Seed Lipids

Seed triglyceride oils are chemically diverse, scientifically interesting, and versatile in utility.

Ivan A. Wolff

The advent of a variety of new and elegant experimental techniques has resulted in exponential proliferation of data on the structures of many natural products. Before describing how research on seed lipids has joined this chemical mainstream, I shall review their present major sources and importance.

The uses of seeds far transcend their natural purpose of species perpetuation (*1*). A large part of the world's food and feed supply consists of seed crops. About a quarter of the calories in our diet and almost a third of the total feed nutrients for livestock are supplied by cereal grains, which represent 90 percent of all seeds cultivated. Generally grains are rich in carbohydrates but poor in lipids (*2*). In this country, despite the relatively low percentage of lipid present, the large volume of corn processed for starch makes sizable quantities of corn oil available for marketing.

Legume seeds are usually rich in protein; many also contain either starch or galactomannan polysaccharides as a principal energy-reserve substance. Even though on the average legume seeds are, like cereal grains, low in oil content (*3*), two of the best-known and most widely used oilseeds, soybeans and peanuts, are legumes. These two legumes supply about a fourth of the world's edible oils and fats. Soybean oil also goes into many nonfood uses, and the beans themselves are man's most important leguminous food.

In commerce, seed lipids, primarily triglycerides at various stages of purification or refinement, are termed vegetable oils. Other major plant raw materials besides soybeans and peanuts that fulfill world needs for vegetable oils include, in decreasing order of total estimated production, cottonseed, sunflower, rapeseed, flaxseed, coconut, sesame, palm, olive, and castor beans (*4*). These other oilseeds are distributed among various plant families.

More than 20 million tons (*4*) of vegetable oils are produced annually. One-fourth of this amount is produced in the United States, and the United States accounts for a third of the world's exports of oilseeds and vegetable oils. The chief use of the oil is in edible products, though 1.6 million tons a year are used for soaps and surfactants, drying oils, and other important industrial applications. Some oils, such as rapeseed, which in the United States are used primarily or exclusively for nonfood purposes, are important elsewhere principally as edible oils. Many authorities (*5*) regard the highly concentrated protein meal which is a coproduct with the lipid from oilseeds as the best readily available and expandable food protein source for countries deficient in protein. The demand for oilseeds for all these uses is still increasing.

New Oilseeds

Only a small proportion—perhaps 1 percent—of the 250,000 known species of higher plants is utilized for human needs. Emphasis in chemical research on natural plant products has been on the cultivated plants, with the possible exception of surveys of wild plants made for narrowly circumscribed reasons, such as a search for alkaloids, steroid precursors, or other biologically active or nutritionally significant constituents. Sir Ewart Jones (*6*) expressed the situation well when he wrote, "There is ample scope for the discovery of new natural products. Hitherto our screening has been coarse—more a pursuit of the exotic in colour, odour, flavour and toxicity than a systematic search for structural types and taxonomic relationships."

As a consequence of the limitations of plant raw materials readily available for study and the selectivity with which subjects for investigation have been chosen, there are large segments of the plant world whose seed lipids (as well as many other plant constituents) have not been chemically characterized. The paucity of data is particularly striking when contrasted with the chemical research in depth devoted to understanding the composition, transformations, and industrial potentials of the world's mineral and petrochemical resources.

It has been estimated (*7*) that from all higher plants lipids of only about 900 species have been studied for their *average* composition of total fatty acids; fewer than 100 seed oils have been investigated for information on their glyceride structures. In both categories much of the work reported has used methods that are no longer considered highly reliable. In 1965 the statement could still be made that although "The pace has quickened in plant lipid chemistry . . . most of the significant problems still await solution. . . . The lipid composition of no plant or plant seed is completely known" (*8*). The pace has indeed quickened, facilitated by significant advances in separations, purification, analysis, identification, and structural elucidation of lipids, which offer greater hope for rewarding discovery than ever before. Scientists of different disciplines have stepped up efforts to discover and develop new crop sources of lipid raw materials for practical use by finding readily grown and harvested seed species with unique chemical composition (*9*). Thus the extensive opportunities for both scientific exploration and development of useful practical applications have attracted many investigators, including our group at the Northern Division, to the study of seed lipids.

We have become aware of many oil-rich seed species that have been neither utilized nor studied (*3*). Of about 6000 seed samples analyzed for gross composition in our laboratories in the past 8 years, 42 percent contained more than 20 percent lipid and 20 percent contained between 10 and 20 percent lipid (*10*). Among these and other seed lipids have been found many of novel composition, including constituents of previously unknown structure.

The author is chief of the Industrial Crops Laboratory at the U.S. Department of Agriculture's Northern Utilization Research and Development Division, Peoria, Illinois 61604.

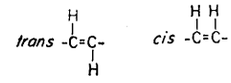
My purpose here is not to provide a complete or exhaustive review of the field, but rather to indicate research trends and newer findings so that those not conversant with current seed-lipid work will recognize the encouraging progress being made. Having mentioned our debt to new instrumental and analytical procedures and our dependence on them, in this article I shall ignore procedural details in citing the research results.

Glyceryl triesters of long-chain fatty acids, or triglycerides, usually comprise the major constituent of the lipid extracted from ground seed by organic solvents. Hence I shall consider the fatty acids of seed triglycerides first.

Unusual Olefinic Acids

The esterified fatty acids most prevalent in seed oils are oleic, linoleic, linolenic, palmitic, and stearic (*11*). For many years it has been known that other acids predominate in some oils; for example, ricinoleic acid in castor oil, lauric acid in coconut oil, and eleostearic acid in tung oil. In the past decade an imposing array of new naturally occurring fatty acids has been demonstrated in seed oils. The newly found acids are frequently major components and have structural features that mark them as quite unusual according to earlier concepts. In our own work at the Northern Division we have by now seen such a variety of structures that the unique is common, and we are no longer surprised by the diverse structures we continue to find.

The familiar, and by far the most prevalent, monoolefinic acids of seed oils are oleic (*cis*-9-octadecenoic), petroselinic (*cis*-6-octadecenoic), and erucic (*cis*-13-docosenoic) (*11*). Oleic acid is quite universally found; the other two occur in a number of species and in substantial quantity, but their distribution is limited to only a few plant families. Until recently *trans* monoenes had not been reported to occur naturally in vegetable oils; *cis* configuration of bonds was generally accepted as the pattern characteristic of unsaturated seed oils. Structural correlations among known acids suggested the empirical generalization that double bonds in the acids are, except for biological rarities, in positions that are $3n$ carbon atoms removed from one end of the chain (n = a small integer) (*12*). As a larger number and variety of seed oils are investigated, more and more monoenes are being found in which either the double bond does not fall into such positional patterns, or *trans* instead of *cis* geometrical isomers are present.



Acids I through VII in Table 1 exemplify some of the newly characterized compounds of this type.

Monounsaturation among 18-carbon seed-oil acids has been found at positions 3, 5, 6, 9, and 11. In the common monounsaturated acids having more than 18 carbon atoms, when the double bond is remote from the carboxyl group it generally occurs 9 carbons from the methyl end of the chain, as in oleic acid, and is *cis*. Thus, usual 20-, 22-, and 24-carbon monoolefinic acids have *cis* unsaturation at positions 11, 13, and 15, respectively, and are at least formally derivable from oleic acid by chain elongation, in 2-carbon units added at the carboxyl end of the molecule. In contrast, monoolefinic acids that have the unsaturation in greater proximity to the acidic function tend to be in the same 3 or 5 position regardless of chain length, and the bond may be *cis* or *trans*. This situation suggests a mechanism of biosynthesis controlled primarily by the carboxyl end after formation of the chain skeleton is complete. Monounsaturation at the 7 position has not so far been demonstrated in a seed lipid. Unsaturation at that location may yet be found, but possibly the 7 position is too far from the carboxyl to come under its positional influence, or precursor acids unsaturated at carbon 5 do not elongate because of a block of some sort in the biosynthetic mechanism. Unsaturation in both *cis* and *trans* forms, and in great positional diversity (including unsaturation at carbon 7), has been reported in sources other than seed lipids, including leaves, microorganisms, and animal products (*13*, and references in *14, 15, 16*).

