Chromatographic Methods in the Determination of Absolute and Relative Configurations of Fatty Acids

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Abstract

Chromatographic techniques for determination of configurations of branched and substituted long-chain fatty acids, both absolute and relative, are reviewed. Gas-liquid chromatographic and thin-layer chromatographic procedures are emphasized.

Introduction

This review is concerned with chromatographic methods for determining configurations, both absolute and relative, of fatty acids and related compounds which have chiral carbon atoms. In this context, it may be helpful to recall that a racemic mixture consists of two optical isomers (antipodes) having one or more chiral centers. Optical antipodes show a mirror-image relationship at all points, and have identical physical properties except for sign of rotation. On the other hand, diastereomers exhibit different physical properties.

Most procedures for resolving racemic mixtures depend upon conversion of their enantiomeric forms into diastereomers, usually by adding new chiral centers through derivatization. Under suitable conditions, diastereomers can be separated because of differences in their physical properties. Chromatographic separations of diastereomers are possible because they differ in boiling point and solubility, and they interact differently with solid adsorbents or stationary liquid phases.

Stern et al. (1) concluded that the distance between the carboalkoxyl function and the nearest chiral center affects the degree of separation of diastereomeric esters, with increased proximity of the two being conducive to better chromatographic resolution. These authors also attached importance to conformational influences of the alcohol moieties of those esters as well as to steric bulk of substituents attached to centers of chirality.

Racemic mixtures of various types have been resolved by chromatography on optically active adsorbents, e.g., on such natural polymers as cellulose and starch; these separations have been reviewed by Losse and Kuntze (2). This approach to chromatographic differentiation of enantiomeric compounds has been developed by using optically active liquid phases in the gas-liquid chromatographic (GLC) analysis of amino acids (3-5). In principle, this technique appears to be broadly applicable, but apparently there are still no recorded examples relating to optically active fatty acids.

In order to determine the stereochemistry of fatty acid derivatives by chromatographic techniques, reference compounds of known configuration must be available for comparison of retention values.

Isoprenoid Acids

One of the most extensive uses of chromatographic techniques to elucidate the stereochemistry of fatty acid derivatives has been in the investigation of isoprenoid acids. Most of this work has been carried out by Ackman and associates (6-12).
They have achieved exceptionally efficient resolutions in GLC analyses of diastereomeric isoprenoid esters by using capillary columns coated with butanediol succinate polyester (BDS) which itself has no optical activity.

Initially, Ackman and Hansen (6) demonstrated partial resolution of diastereomeric mixtures of phytanic (3,7,11,15-tetramethylhexadecanoic) and pristanic (2,6,10,14-tetramethylpentadecanoic) acids from natural sources by GLC of their methyl esters. They observed partial resolution of some of the samples into two peaks and concluded that two diastereomers were present (Figure 1). Since samples of methyl phytanate and methyl pristanate with the all-D-configuration had a retention time coincident with the slower-moving peaks from the mixtures under investigation, they considered the faster-moving component to be the LDD isomer. This assignment was supported by the GLC elution pattern exhibited by phytol-derived samples (Figure 1A and B) which were known to be mixtures of LDD and DDD isomers (6,7).

Ackman and coworkers (8,9) subsequently developed the use of methyl esters in the GLC analysis of isoprenoid acids, and thereby greatly augmented their GLC resolutions. Since (-)-menthol is a natural product consisting of only one enantiomeric form, the derived isoprenoid esters contain twice as many potentially separable diastereomers as the corresponding methyl esters. Comparison of GLC retention patterns of (-)-menthyl esters of isoprenoid acids from natural sources with synthetic esters led to a more confident assignment of the absolute configuration of many components than was possible by analyzing methyl esters (Figure 2). Ackman et al. (9) noted certain differences between the methyl and (-)-menthyl ester series in the elution sequence for various mixtures of diastereomers. In the order of elution of methyl esters with three chiral centers, they observed the maximum separation between an RRR [SSS] isomer and an RSR [SRS] or RSS [SSR] isomer. In the (-)-menthyl ester series, the RRR isomers eluted last except for compounds with the first methyl branch at the 4-position.

Figure 1. Portions of gas chromatograms of methyl esters of phytanic acid (A-F) and pristanic acid (G and H). Sources of samples: A, B, synthesized from phytol; C, ox fat; D, E, butterfat; F, patient with Refsum’s Syndrome; G, sheep fat; H, butterfat. (From Ackman and Hansen (6). Reproduced with the consent of the American Oil Chemists’ Society.)

