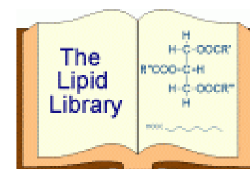
Chiral chromatography of lipids



This article appeared first in *Lipid Technology*, **4**, 21-23 (1992). Minor improvements only were required to update it. However some key references were still in press when it was published, and readers may wish to consult the following review.

Christie, W.W. The chromatographic resolution of chiral lipids. in *Advances in Lipid Methodology - One*, pp. 121-148 (1992) (edited by W.W. Christie, Oily Press, Dundee).

CHROMATOGRAPHIC RESOLUTION OF CHIRAL LIPIDS

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Background

I hope my regular readers will forgive me if I start this contribution with some textbook material. Any molecule has the property of chirality or asymmetry when its mirror image cannot be superimposed on itself. The two forms are termed 'enantiomers' or 'optical isomers', and have the same chemical bonds but differ in their arrangement in space and thus in their three-dimensional structure. With substances with two or more chiral centres, those in which the configuration of one or more of the asymmetric centres differs are termed 'diastereomers'. With a given compound, diastereomers may have slightly different conformations and for example small changes in intra-molecular hydrogen bonding and thus different internal energies. They have distinct chemical and physical properties and thus have the potential to be separated in non-chiral chromatographic environments.

Does this have any relevance to lipids? The lipid analyst in a routine analytical laboratory will perhaps doubt it at the moment, but most specialist laboratories will have to take account of developments. Lipids like all natural compounds are synthesised by enzyme systems, which are themselves chiral and invariably produce chiral products. The asymmetry may be inserted directly during the biosynthesis of the aliphatic chains of lipids, for example in isoprenoid and other branched-chain lipids where the precursors are chiral. Alternatively, the chirality may be introduced to the aliphatic chain later, as in the synthesis of oxygenated or cyclopropane fatty acids. Similarly, glycerolipids have a centre of asymmetry at the second carbon atom, and the three positions on the glycerol moiety of triglycerides for example may be occupied by different fatty acids, *i.e.* the two primary positions are not identical. Chirality is important to biochemists who wish to understand the mechanisms of lipid biosynthesis. In addition, pharmaceutical companies have been well aware for many years of the importance of chirality in specific drugs, and increasingly lipid molecules are finding such uses, including the prostaglandins and eicosanoids. Purified lipids are being used in liposome preparations to target drugs to particular organs. Analysts with interests in these areas will be easily convinced of the importance of lipid chirality. Similarly, chirality may one day provide a means of detecting adulteration of fats and oils.

Determination of chirality

The primary method for the determination of the stereochemical configuration of a chiral molecule is chemical synthesis from a precursor of defined stereochemistry. With lipids, the

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secondary classical method of measuring optical activity is often of little value, because the effect is so small and the same is true of modern techniques such as nuclear magnetic resonance (NMR) spectroscopy in the presence of chiral shift reagents. Often it is possible to use enzymic methods. In recent years, however, the chromatographic behaviour of chiral compounds or their derivatives in suitable systems has begun to contribute to the solution of stereochemical problems. The development of methods for the chromatographic resolution of chiral compounds in general has also made it much easier to obtain the pure enantiomers that may be required for studies of biological activity. The pharmaceutical industry has been a major beneficiary and has stimulated much of the published work, but lipid analysts have been quick to take advantage of new opportunities.

Chromatographic methods

Many factors have contributed to the improved resolution of enantiomeric lipids by chromatographic means. These include technical developments such as the construction of the many components of high-performance liquid chromatography (HPLC) systems, including pumps, injection systems and detectors, which have lead to such enormous improvements in the capabilities and efficiency of this technique. It is even possible to purchase an HPLC detector that senses optical activity in the eluent, though applications to lipids have yet to be described. In gas chromatography (GC), the advent of highly efficient capillary columns of fused silica has been a major advance. However, it may be argued that most important of all has been the knowledge gained of the effects of chirality on chromatographic selection mechanisms, especially in the laboratory of W.H. Pirkle. In particular, this has lead to the discovery of suitable derivatives for the resolution of diastereomers and to the development of novel stationary phases for HPLC with chiral moieties bonded chemically to inert supports. HPLC offers special opportunities in that there is flexibility in the choice of both the mobile and stationary phases to enhance resolution, and the separation is carried out at ambient temperature.