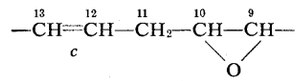
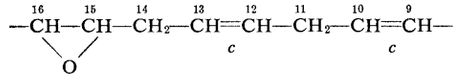
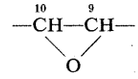
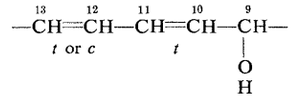
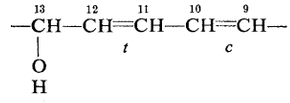
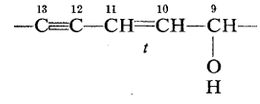
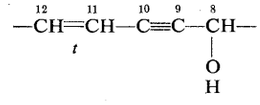
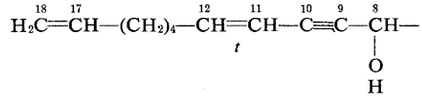
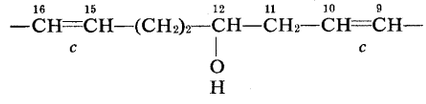
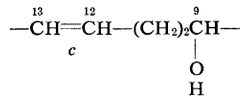
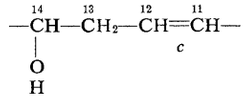
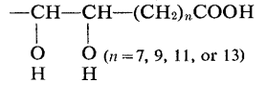
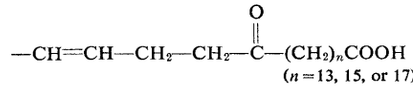
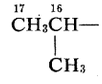
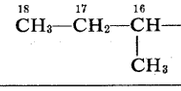
Polyolefinic unsaturation in all known seed-oil fatty acids classically follows one of two patterns: Either the unsaturation is conjugated (—CH=CH—CH=CH—), or any double bonds present are separated by a single methylene group in the hydrocarbon chain (—CH=CH—CH_2—CH=CH—). The first well-authenticated examples of structures in seed oils that deviate from this pattern in having more widely separated double bonds (—CH=CH—$[CH_2]_{n>1}$—CH=CH—) have been found only in the past few years. These polyunsaturated acids with "remote" or "lonely" double bonds, however, appear to be more than biogenetic oddities, for they have been discovered in several widely separated families of the plant kingdom (Nos. VIII through XIV, Table 1).

In this new class of fatty acids may be found isolated double bonds separated by 2, 4, or 6 methylene groups from other olefinic linkages. The isolated bonds nearest the carboxyl end of the acid so far encountered are either in the 3 or the 5 position and may be *cis* or *trans* in geometrical configuration; other centers of unsaturation in the molecules are in conventional positions. Since monoenoic acids having unsaturation in the unusual 3 or 5 positions frequently coexist in the oils with their corresponding polyunsaturates, we can speculate, though without experimental evidence at this time, that some type of dual biosynthetic mechanism is operative. Such a mechanism may be responsible for independent synthesis of different centers of unsaturation in these polyunsaturated fatty acids containing both usual and unusual positions of unsaturation, widely separated from one another in the acid molecules. Possible support for such a postulate comes from research on biosynthesis of similar polyunsaturated acids in slime molds, in which evidence is presented for specific and sequential reactions to desaturate long-chain acids first at position 9 and then at 5 (*17*).

Both positional and geometric isomers are known for the usual all-*cis* polyenoic acids with one methylene group separating neighboring double bonds (also called the divinylmethane arrangement of double bonds). Positional isomers are typified by γ-linolenic (all-*cis* 6,9,12-octadecatrienoic) acid and related compounds in which the unsaturation sequence begins at the 6 instead of the 9 position. Recently reported seed sources of such acids include hops (*18*) and various members of the borage family (*19*). Gamma-linolenic acid has long been known as a constituent of seed oil of the evening primrose (*11*). These isomeric polyenoic acids are currently of some interest as possible precursors of the physiologically active prostaglandins (*20*). The all-*trans* geometrical isomer of linoleic acid has recently been shown to comprise 15 percent of the seed-oil acids of a plant of the Bignoniaceae. In

Table 1. Examples of recently discovered seed-oil fatty acids having structures unusual for vegetable oils (Numbers on carbon atoms refer to chain position, numbering from carboxyl group; *c* = *cis*, *t* = *trans*). The structural parts listed have been found in only one species in a family, except where otherwise noted.