![Gas-liquid chromatograms of (-)-menthyl esters of some 4,8,12-trimethyltridecanoic acids at 150° on a 150-ft BDS column, 40 psig He. (From Ackman et al. (9). Reproduced with the permission of the Journal of Chromatographic Science.)](image)

Figure 2. Gas-liquid chromatograms of (-)-menthyl esters of some 4,8,12-trimethyltridecanoic acids at 150° on a 150-ft BDS column, 40 psig He. (From Ackman et al. (9). Reproduced with the permission of the Journal of Chromatographic Science.)

Ackman and coworkers have applied their GLC method to the stereochemical analysis of isoprenoid acids from various natural sources, including marine fats (6,8,10) terrestrial fats (6,8,10), bacteria (6,10), and oil shale (11,12). They ascertained that both marine and terrestrial fats are, in general, mixtures of diastereomers and that samples from various sources differ somewhat in the proportions of these isomers. Isoprenoid acids from oil shale resemble those from living organisms in their isomeric composition, probably as a consequence of having a common origin in chlorophyll-derived phytol (12). Isoprenoid alcohols prepared from glyceryl ethers of the extremely halophilic bacterium Halobacterium cutirubrum appear to be unique in being optically pure—exclusively the DDD isomer (6,10).

**Feather-Wax Constituents**

Birds secrete green gland liquids called feather waxes which, upon hydrolysis, yield a variety of long-chain acids and alcohols [for further details see (59)]. The acids usually have from one to four methyl branches, but they are polypropionate rather than isoprenoids since they have methyl groups at even-numbered positions along the main carbon chain. GLC procedures have been used extensively by Odham in determining the configurations of acids derived from feather waxes (13).

In his study of 2,4-dimethylheptanoic and related acids from feather waxes, Odham (14,15) synthesized methyl 2D,4D- and 2L,4D-dimethylheptanoate by electrolytic (Kolbe) coupling. He used preparative GLC to resolve the two diastereomeric forms and measured their rotations. The retention time of the synthetic levorotatory 2D,4D-isomer, the shorter of the two on polypropylene glycol or Versamid 900 columns, was identical with that of the natural product. Odham homologated the 2D,4D-dimethylheptanoate by a scheme involving further electrolytic coupling to provide a mixture of 2D,4D,6D- and 2L,4D,6D-trimethylheptanoate; this product likewise was resolved by preparative GLC. The two isomers were identified by optical rotation, since it was known that 2D-methyl substituted methyl esters are levorotatory.

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2 The configuration of these isomers could be designated by the Fischer convention as DDD [LLL], DLD [LDD], etc.
By a series of nonstereospecific Kolbe coupling reactions, Odham (16) synthesized all eight possible diastereomers (four racemic mixtures) of methyl 2,4,6-trimethylheptanoate. The four racemates were resolved by preparative GLC and configurations were assigned on the basis of optical rotations and GLC elution sequence. The natural isomer again was shown to have the all-D configuration. Odham (17) likewise deduced all-D stereochemistry for 2,4,6,8-tetramethyldecanonic and -tetramethylnonanoic acids derived from feather waxes.

A salient point that emerged from Odham's investigations of feather-wax acids was the conclusion that methyl esters of all-D isomers (plus all-L isomers, if present) are eluted first in GLC analyses of fatty acids with two or more methyl substituents. This observation facilitated assignments of configurations to other components. Also noteworthy is Odham's use of preparative GLC to resolve diastereomeric mixtures, primarily made possible by the powerful effect of the methyl substituent in the 2-position.

Acids from Bacterial Lipids

The tubercle bacillus and some related organisms elaborate a wide array of complex lipids which yield a variety of fatty acids with alkyl branches (18,19). Chromatographic techniques have been employed to a limited extent in the determination of configurations of such acids.

Odham and coworkers (20,21) synthesized and resolved two of the mycocerosic acids by procedures similar to those used in their work on feather-wax constituents. By preparative and analytical GLC in combination with measurements of rotation, they demonstrated that 2,4,6-trimethylheptanoic and 2,4,6,8-tetramethyldecanonic acids have all-D configurations.