In 1979, Pirkle and House (1) first put chiral chromatography on a systematic basis with a theoretical explanation of the "three-point rule", *i.e.* there must be at least three simultaneous interactions between a chiral stationary phase and a solute enantiomer and one of these must be stereochemically dependent, if chiral resolution is to be effected. In effect, the solute and the stationary phase form a transient diastereomeric complex, and the configurations of the substituents in each enantiomer determine the nature of the separation. One advantageous strategy for effecting a separation, for example, can be to include a naphthyl or anthryl group (electron-rich) in the stationary phase to cause it to interact via the *pi* bonds with an electron-deficient aromatic moiety (*e.g.* dinitrophenyl) in the solute, perhaps after suitable derivatization of the latter. Similarly, the hydrogen atom of a secondary amide can interact strongly with the oxygen of a carboxyl group via hydrogen bonding.

Chiral chromatography

This approach has been followed in the laboratory of Professor Toru Takagi in Japan, who has recently reviewed application of chiral chromatography to lipids (2). He and his coworkers have been able to resolve a number of chiral glycerol derivatives by preparing the dinitrophenylurethane derivatives for separation on special HPLC columns packed with a silica-based stationary phase to which chiral organic moieties were bound by chemical means. Until recently, such columns were only sold in Japan at very high cost, but they are now much more readily available and the cost has dropped. Unfortunately, the derivatizing agent still only appears to be available in Japan. Similar methods have been used for the resolution of prostaglandin derivatives (3).

My colleagues and I have been using a different approach to the problem (4,5). Diastereomeric naphthylethyl and other urethanes especially have been used for the resolution of alcohols and amines of many kinds by column chromatography on non-chiral adsorbents such as silica gel or

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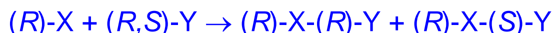
alumina. This is possible because diastereomers have different physical and chemical properties as discussed briefly above. Thus a chiral derivatizing agent (*R*)-X will react with an enantiomeric substance (*R,S*)-Y as -



$$(R)\text{-X} + (R,S)\text{-Y} \rightarrow (R)\text{-X-}(R)\text{-Y} + (R)\text{-X-}(S)\text{-Y}$$

In a given chromatographic system, the degree of separation obtained of the two diastereomers will depend on the chiral structures of X and Y and the manner of their interactions with the mobile and stationary phases. Another useful feature is that the order of elution of the diastereomeric derivatives is reversed in the same chromatographic system if the other enantiomer of the reagent, *i.e*. (*S*)-X, can be employed. We, therefore, derivatized glycerol compounds, diacyl-*sn*-glycerols especially, with a chiral naphthylethyl isocyanate reagent to form diastereomeric urethane compounds. It was then possible to resolve them easily by HPLC on columns of silica gel. Others have used analogous methodology with prostaglandins (3).

Both approaches to the resolution of chiral lipids have lead to methods for determining the fatty acid compositions of all three positions of triacyl-*sn*-glycerols (*i.e*. for stereospecific analysis) by chromatographic means only without any requirement for the use of enzymes. These methods will perhaps be described in more detail in a later article. Meantime, anyone wishing further information on chiral chromatography of lipids might wish to consult a more comprehensive review of mine (6).

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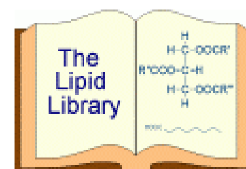

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