Compound	No. of carbon atoms	Unique structural part of fatty acid molecule	Plant family and references
		Monoolefinic acids	
I	16	$CH_3(CH_2)_{11}-\overset{4}{C}H=\underset{t}{\overset{3}{C}H}-\overset{2}{C}H_2-COOH$	Compositae (29 species) (*10, 14, 63*)
II	18	$-\overset{4}{C}H=\underset{t}{\overset{3}{C}H}-$	Compositae (7 species) (*10, 63*)
III	16, 18	$-\overset{6}{C}H=\underset{t \text{ or } c}{\overset{5}{C}H}-$	Ranunculaceae (2 species) (*15, 64*)
IV	20	$-\overset{6}{C}H=\underset{c}{\overset{5}{C}H}-$	Limnanthaceae (7 species), Ranunculaceae (*43, 44, 64*)
V	22	$-\overset{6}{C}H=\underset{c}{\overset{5}{C}H}-$	Limnanthaceae (7 species), (*43, 44*)
VI	16	$-\overset{12}{C}H=\underset{c}{\overset{11}{C}H}-$	Proteaceae (*65*)
VII	18	$-\overset{12}{C}H=\underset{c}{\overset{11}{C}H}-$	Asclepidaceae, Bignoniaceae (*16, 66*)
		Polyolefinic acids	
VIII	18	$-\overset{10}{\underset{c}{C}}H=\overset{9}{\underset{c}{C}}H(CH_2)_2\overset{6}{\underset{t}{C}}H=\overset{5}{C}H-$	Ranunculaceae (*64*)
IX	18	$-\overset{13}{\underset{c}{C}}H=\overset{12}{\underset{c}{C}}H-\overset{11}{C}H_2-\overset{10}{\underset{c}{C}}H=\overset{9}{\underset{c}{C}}H-(CH_2)_2-\overset{6}{\underset{t}{C}}H=\overset{5}{C}H-$	Ranunculaceae (11 species) (*10, 15, 64, 67*)
X	18	$-\overset{13}{\underset{c}{C}}H=\overset{12}{\underset{c}{C}}H-\overset{11}{C}H_2-\overset{10}{\underset{c}{C}}H=\overset{9}{\underset{c}{C}}H-(CH_2)_2-\overset{6}{\underset{c}{C}}H=\overset{5}{C}H-$	Pinaceae, Compositae (*68*)
XI	18	$-\overset{13}{\underset{c}{C}}H=\overset{12}{\underset{c}{C}}H-\overset{11}{C}H_2-\overset{10}{\underset{c}{C}}H=\overset{9}{\underset{c}{C}}H-(CH_2)_4-\overset{4}{\underset{t}{C}}H=\overset{3}{C}H-$	Compositae (22 species) (*69*)
XII	18	$-\overset{16}{\underset{c}{C}}H=\overset{15}{\underset{c}{C}}H-\overset{14}{C}H_2-\overset{13}{\underset{c}{C}}H=\overset{12}{\underset{c}{C}}H-\overset{11}{C}H_2-\overset{10}{\underset{c}{C}}H=\overset{9}{\underset{c}{C}}H-(CH_2)_4-\overset{4}{\underset{t}{C}}H=\overset{3}{C}H-$	Bignoniaceae (*70*)
XIII	20	$-\overset{15}{\underset{c}{C}}H=\overset{14}{\underset{c}{C}}H-\overset{13}{C}H_2-\overset{12}{\underset{c}{C}}H=\overset{11}{\underset{c}{C}}H-(CH_2)_4-\overset{6}{\underset{c}{C}}H=\overset{5}{C}H-$	Taxaceae, Equisetaceae (2 species), Ginkoaceae (*71*)
XIV	22	$-\overset{14}{\underset{c}{C}}H=\overset{13}{\underset{c}{C}}H-(CH_2)_6-\overset{6}{\underset{c}{C}}H=\overset{5}{\underset{c}{C}}H-$	Limnanthaceae (7 species) (*43, 45*)
		Acetylenic and allenic acids	
XV	18	$-\overset{13}{C}\equiv\overset{12}{C}-\overset{11}{C}H_2-\overset{10}{\underset{c}{C}}H=\overset{9}{\underset{c}{C}}H-$	Compositae (22 species) (*10, 72*)
XVI	18	$-\overset{10}{C}\equiv\overset{9}{C}-$	Santalaceae (*73*)
XVII	18	$H_2\overset{18}{C}=\overset{17}{C}H-(CH_2)_6-\overset{10}{C}\equiv\overset{9}{C}-$	Santalaceae (*39*)
XVIII	18	$-\overset{7}{C}H=\overset{6}{C}=\overset{5}{C}H-$	Labiatae (53 species) (*26*)
XIX	8	$HO\overset{8}{C}H_2-\overset{7}{C}H=\overset{6}{C}=\overset{5}{C}H-$	Euphorbiaceae (*27*)
		Oxygenated acids	
XX	18	$-\overset{13}{C}H-\overset{12}{C}H-\overset{11}{C}H_2-\underset{c}{\overset{10}{C}}H=\overset{9}{C}H-$ (O bridge between 13 and 12)	Compositae (9 species), Malvaceae (8 species), Euphorbiaceae (3 species), Valerianaceae, Onagraceae, Dipsacaceae (2 species) (*10, 11, 28*)

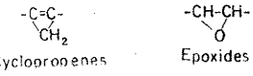
Compound	No. of carbon atoms	Unique structural part of fatty acid molecule	Plant family and references
XXI	18		Compositae (3 species) (*10, 74*)
XXII	18		Cruciferae (*75*)
XXIII	18		Compositae (*76*)
XXIV	18		Compositae (7 species) (*10, 77, 78*)
XXV	18		Compositae (3 species), Coriariaceae (*10, 78*)
XXVI	18		Compositae (*79*)
XXVII	18		Santalaceae, Olacaceae (*40, 80*)
XXVIII	18		Santalaceae (*40*)
XXIX	18		Cruciferae (5 species) (*81, 82*)
XXX	18		Apocynaceae (13 species) (*83*)
XXXI	20		Cruciferae (15 species) (*82, 84*)
XXXII	18, 20, 22, 24		Cruciferae (*85*)
XXXIII	24, 26, 28		Bignoniaceae (*86*)
		Fatty acids with novel carbon chain skeletons	
XXXIV	18		Scrophulariaceae (*38*)
XXXV	19		Scrophulariaceae (*38*)

the same seed oil the major component (*21*) is another isomer of linoleic acid, the conjugated *trans*-10, *trans*-12 isomer.

Acetylenes and Allenes

Until 1964 only one acetylenic seed-oil fatty acid was known in which the triple bond was not conjugated with other olefinic or acetylenic groupings. This acid was tariric (octadec-6-ynoic). Compounds XV through XVII in Table 1 are newly characterized additional nonconjugated acetylenes. Tariric acid and acids XV and XVI have structural similarity in position of unsaturation to the olefinic acids petroselinic, linoleic, and oleic, respectively. As a natural sequel to this relation to known acids, hypotheses concerning biosynthetic ties are appearing (*22–25*). While this article was being prepared, it was proposed that the methylene-interrupted enyne XV, called crepenynic acid, occupies a critically important position as an intermediate in the biosynthesis of polyacetylenes and other polyunsaturated acids (*22–25*). Recent experimental data seem consistent with the formulation of some type of desaturation mechanism for formation of the various acetylenic acids and other natural acetylenes, but details have yet to be worked out. Crepenynic acid is present in the fungus *Tricholoma grammopodium* as well as in higher plants.

Acetylenic functions coexist in the same seed oils as cyclopropenes and epoxides in amounts and positions such as to suggest strongly that these various groups are related biosynthetically.



Thus, when stearolic acid (XVI) is the principal acetylenic compound found, the major cyclopropenoid acid has the three-membered ring in the 9,10 position, and when heptadec-8-ynoic acid ($CH_3-(CH_2)_7-C\equiv C-(CH_2)_6-COOH$) is the predominant acetylene, the cyclopropenoid group is primarily in the 8,9 position. The presumption is that a one-carbon unit was added across the triple bond (*23*) to form the three-membered ring. The alkylation may be analogous to that in bacteria, in which the methyl group of methionine (in the form of *S*-adenosylmethionine) reacts with a monoolefinic acid and furnishes the ring carbon source for the biosynthesis of cyclopropane fatty acids; the mechanism and intermediates of the transmethylation process are not known (*24*). Alternatively, the cyclopropenoid acids may originate by desaturation of the corresponding cyclopropanes (*25*), in which case their biosynthetic relationship to coexisting acetylenes would be obscure at this time.

In comparing different seed oils from species of *Crepis* (family Compositae) we find (*10*) that the sum of the acetylenic acid XV plus epoxy acid XX bears an inverse linear relationship to the amount of linoleic acid present, again strongly suggesting biosynthetic interrelationships. Since XV makes up as much as 60 percent of the acids of some seed oils, quantities may become available for further needed studies of its synthesis in plants as well as of its metabolic fate in animals. In both respects, comparison with the formally related linoleic acid will prove interesting.

In addition to its nonconjugated acetylene group, compound XVII has a terminal double bond, also a rare occurrence in seed lipids.

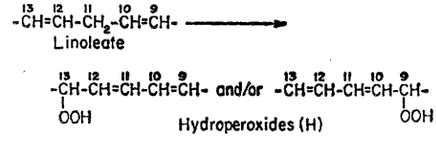
Allene functions are uncommon in nature, and only recently have they been discovered in seed lipids (*26, 27*). Like the acetylenes, natural allenic groups have usually been part of a conjugated system. Most known ones are fungal metabolites. The two seed-oil acids XVIII and XIX, both optically active as a result of molecular asymmetry conferred by the allene group, are the only known naturally occurring nonconjugated allenes. The ease of interconversion of some allenes and acetylenes in the laboratory suggests that XVIII could have a biogenetic relationship to tariric acid. Hydroxy acid XIX exists naturally, acylated on the hydroxyl group with 2,4-decadienoic acid and esterified on the carboxyl group with one of the primary hydroxyls of glycerol. Thus the glyceride that contains XIX yields upon hydrolysis four instead of the usual three fatty acid moieties.