In a few cases, thin-layer chromatography (TLC) or column chromatography has been employed to determine configurations of branched-chain fatty acids from bacterial lipids. These techniques have been useful in establishing relative configurations in the mycoceric acids, a group of acids with 2-alkyl-3-hydroxy structures.

Asselineau and coworkers (22) prepared a mixture of the 2,3-erythro and three isomers of corynomycolic acids and resolved them by column chromatography on alumina; they noted a reversal in elution order of the two isomers if they chromatographed methyl esters rather than the free acids. After infrared (IR) studies of the two methyl esters, they inferred that natural corynomycolic acid has the 2R,3R-configuration. In a related study, Tocanne and Asselineau (23) prepared a mixture of erythro and three-corynomycolanediol isomers (reduction products of corynomycolic acids), resolved them by preparative TLC, and studied their IR spectra to reinforce their previous conclusions. A similar approach was taken by Coles and Polgar (24) in an investigation of mycolipanic acids. They converted the natural compound into a mixture of 2,3-erythro and three isomers which they resolved by preparative TLC. They demonstrated by IR studies that the natural isomer has the erythro configuration.

In their study of the configuration of C30-mycocerosic acid, Asselineau and coworkers (25) resolved a mixture of two diastereomeric trimethylnonanoates, and identified the all-D isomer by rotational measurements.

Monohydroxy Acids

For some time, it has been recognized that a wide range of positional isomers of long-chain monohydroxy acids can be differentiated by TLC on Silica Gel G (26,27). Comparable separations of these positional isomers can be achieved by GLC on a variety of liquid phases (28). In contrast, the determination of configurations of these acids by chromatographic methods appears to have been applied only to the more unsymmetrical isomers.

Westley and Halpern (29) introduced the use of (+)-methylhydroxycarbonyl derivatives of 2-hydroxy acid methyl esters to facilitate GLC resolution of enantiomers. Hammarström (30) utilized such derivatives to elucidate the stereochemistry of 2-hydroxy acids from brain cerebrosides. He resolved the D and L forms by GLC on an OV-210 column and demonstrated that the 2-hydroxy acids derived from cerebrosides have the D-configuration exclusively. Brooks, Gilbert, and Gilbert (30a) devised a similar procedure for determining the chirality of methyl 12- and 13-hydroxyoctadecanoates. The hydroxyl functions of these esters were acetylated with dimanoyl chloride or chrysanthemoyl chloride, and the resulting diastereomers were resolved by GLC with an SE-30 column. Hamberg (30b) resolved the enantiomeric forms of 2- and 17-hydroxyoctadecanoates by GLC of their (R)-1-phenylethyl urethanes on a 1% QF-1 column. Hammarström and Hamberg (30c) extended this technique to a wider range of isomers by acylating their hydroxyl functions with D-phenylpropanoyl chloride. The resulting derivatives could be clearly resolved on a QF-1 column in the case of the methyl 3-, 15-, 16-, and 17-hydroxyoctadecanoates; the 2- and 14-isomers showed some separation but the 4-, 7-, and 13-isomers were not perceptibly resolved.

Morris and Hitchcock (31) investigated the oxidation of palmitic acid, doubly labeled in a stereospecific manner, by enzyme systems from pea seedlings. By TLC and gas-liquid radiochromatographic techniques, they demonstrated that the 2D hydroxyl is introduced by replacement of hydrogen with retention of configuration. Tsai and coworkers (32) prepared four diastereomers of 2-hydroxyphytanic acid (2D,3D; 2D,3L; 2L,3D; and 2L,3L) and were unable to resolve the two racemates by GLC; however, they succeeded in doing so by TLC on silica (solvent: hexane-ethyl acetate, 90:10).

Epoxy Acids, Polydroxy Acids, and Related Derivatives

Determination of configurations of fatty acid epoxides is likely to be associated with the stereospecific conversion of these compounds into vicinal diols, halohydrins, or comparable derivatives; conversely, new reversals of these reactions may be studied. As with the monohydroxy acid series, isomeric fatty acids with one epoxy function vary in TLC mobility according to the position of the oxygen substituent (33–37). Vicinal diols show comparable variations in Rp values; more important, however, is the fact that erythro and three forms of each positional isomer have markedly different Rp values when chromatographed on silica impregnated with boric acid, sodium borate, or sodium arsenite (33,34,38). TLC separations facilitated by these complexing agents have made numerous configurational assignments possible. Similarly, GLC separations of erythro and three isomers of fatty acid vicinal diols can be achieved; trifluoroacetyl or isopropylidene derivatives have been employed for this purpose (39–41).

