice for accurate and convenient resolution of enantiomers. The normal high-resolving power of HPLC is now amplified by the utilization of improved spatial surface interactions between stationary phase and chiral solutes. Even a brief scan of the literature reveals a variety of promising procedures: chiral stationary phases, chiral mobile phases, transient selective interactions (hydrogen bonding and $\pi-\pi$ interactions), ligand exchange resolution, and chromatographic separation of chiral derivatives.

A survey of the literature is likely to result in more confusion than enlightenment to an investigator searching for a separation technique to resolve a specific enantiomeric mixture. This occurs because the early literature is largely empirical. More recently serious attempts have been made to understand in a systematic way the specific interactions that result in chiral separation.

An understanding of the mechanisms involved and consequent prediction of separation with any given chromatographic technique becomes important to analytical and synthetic chemists as the biological significance of enantiomeric purity (or optical activity) becomes more and more apparent. Many pharmaceutical as well as agricultural compounds of interest are optically active. The opposite enantiomer is frequently inhibitory or toxic. Therefore, the need is steadily increasing for rapid, convenient methods for analysis and purification of these physiologically interesting compounds.

With the needs of analytical and synthetic chemists and pharmacologists in mind, this book serves two purposes:

1. To bring together in one volume the wide variety of liquid chromatographic chiral separation techniques
2. To elucidate the separation mechanisms involved, whenever possible

The success of this book rests on the splendid cooperation of the contributors who have devoted considerable effort to the preparation of their chapters. We are also grateful for the assistance and encouragement provided by the J. T. Baker Chemical Company.

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Contents

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The History of Enantiomeric Resolution

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In a letter addressed to E. Gil-Av after the publication of the first separation of enantiomers on a chiral gas chromatography (GC) stationary phase (1), A. J. P. Martin made the following remark: "As you no doubt know, I had not expected such attempts to lead to much success, believing that the substrate-solvent association would normally be too loose to distinguish between enantiomers." Actually, only a few reports existed concerning the separation of enantiomers by other chromatographic methods.

The pioneering work of Pasteur (2) marked the beginning of chiral separation. He discovered that the spontaneous resolution of racemic ammonium sodium tartrate yielded two enantiomorphic crystals. Solutions of hand-picked crystals gave a levo or dextro rotation of polarized light. Because the difference of the optical rotation was observed in solution, he proposed that, like the two sets of crystals, the molecules themselves are mirror images of each other. Furthermore, this discovery motivated Pasteur to study the influence of one asymmetric compound upon another and introduced the methodology of resolution via diastereoisomer formation. In a lecture addressed to the Chemical Society of Paris in the winter of 1860 he said (3):

> It has been determined that the identity of properties in the case of the two tartaric acids and their derivatives persists

as long as they are brought together with bodies of the first category (nonchiral), for example: potash, soda, ammonia, lime, baryta, aniline, alcohol, the ethers; in short with all the bodies without asymmetry, ..., without action upon polarized light. On the contrary, if they are subjected to the action of bodies of the second class (chiral); for example: asparagine, quinine, strychnine, brucine, albumens, sugars, etc., or other asymmetric bodies like itself, then entirely different properties appear.

The solubility is different. When compounds are formed, the products differ in crystal form, in specific gravity, in their amount of water of crystallization, in their action toward heat, and indeed may differ from one another quite as much as the more distant isomers. Thus it appears that the molecular asymmetry of a body is an important agent for changing the affinity. The two tartaric acids do not behave toward quinine as they do toward potash, because quinine is asymmetric and potash is not.

In his quest to understand the asymmetry and the specificity of biological systems, he proved that only the dextro isomer of racemic tartrate was totally consumed by fermentation while the levo form remained intact. These three techniques: spontaneous resolution, diastereomeric separation and differential enzymatic reactivity were, for more than a century, the only methods employed for enantiomeric purifications.

However Pasteur's initial discovery of spontaneous resolution could only be applied to unique examples in which each of the isomers crystallized separately and in a recognizable morphologic form. Even in those selected cases, the separation of single crystals is tedious. Recently, Addadi et al. (4) showed that many of these difficulties, such as obtaining big single crystals or recognizable morphology, and/or the need for hand picking, might eventually be overcome by controlling the crystal growth of one of the enantiomers using chiral growth inhibitors.

The introduction by Schlenk (5) of urea inclusion compounds for resolution of enantiomers was a step forward in the field of separation. Urea forms inclusion compounds with a wide variety of straight-chain aliphatic compounds of suitable length. The host molecules form a lattice in which channellike hollow spaces are occupied by long-chain organic molecules. The urea molecules are arranged in a left-handed or right-handed screw along the channel, forming two enantiomorphous lattices. Each lattice

has a different affinity to enclose one of the enantiomers. In addition, trace amounts of certain chiral compounds induce the formation of only one of the chiral forms. Schlenk utilized these properties of urea to resolve enantiomers; for example, 2-chlorooctane was resolved up to 95% optical purity. Cyclodextrins, deoxycholic acid, Cram's crown ethers, and other compounds form, similar to urea, chiral inclusion compounds in which guest molecules are enclosed in a chiral cavity. Urea differs from those asymmetric molecules in which only the inclusion lattice is chiral. This, and other unique properties of urea, could possibly be of relevance in the prebiotic conditions regarding the initiation of chiral activity.