New Oxygenated Fatty Acids

Another facet of chemical work on seed-oil fatty acids is the unexpectedly wide distribution of some of the newly found compounds which so frequently becomes apparent soon after their initial discovery. Only in 1954 was the first natural epoxy acid isolated and structurally characterized as XX, *cis*-12,13-epoxy-*cis*-9-octadecenoic acid. Not only is this substance prevalent in many seed oils in different plant families, but both of its enantiomorphic forms (*28*), as well as three additional new epoxy acids, have been characterized. These acids, XXIII, XX and XXI, and XXII, may be regarded, formally at least, as derivatives of oleic, linoleic, and linolenic acids, respectively, in which one of the usually present double bonds is epoxidized. It is probably only a matter of time until plant sources of the two remaining epoxy acids corresponding to epoxidation of the 9 or 12 olefinic bond of linolenic acid are discovered. Chromatographic and chemical evidence suggests the presence of XX, XXI, or similar acids in several additional Compositae and Leguminosae seed oils from which their isolation in pure form has not been undertaken (*11, 29*).

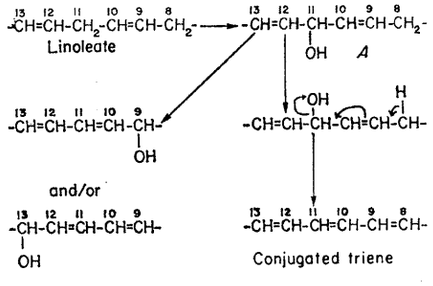
Again, our knowledge of the sequential synthetic relationships of these new epoxy acids is deficient. Are the epoxides precursors or end products of other coexisting olefinic or acetylenic acids, or are they side products of common precursors? Are they related to the unsaturated acids through intermediation of natural mono- or dihydroxy acids? This is a fertile field for investigation.

In 1960 the first representative (XXIV) of a unique new class of naturally occurring seed-oil acids was reported in which a hydroxyl group is alpha to conjugated diolefinic unsaturation. Since that time the more general occurrence of this type of compound has been recognized (*30*). In some species the hydroxyl groups are all at carbon 9, in others exclusively at carbon 13, and in some species both positional isomers (XXIV, XXV) are in the same oil. Unsaturation nearest the hydroxyl group in the compounds examined has *trans* geometric configuration; the other olefinic bond may be either *cis* or *trans*. Biosynthesis of these compounds from linoleate can easily be rationalized through the reactions of the sort shown below, though direct experimental evidence is lacking. A conjugated dienoid hydroperoxide (H), which is formed from linoleate either upon autoxidation or lipoxidase-catalyzed oxidation, can be reduced in vitro to the α-hydroxy conjugated diene.



Autoxidation provides a mixture of 9- and 13-hydroxy isomers, whereas some lipoxidase systems result in high positional specificity (*10, 31*).

Conjugated trienoic acids frequently occur in seed oils of species of Compositae closely related to those which contain the conjugated diene-ols. Gunstone (*32*) has proposed a plausible scheme for rationalizing a biosynthetic pathway for both these classes of compounds, which involves their derivation from a common precursor intermediate such as the 11-hydroxylated compound (*A*), formed from linoleate:



Although there is no direct evidence for intermediate *A*, the observed hydroxy acids may be readily derived from it (diagonal arrow) by the anionotropic rearrangement so familiar to organic chemists. Also, conjugated trienes might easily be formed by 1,4 dehydration, through the types of shifts indicated, to either the 8,10,12 isomer shown or to the 9,11,13 isomer. Other investigators (*33*) agree that linoleic acid is the likely precursor of conjugated trienes but consider intermediate *A* "neither necessary nor likely." Compounds XXVI through XXVIII, in which the unsaturated system vicinal to the hydroxyl group contains one acetylenic bond, may be formed in a similar manner through an enyne, like XV, or by further desaturation of a conjugated dien-ol. Compound XXVIII, like XVII, contains the terminal olefinic bond infrequently found in seed oils.

Compound XXIX, which is unique among seed-oil acids in having its hydroxyl group between centers of unsaturation, is related formally in structure to linolenic acid, from which it might be derived by the addition of elements of water to the double bond in the 12 position. Compound XXX may, like ricinoleic acid, be formed from a monoolefinic acid as an immediate precursor (*34*). It could also be an interconversion product with linoleic acid, derived either from it or as an intermediate in its synthesis. Acid XXXI, a higher homolog of ricinoleic acid, is probably formed from it in the seed by chain lengthening. This elongation reaction has been demonstrated by use of an enzyme system of avocado mesocarp (*35*).

Dihydroxy acids are uncommon in seed oils and if present normally occur only in low percentages. However, the cruciferous plant *Cardamine impatiens* contains in its seed lipids some 25 percent of saturated long-chain vicinal dihydroxy acids of varying chain lengths (XXXII). The hydroxyl groups are located on carbons 9 and 10, *counting from the methyl end* of each acid. Since the hydroxyl positions correspond to locations of the double bonds in oleic, 11-eicosenoic, erucic, and 15-tetracosenoic acids—all commonly found in oilseeds of Cruciferae—the *Cardamine* dihydroxy acids and those monoolefinic acids probably have a biosynthetic relationship. In no vicinal pair in the original *Cardamine* oil are both hydroxyl groups free (*10*). Possibly each glyceride molecule contains more than three acyl groups, in an estolide (polyester-type) structure like that proposed for some other seed oils that contain hydroxy acids (*36*).

The three compounds represented by XXXIII contain two structural features rare in seed lipids—the keto group and chains longer than 22 carbon atoms. The only two previously known long-chain keto acids of seeds are the long-known licanic acid of oiticica oil (*11*) and a small percentage of the keto compound corresponding to XXIV that occurs with it in *Dimorphotheca sinuata* seed oil (*37*). Both of these other keto acids are 18-carbon compounds. The homologous relationship of the three keto acids XXXIII suggests the possibility of a chain-lengthening mechanism for elaboration of the 26- and 28-carbon compounds.

Acids with Novel Chain Skeletons

Although the great preponderance of known seed-oil acids have skeletal structures that are unbranched and that contain an even number of carbon atoms, usually 16 or 18, striking exceptions are being discovered as more plants are examined. The seed oil of the snapdragon, *Antirrhinum majus,* has a homologous series of fatty acids with methyl branches on the chain (XXXIV and XXXV) (*38*). *Iso* and *anteiso* acids (*38*) are quite prevalent in some animal and bacterial lipids but were not definitely identified in a seed until 1965. In the snapdragon these acids are present in quite small percentage, particularly the *anteiso* compounds that have an odd number of carbon atoms.

In contrast, seed oil of the South American tree *Acanthosyris spinescens* contains 34 percent of normal-chain 17-carbon acids, by far the highest concentration of acids with an odd number of carbon atoms in a straight chain for any higher plant source (*39, 40*). Since the 17-carbon compounds are homologous with co-occurring 18-carbon acids, like XVII and XXVIII, except for the number of methylene groups between the carboxyl and the first other functional group (either OH or unsaturation), 1-carbon degradation by alpha-oxidation is a probable biogenetic mechanism for formation of the odd-numbered chain compounds (*41*). A similar relationship exists between the better-known cyclopropenoid acids, malvalic and sterculic, which differ by a single carbon atom between the carboxyl and cyclopropenoid functions. Sterculic acid, itself odd-numbered but undoubtedly formed by addition of 1 carbon to an even-numbered precursor, gives rise to malvalic acid by alpha-oxidation (*42*).