TLC with borated silica has been used to establish relative configurations of (±)-three-9,10-dihydroxyoctadecanoic acid isolated from ergot lipids (42), erythro-5,6-dihydroxyhexadecanoic acid from a mosquito pheromone (43), and erythro-2,3-dihydroxytetraocanoic and -dihydroxyhexadecanoic acids from baker's yeast (44). Diastereomers of these 2,3-dihydroxy acids also can be resolved by TLC on plain silica (45).
Morris made extensive use of TLC techniques in establishing the absolute configuration of (+)-vernolic (cis-12,13-epoxy-cis-9-octadecenoic) acid. The epoxy group of (+)-vernolic acid was correlated configurationally with the 12-D-hydroxy of ricinoleic acid by establishing the identity of certain tetrols derived from each (46). This feat required isolation of four individual diastereomeric tetrols by successive TLC separations with borated silica or with arsenite-impregnated silica. Morris and Crouchman (47) similarly correlated the configurations of the (+)- and (-)-12,13-dihydroxyacids derived from (+)-vernolic acid by a series of interconversions. Key intermediates were two pairs of diastereomeric hydroxyoxysloxylates which were inseparable on plain silica; in this case, TLC with silver nitrate-impregnated silica was efficacious, presumably because of the proximity of the double bond to the oxygen functions. Morris and Wharry (46) carried out a similar correlation through chlorohydrins derived from (+)-methyl vernolate. The isomeric chlorohydrins were separated on ordinary silica, and the configuration of each hydroxyl group was inferred from considerations of the reaction mechanism (preferential attack of chloride at C-13 was assumed).

Wood, Bever, and Snyder (48) compared the efficacy of various TLC and GLC conditions in separating diastereomeric 9,10,12-triols (two pairs) derived from methyl ricinoleate as well as the structurally related 9,10,12,13-tetrols (Figure 3).

Morris and Crouchman (50) also confirmed the conclusions of Wood, Bever, and Snyder (48) regarding the configurations of the 9,10,12-triols and 9,10,12,13-tetrols mentioned above. It was noted that all isomers with a 10,12-threo relationship ran slower on plain silica than did the corresponding erythro isomers, but considerably faster on arsenite-impregnated silica.

Eliasson, Odham, and Petterson (51) prepared methyl 2D,4D- and 2L,4D-dimethoxyheptacosanoate by procedures resembling those used in Odham’s previous work on feather-wax constituents (13-17). 2D,4D- and 2L,4D-dimethoxytridec-12-enoate were important intermediates that were separated by silicic acid chromatography; they were also resolved by GLC.

Hydroperoxides Derived from Fatty Acids

The familiar 9- and 13-hydroperoxydienes derived from linoleic acid (or other polyunsaturated acids) are formed stereospecifically by a variety of plant enzymes, and their various configurations have been studied extensively. Hamberg (52) devised a GLC procedure for determining their absolute configurations. The hydroperoxides are reduced with stannous chloride to give hydroxydienes (separable by TLC if positional isomers are present). The hydroxyl functions are acylated with (-)-menthyl chloroformate, and the resulting derivatives are cleaved by ozonolysis. The final products contain methyl esters of either 2-menthylhydroxyoctadecanoate or -sebacate, the diastereomeric forms of which have distinctly different GLC retention times. Earlier, Hamberg and Samuelsson (53) made use of radiocromatography to establish the stereochemistry and mechanism of formation of 15L-hydroperoxy-cis-8, cis-11,trans-13-eicosatrienoic acid.

A variety of decomposition products are generated from the fatty acid hydroperoxydienes (54). TLC has been used to identify threo or erythro isomers of certain of these products, e.g., methyl erythro-11-hydroxy-12,13-epoxy-9-octadecenoate (55-57).

Prostaglandins

The exceptionally voluminous literature on prostaglandins places them beyond the scope of this review. However, we wish to draw attention to one leading reference on TLC of prostaglandins and intermediates in their biosynthesis. A paper by Nugteren and Hazeloof (58) gives considerable data for such compounds, including separation of some pairs of diastereomers.

References