Another, less known approach is a surface-imprinting technique introduced by Dickey (6). Silica gel is produced in the presence of organic molecules. These molecules are later extracted, leaving "footprints," or imprints of their shapes on the silica surface. Such a surface may have a higher affinity to adsorb compounds of similar structures. In the same way, silica gel imprinted with asymmetric molecules could resolve racemates of the same or related compounds. For example, imprinting with (+)-*N*-methyl-3-methoxymorphinan caused partial resolution (30% optical enrichment) of its racemate (7).

Most of the methods mentioned before, including enantiomeric separation through diastereomeric compounds, were characterized by multiple steps and low efficiency. The introduction of chromatography as a new, fascinating separation tool also created the opportunity for efficient enantiomeric separation. Obviously, it was understood that a necessary condition to accomplish resolution is chirality of at least one of the two phases, the stationary or the mobile phase. A review of the early chromatographic literature showed that complete separation was achieved only on paper chromatography, while other asymmetric supports showed partial or no resolution.

In 1939 Henderson and Rule demonstrated the separation of *d,l*-*p*-phenylenediiminocamphor on *d*-lactose (8). In 1951 Kotake et al. (9) studied the influence of a chiral mobile phase on the resolution of several amino acids on paper chromatography and found that the separation could be ascribed to the chirality of the support (cellulose). They obtained full resolution for tyrosine-3-sulfonic acid and partial resolution for glutamic acid and tyrosine. After this report other amino acids were resolved on the same support: 3,4-dihydroxy-2-methyl- and 2,3-dihydroxyphenylalanine (10); tryptophan (11); α,ε-diaminopimelic acid (12)

and 5-hydroxy- and 6-hydroxytryptophan (13). The apparently strange results, e.g., 3,4-dihydroxyphenylalanine showed no resolution while closely related isomers were resolved, brought Dalgliesh (10) to suggest a necessary three-point simultaneous attachment between the resolvable amino acids and the cellulose surface.

Starch, another polysaccharide, was used by Prelog et al. to separate Troeger's base (14), and by Krebs et al. (15) to separate mandelic acid, tartaric acid, camphor sulfonic acid and *N*-acetyl-α-amino acids.

Klemn and Reed (16) were the first to use a chromatographic support according to the purpose of their separation. They used the property of nitroaromatic compounds to form molecular complexes with other aromatic hydrocarbons via π-π interactions. The impregnation of silica with (+)-α-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid, a compound engineered by Newman and Lednicer (17), led to a partial resolution of 1-naphthyl-2-butyl ether and 2,4,5,6-dibenzo-9,10-dihydrophenanthrene. When more efficient high-performance liquid chromatography (HPLC) systems became available, complete separation of the same and other analogues was reported (18).

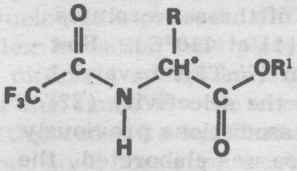
In the 1950s through the 1960s, development in the separation techniques was dominated by gas chromatography (GC). Difficult separations such as isotopic separation of organic and inorganic compounds, diastereoisomers, etc., were now made possible by the more efficient GC systems. Nevertheless, direct enantiomeric separations failed. Although a number of communications claimed such separations, these could not be reproduced, and some were even shown to be wrong, or artifacts. In fact, most scientists in the field were convinced that a sufficiently large energy difference in the interaction between the D- and L-solute and an asymmetric solvent could not possibly occur. Thus when one of us had to defend his proposal for the Ph.D. thesis: *The Separation of Optical Enantiomers by GC on Optical Active Stationary Phases*, the experienced committee advised him, in a fatherly manner, to choose another subject.

In 1966, Gil-Av, Feibush, and Charles (1) reported the first successful direct enantiomeric separation, a step that had a large echo in the field. The model of this achievement was summarized as follows (19):

> There should be a special correlation between the solvent and the solute in order to establish a difference in the behavior of

the enantiomers tend to separate them in the GC column: (a) strong interactions such as π-π interactions, [coordinate-covalent] bonds, hydrogen bonds, etc., to yield short-lived diastereoisomers; (b) close proximity of the bonds to the respective asymmetric carbons; (c) more than a single bond to prevent free rotation and to increase the interaction between the adjacent solvent-solute molecules, while the two respective asymmetric carbons are brought to proximity; (d) to minimize the noncontributing associative forms which do not bring the respective asymmetric centers to proximity.

Once the principle of separation was formulated, the next step was to choose the system that could fulfill the demands as just described. The amino acids were selected because they were available, inexpensive and provided, after derivatization, a simple model able to form hydrogen bonds flanking the chiral centers of both solvent-solute associating partners. The first stationary phase, *N*-TFA-L-isoleucine lauryl ester*, resolved derivatized amino acids as *N*-TFA isopropyl, 2-butyl, and *t*-butyl esters



R = 2-butyl, benzyl

R¹ = dodecyl, cyclohexyl

[1]

while the *n*-alkyl esters showed only partial resolution. Because the resolution of optical isomers by GC was then a controversial matter, additional evidence had to be furnished to establish that the observed separation of enantiomers was not an artifact. As we worked with capillaries, we could not isolate material from the column, and we turned to chromatographic supportive data. By injecting into the GC system an enantiomeric mixture in which the L isomer was enriched, first on the L stationary phase and then on its enantiomeric D phase, a reversal of order of the enriched larger peak was obtained. Similar behavior was observed for all the amino acids studied and resolved (20). Later, when a more selective phase was developed, enantiomeric separation was performed on a packed column. Material was isolated from each peak and proved to be two separable enantiomers (21).

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*TFA = trifluoroacetyl.