Though only normal, even-numbered chains are present, seed oil of the meadowfoam plant, *Limnanthes douglasii,* is sufficiently unusual to merit mention. Over 95 percent of the acids in that seed oil contain more than 18 carbon atoms, predominantly IV (*43–45*).

Fine Structure of Triglycerides

Seed-oil triglycerides are complex mixtures of individual compounds in which the different esterified fatty acyl groups may be arranged in various permutations and combinations among the three hydroxyl groups of glycerol molecules. Investigators have frequently been content to identify the fatty acids obtained by hydrolysis of an oil and to report the average percentage of each component acid. Only a relatively few have attempted to define the distribution of fatty acids in individual molecular species, and most often those species were not completely separated from one another and characterized as molecular entities. Now, with better methodology and instrumentation to facilitate identification of fatty acids, an important trend in seed-lipid re-

search is to direct more attention toward determination (i) of positional distribution of acyl groups between primary and secondary hydroxyls, (ii) of intermolecular distribution patterns of acyl groups among various triglycerides, and (iii) of any positional specificity of different fatty acids for one of the two primary hydroxyl groups, now known to be biochemically different, which would cause the beta-carbon atom of the glycerol moiety to be asymmetric.

Hydrolysis of a triglyceride with pancreatic lipase under suitably controlled conditions causes selective removal of fatty acyl groups from the 1 and 3 positions of a triglyceride (*46*). Isolation of residual monoglycerides and identification of the acid (or acids) present then provide data on the groups esterified onto the beta position. Application of this technique shows that acyl groups are usually *not* randomly distributed among the three glyceridic hydroxyl groups. Rather, researchers agree that there is a distinct preference of saturated fatty acids and of those having more than 18 carbon atoms for the alpha positions. For example, the 22-carbon erucic acid, which comprises 55 to 60 percent of the acids of *Crambe abyssinica* seed oil, is almost entirely esterified onto positions 1 and 3 of the glycerol (*47*). Conversely, the beta position of glycerol in seed oils is preferentially acylated by the usual 18-carbon unsaturated fatty acids. However, these positional preferences are relative rather than absolute. Empirical examination of data from a number of seed oils suggests a pattern of priority differences among unsaturated acids in their selectivity for the beta position (*48*), and semiquantitative concepts of "enrichment factors" and "selectivity factors" have been devised to express these differences in affinity. Numerous other attempts have been made to quantitate data on distribution of fatty acyl groups among natural triglycerides, but mathematical expression of results must be regarded as still in a transitional phase where further changes are likely.

Despite the apparent qualitative adherence to positional preferences as mentioned, one cannot yet predict a priori with any confidence the disposition of acyl groups in previously uninvestigated seed oils, especially when an acid containing unusual structures is present. For example, the vernolic (*cis*-12,13-epoxyoleic) acid (about 70 percent) of *Vernonia anthelmintica* seed oil is there almost exclusively as the single compound trivernolin, its triglyceride ester (*49*). The same epoxy acid is distributed quite differently among the triglyceride molecules of *Euphorbia lagascae* seed oil (57 percent vernolic acid), which contains only 18.5 percent trivernolin (*50*).

Modern lipid chemists also want to know whether in the synthesis of triglycerides nature distinguishes between the two primary hydroxyl groups of glycerol. This stereochemical aspect of glyceride structure was bypassed for many years because of two difficulties. First, a homogeneous triglyceride molecular species is hard to obtain from a complex natural mixture. Second, asymmetry (*51*) at carbon 2, even if present, is so slight in triglycerides of usual fatty acids that no measurable optical activity has been observed. Authentically asymmetric triglycerides, like (R)-1-stearo-dipalmitin, have been synthesized by unequivocal organic chemical procedures and shown to have optical rotations at the sodium D-line of 0.0° (*52*); instrumental or other experimental restrictions still limit our ability to obtain a finite rotation for such compounds. A very small, but measurable, rotation at shorter wavelengths was recently obtained for synthetic lauro-dipalmitin (*52*). Several physical procedures, such as x-ray diffraction analysis and measurement of piezoelectric effect, can demonstrate asymmetry in a triglyceride but are of very restricted usefulness since pure crystalline triglycerides for such analyses are so difficult to isolate.

Now effective chemical methods have been devised for detecting stereochemical asymmetry in seed-oil glycerides (*52, 53*). That is, one can determine individually the fatty acyl groups on glycerol oxygens 1, 2, and 3. The basis common to several approaches used is shown in Fig. 1, in which we assume that we start with a triglyceride in which the middle carbon of the glycerol moiety is asymmetric. Pancreatic lipase attacks positions 1 and 3 substantially at random. This enzyme is allowed to act on the triglyceride long enough to produce the maximum content of diglyceride in the product. Free hydroxyl groups in the diglyceride fraction are then converted to a phosphate or phenylphosphate ester, to a trimethylsilyl ether, or to an acetate ester. Such replacement accentuates the asymmetry as compared to the original molecule. Further chemical treatments, separations, or specific enzymatic reactions are then applied to the new derivatives. Such a reaction sequence has in some cases permitted isolation or detection of optically active materials if the starting triglyceride was asymmetric. In other cases, though optical activity was not shown, the stereospecific reactions utilized did permit determination of the fatty acid composition at each position of a triglyceride. If the original triglyceride in Fig. 1 had been racemic, each diglyceride and its derivative would have comprised a racemic pair, devoid of optical activity.

Thus, starting with purified triglyceride fractions from selected natural fats, measurable optical activity was demonstrated in the separated trimethylsilyl derivatives of the diglycerides obtained by pancreatic lipolysis. The original triglycerides were therefore composed, predominantly or exclusively, of one optical isomer (*54*).

When Y in Fig. 1 is phenylphosphate, L- and D-forms of phosphatidyl phenol are present; only the L-phosphatide is selectively attacked by phospholipase A from snake venom to remove the acyl group from the 2 position. The residual acyl groups on the 1 position of the molecules attacked by the phospholipase can be identified and are derived from the original 1 position of the starting triglyceride. Fatty acid composition of acyl groups in the 2 position is known from pancreatic lipase studies, and overall composition for all 3 positions is known by identification of acids in a total hydrolyzate. Acids in the 3 position are obtained by difference. Thus it is possible by this scheme to specify the fatty acyl that was on each individual position in the original triglyceride.

This approach is so new that it has not yet been applied to many triglycerides. Some triglycerides tested appear to be asymmetric or at least partially so, and in others positional preference between the two alpha carbons was not so evident (*54, 55*). Optimum interpretation demands application to a single triglyceride. The problem of isolating a pure triglyceride has been simplified by development of several new methods of separation: gas-liquid chromatography, which separates according to total number of carbon atoms (*56*); thin-layer or column chromatography, which separates fractions that differ in amount and geometric configuration of unsaturation (*57*); and various crystallization (*49*) and solvent-distribution methods (*58*). Frequently,

however, even these methods and combinations of them fail to yield products of desired purity. Still the ability, with some limitations (*52, 59*), to pinpoint the fatty acyl radical on each individual position of glycerol constitutes an important scientific advance that will contribute significantly to our knowledge of lipid structures.

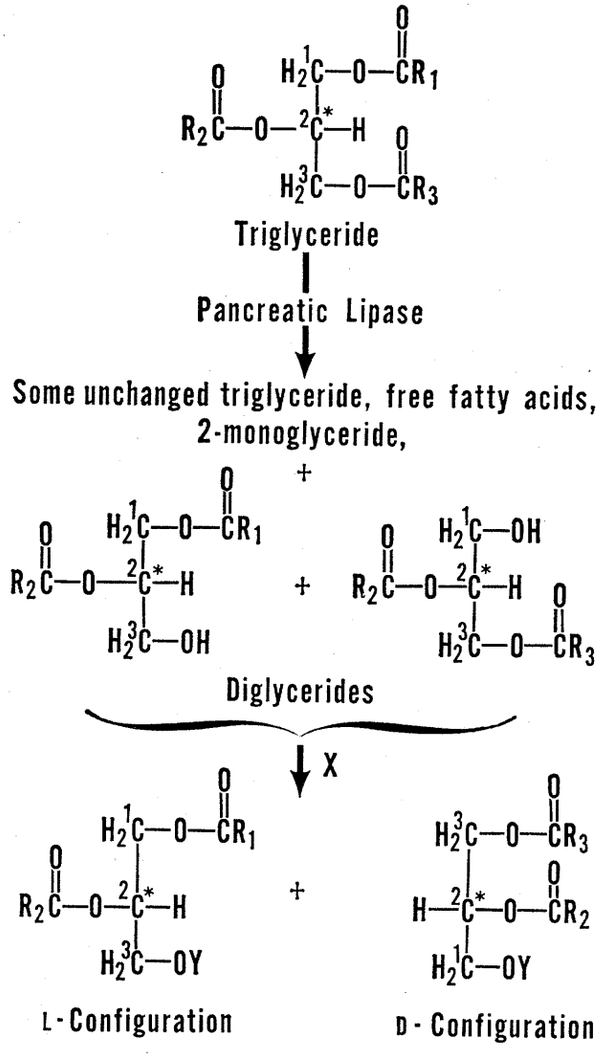
The first direct isolation of a naturally asymmetric seed-lipid triglyceride, without any structural modifications, has been achieved (*60*). The optically active fraction obtained was an *(S)*-α-acetotriglyceride containing C_{16} and C_{18} acids. Such a glyceride has greater asymmetry than those of seed oils containing *only* long-chain acids.

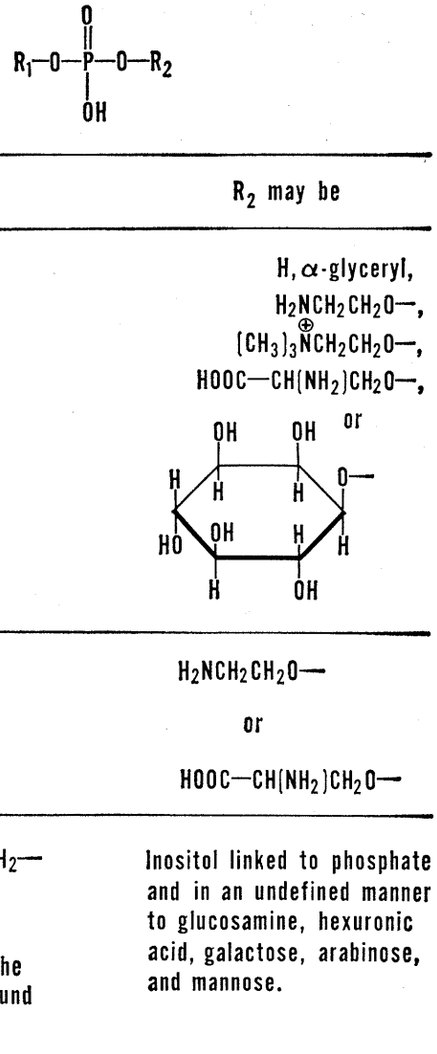
Other Seed-Lipid Constituents

Ubiquitously present with the triglycerides in seed lipids, but usually as only a few percent of the total amount of lipid extracted by solvent, is a complex mixture of phospholipids, hydrocarbons, sterols, triterpene alcohols, carotenoid and other pigments, tocopherols, aliphatic alcohols, glycolipids, proteolipids, and related materials. These should be mentioned for the sake of completeness in describing seed-lipid composition, though the treatment will not be exhaustive.

In phospholipids long hydrocarbon chains are present as fatty acyl groups, enol ether groups, or the backbone chain of the sphingosine molecule. The phosphoric acid is esterified to glycerol or to phytosphingosine. Typical structures are shown in Fig. 2. Despite the universal distribution of the phospholipids in living cells, definition of their specific function (or functions) and explanation for their occurrence in different structural forms are still to be made.

Squalene (Fig. 3), the most widely prevalent hydrocarbon in seed lipids, serves as a precursor of sterols and polycyclic terpenes. These two classes of compounds exist in many seed lipids either in free or esterified form or as glycosides or esterified glycosides. Typical examples of a triterpene alcohol



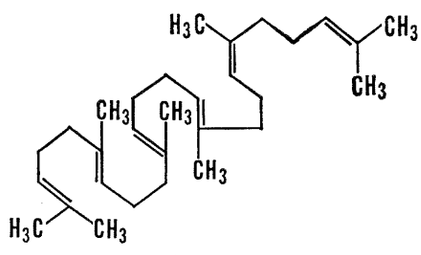


Figs. 1 and 2. Fig. 1 (left). Reactions used to demonstrate stereochemical asymmetry in seed-oil triglycerides. The starred carbon atom of the triglyceride is asymmetric when R_1 and R_3 are different. Y = phenylphosphate, trimethylsilyl, acetyl, or phosphate when, respectively, X = $Cl_2(C_6H_5O)P{=}O$, $[(CH_3)_3{-}Si{-}]_2NH$, Ac_2O + $HClO_4$, or adenosine triphosphate + diglyceride-kinase of *Escherichia coli*. Numbering on carbon atoms refers to their origin in the starting triglyceride. Fig. 2 (right). Typical seed phospholipid structures. Stereochemistry is unspecified except for inositol. R_3 and R_4 represent long-chain fatty chains, either the same or different, corresponding to residues of the common fatty acids such as palmitic, stearic, oleic, and linoleic. R_5 = $CH_3(CH_2)_{21\text{ or }23}CHOHC(=O)$— plus other long-chain fatty acyl groups (*87*).

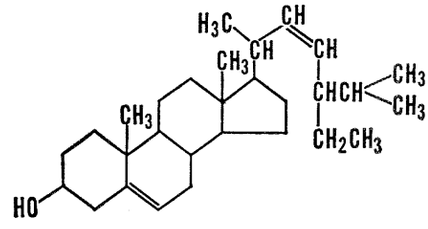
and of seed-lipid sterols are provided by β-amyrin, stigmasterol, and β-sitosterol (Fig. 3). Soybean oil stigmasterol is an important commercial precursor for conversion to steroid hormones.

Isomeric tocopherols (Fig. 3) of seed lipids function as antioxidants which retard oxidative deterioration of vegetable oils. The carotenoid, chlorophyll, and other pigments are trace components, which are, however, important in accounting for the color of many seed oils. Commerical refiners of seed oil, when concerned with the color of their edible oils, modify bleaching procedures on the basis of types of pigments in the oil.

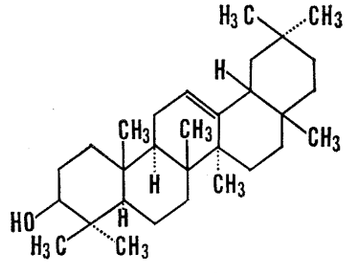
In rare instances nontriglyceride constituents of seed lipids are major components. In seed of the grass *Briza spicata,* the galactosyl glycerides, found earlier as trace constituents in other seeds, comprise a major portion of the lipids (Fig. 3) (*61*). The *Briza* lipid is a waxy solid instead of the fluid yellow oil usually obtained from seeds, and it contains only a minor quantity of conventional triglycerides. Actually there are other oilseeds in which components usually present in minor or trace amounts exist in substantial percentages of the seed lipid isolated (*10, 62*). Very few of such lipids have been examined chemically. In general, little is known about the functions in the plant of most of these various classes of compounds that coexist with the triglycerides in seeds.



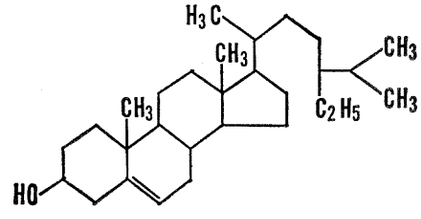
Squalene



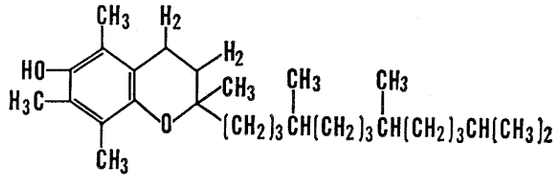
Stigmasterol



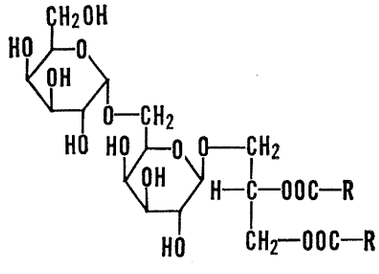
β-Amyrin



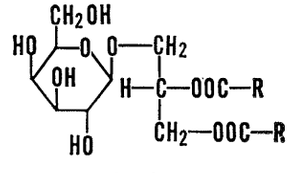
β-Sitosterol



α-Tocopherol



2,3-Diacyl-1-[α-D-galactopyranosyl-1,6-β-D-galactopyranosyl]-D-glycerol



2,3-Diacyl-1-[β-D-galactopyranosyl]-D-glycerol

Fig. 3. Prevalent seed hydrocarbon, sterol, triterpene alcohol, tocopherol, and glycolipid constituents.

Summary and Significance

Many of the newly discovered seed-oil acids have reactive or unusual functional groups or other facets of molecular structure that permit their ready differentiation from oleic, linoleic, linolenic, and the other most prevalent saturated and unsaturated long-chain fatty acids. The recognition and availability of the new acids, coupled with methods that make detection and determination easy, will help studies of lipid biosynthesis in the plant and of lipid metabolism and utilization in animals, and will stimulate more studies in depth on the fine points of seed-lipid structure. Correlations of structural patterns in seed lipids of particular groups of plants with classical taxonomic categories will permit clarifications, raise needed questions concerning classifications, and accelerate research in chemotaxonomy and phylogenetics. Seed lipids are particularly well suited for establishing relationships among plants because of their great variety in structure compared to the more limited structural types of amino acids, sugars, purines, and many other plant substances. The newly characterized seed oils are potentially important industrial raw materials whenever they come from agronomically promising plant species.

The molecular structures of seed triglycerides have major influence on their physical properties and therefore advances in knowledge in that sphere have practical implications. For example, the unusual characteristics of cocoa butter that make it so valuable for food and confectionery use are attributed to the specific *arrangement* of fatty acids it its triglycerides. The glycerides are almost all 2-oleic-1,3-disaturated acid triglycerides. The physical characteristics of lard are advantageously changed by catalytically rearranging fatty acyl groups among the glycerides initially in the fat to achieve a more nearly random distribution, followed sometimes by further fractionation to remove more saturated glycerides. Through this change of glyceride structures a preferred, less grainy texture is achieved. Future studies to understand, unravel, and control seed-oil tri-

glyceride structures will be significant in developing margarines of improved texture and "feel," cocoa-butter substitutes, and many other products. I expect rapid, fruitful progress in seed-lipid research and utilization to continue. Such progress will be aided by investigation of seed lipids from a large number and variety of different plants to find new types of fatty acids, to find new sources of familiar oils, and to obtain more data regarding their glyceride structures.

References and Notes

1. "Seeds," *Yearbook Agr. U.S. Dept. Agr.* (1961).
2. The terms "lipid," "oil," and "fat" are used interchangeably in this article to refer to seed substances soluble in nonpolar organic solvents.
3. F. R. Earle and Q. Jones, *Econ. Botany* **16**, 221 (1962).
4. "The World Agricultural Situation, Review of 1965 and Outlook for 1966," *U.S. Dept. Agr. Foreign Agr. Econ. Rept. No. 28* (1966), pp. 18–19.
5. A. M. Altschul, *Proteins, Their Chemistry and Politics* (Basic Books, New York, 1965).
6. E. Jones, *Chem. Brit.* **2**, 6 (1966).
7. T. P. Hilditch, *J. Am. Oil Chemists' Soc.* **42**, 745 (1965).
8. C. F. Allen and P. Good, *ibid.*, p. 610.
9. I. A. Wolff and Q. Jones, in "After a Hundred Years," *Yearbook Agr. U.S. Dept. Agr.* (1962).
10. Unpublished results, U.S. Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois.
11. T. P. Hilditch and P. N. Williams, *The Chemical Constitution of Natural Fats* (Wiley, New York, ed. 4, 1964).
12. J. A. Lovern, *J. Sci Food Agr.* **9**, 773 (1958).
13. H. P. Kaufmann and G. Mankel, *Fette Seifen Anstrichmittel* **66**, 6 (1964); E. D. Korn, *J. Lipid Res.* **5**, 352 (1964); G. Scheuerbrandt and K. Bloch, *J. Biol. Chem.* **237**, 2064 (1962).
14. C. Y. Hopkins and M. J. Chisholm, *Can. J. Chem.* **42**, 2224 (1964).
15. M. O. Bagby, C. R. Smith, Jr., K. L. Mikolajczak, I. A. Wolff, *Biochemistry* **1**, 632 (1962).
16. M. J. Chisholm and C. Y. Hopkins, *J. Am. Oil Chemists' Soc.* **42**, 49 (1965).
17. F. Davidoff and E. D. Korn, *J. Biol. Chem.* **238**, 3199, 3210 (1963).
18. J. B. Roberts and R. Stevens, *Chem. Ind. London* **1963**, 608 (1963).
19. R. Kleiman, F. R. Earle, I. A. Wolff, Q. Jones, *J. Am. Oil Chemists' Soc.* **41**, 459 (1964).
20. B. Samuelsson, *Angew. Chem. Intern. Ed. Engl.* **4**, 410 (1965).
21. M. J. Chisholm and C. Y. Hopkins, *Can. J. Chem.* **41**, 1888 (1963).
22. J. D. Bu'Lock and G. N. Smith, *Biochem. J.* **98**, 6P (1966); J. D. Bu'Lock, in "Phytochemical Group Symposium on Comparative Phytochemistry," T. Swain, Ed. (Academic Press, New York, in press); F. Bohlmann, K. Kleine, H. Bornowski, *Chem. Ber.* **99**, 142 (1966).
23. G. N. Smith and J. D. Bu'Lock, *Chem. Ind. London* **1965**, 1840 (1965).
24. W. M. O'Leary, in *Transmethylation and Methionine Biosynthesis*, S. K. Shapiro and F. Schlenk, Eds. (Univ. of Chicago Press, Chicago, 1965).
25. N. K. Hooper and J. H. Law, *Biochem. Biophys. Res. Commun.* **18**, 426 (1965).
26. M. O. Bagby, C. R. Smith, Jr., I. A. Wolff, *J. Org. Chem.* **30**, 4227 (1965).
27. H. W. Sprecher, R. Maier, M. Barber, R. T. Holman, *Biochemistry* **4**, 1856 (1965).
28. F. D. Gunstone, *J. Chem. Soc.* **1954**, 1611 (1954); C. Y. Hopkins and M. J. Chisholm, *J. Am. Oil Chemists' Soc.* **37**, 682 (1960).
29. L. J. Morris, R. T. Holman, K. Fontell, *J. Lipid Res.* **2**, 68 (1961).
30. L. J. Morris, R. T. Holman, K. Fontell, *J. Am. Oil Chemists' Soc.* **37**, 323 (1960).
31. M. Hamberg and B. Samuelsson, *Biochem. Biophys. Res. Commun.* **21**, 531 (1965).
32. F. D. Gunstone, *Chem. Ind. London* **1965**, 1033 (1965).
33. R. C. Badami and L. J. Morris, *J. Am. Oil Chemists' Soc.* **42**, 1119 (1965).
34. A. T. James, H. C. Hadaway, J. P. W. Webb, *Biochem. J.* **95**, 448 (1965).
35. S. F. Yang and P. K. Stumpf, *Biochim. Biophys. Acta* **98**, 27 (1965).
36. K. T. Achaya and J. S. Aggarwal, *Chem. Ind. London* **1962**, 1616 (1962).
37. R. G. Binder, T. H. Applewhite, M. J. Diamond, L. A. Goldblatt, *J. Am. Oil Chemists' Soc.* **41**, 108 (1964).
38. A. Radunz, *Z. Physiol. Chem.* **341**, 192 (1965); the terms "iso" and "anteiso" are used in fatty-acid terminology to indicate branching by a methyl group either one or two carbons removed from the noncarboxyl end of the chain.
39. R. G. Powell and C. R. Smith, Jr., *Biochemistry* **5**, 625 (1966).
40. R. G. Powell, C. R. Smith, Jr., C. A. Glass, I. A. Wolff, *J. Org. Chem.* **31**, 528 (1966).
41. C. Hitchcock and A. T. James, *J. Lipid Res.* **5**, 593 (1964).
42. G. N. Smith and J. D. Bu'Lock, *Biochem. Biophys. Res. Commun.* **17**, 433 (1964).
43. R. W. Miller, M. E. Daxenbichler, F. R. Earle, H. S. Gentry, *J. Am. Oil Chemists' Soc.* **41**, 167 (1964).
44. C. R. Smith, Jr., M. O. Bagby, T. K. Miwa, R. L. Lohmar, I. A. Wolff, *J. Org. Chem.* **25**, 1770 (1960).
44. C. R. Smith, Jr., M. O. Bagby, T. K. Miwa, R. L. Lohmar, I. A. Wolff, *ibid.*, **26**, 1261 (1961).
46. F. H. Mattson and L. W. Beck, *J. Biol. Chem.* **219**, 735 (1956); P. Savary and P. Desnuelle, *Biochim. Biophys. Acta* **21**, 349 (1956).
47. F. H. Mattson and R. A. Volpenhein, *J. Biol. Chem.* **236**, 1891 (1961).
48. F. D. Gunstone, R. J. Hamilton, F. B. Padley, M. I. Qureshi, *J. Am. Oil Chemists' Soc.* **42**, 965 (1965).
49. C. F. Krewson, J. S. Ard, R. W. Riemenschneider, *ibid.* **39**, 334 (1962).
50. R. Kleiman, C. R. Smith, Jr., S. G. Yates, Q. Jones, *ibid.* **42**, 169 (1965).
51. The excellent article of H. Hirschmann [*J. Biol. Chem.* **235**, 2762 (1960)] is recommended for a more detailed discussion and differentiation of the different types of asymmetry than can be provided here; Hirschmann's article will be especially useful to those who read further in the current literature on triglyceride stereochemistry.
52. W. Schlenk, Jr., *J. Am. Oil Chemists' Soc.* **42**, 945 (1965).
53. M. H. Coleman, *ibid.* **42**, 1040 (1965); P. M. Slakey, W. E. M. Lands, R. A. Pieringer, *Federation Proc.* **25**, 521 (1966); H. Brockerhoff, *J. Lipid Res.* **6**, 10 (1965).
54. L. J. Morris, *Biochem. Biophys. Res. Commun.* **20**, 340 (1965).
55. H. Brockerhoff and M. Yurkowski, *J. Lipid Res.* **7**, 72 (1966).
56. C. Litchfield, R. D. Harlow, R. Reiser, *J. Am. Oil Chemists' Soc.* **42**, 849 (1965).
57. B. De Vries, *Chem. Ind. London* **1962**, 1049 (1962); C. B. Barrett, M. S. J. Dallas, F. P. Padley, *ibid.*, p. 1050.
58. H. J. Dutton and C. R. Scholfield, *Progr. Chem. Fats Lipids* **6**, 313 (1963).
59. H. Brockerhoff, *Lipids* **1**, 162 (1966).
60. R. Kleiman, R. W. Miller, F. R. Earle, I. A. Wolff, paper presented to Am. Oil Chemists' Soc., Los Angeles, Calif., April 1966; abstract, *J. Am. Oil Chemists' Soc.* **43**, 102A (1966); *Lipids* **1**, 286 (1966).
61. C. R. Smith, Jr., and I. A. Wolff, *Lipids* **1**, 123 (1966).
62. F. R. Earle, C. A. Glass, G. C. Geisinger, I. A. Wolff, Q. Jones, *J. Am. Oil Chemists' Soc.* **37**, 440 (1960).
63. R. Kleiman, F. R. Earle, I. A. Wolff, paper presented at national meeting, Am. Oil Chemists' Soc., Houston, Texas, April 1965; abstract, *J. Am. Oil Chemists' Soc.* **42**, 147A (1965).
64. M. K. Bhatty and B. M. Craig, *Can. J. Biochem.* **44**, 311 (1966).
65. M. E. deTomas, R. R. Brenner, P. Caltanes, *Rev. Arg. Grasas Aceites* **5**, 53 (1963).
66. M. J. Chisholm and C. Y. Hopkins, *Can. J. Chem.* **38**, 805 (1960).
67. H. P. Kaufmann and J. Barve, *Fette Seifen Anstrichmittel* **67**, 14 (1965).
68. E. Elomaa, T. Lehtinen, J. Alhojärvi, *Suomen Kemistilehti* **B36**, 52 (1963); R. G. Powell, C. R. Smith, Jr., I. A. Wolff, paper presented at national meeting, Am. Oil Chemists' Soc., Cincinnati, Ohio, Oct. 1965; abstract, *J. Am. Oil Chemists' Soc.* **42**, 450A (1965).
69. M. O. Bagby, W. O. Seigl, I. A. Wolff, *J. Am. Oil Chemists' Soc.* **42**, 50 (1965).
70. C. Y. Hopkins and M. J. Chisholm, *J. Chem. Soc.* **1965**, 907 (1965).
71. T. Takagi, *J. Am. Oil Chemists' Soc.* **41**, 516 (1964); H. Schlenk and J. L. Gellerman, *ibid.* **42**, 504 (1965).
72. K. L. Mikolajczak, C. R. Smith, Jr., M. O. Bagby, I. A. Wolff, *J. Org. Chem.* **29**, 318 (1964).
73. C. Y. Hopkins and M. J. Chisholm, *Tetrahedron Letters No. 40*, 3011 (1964).
74. C. R. Smith, Jr., M. O. Bagby, R. L. Lohmar, C. A. Glass, I. A. Wolff, *J. Org. Chem.* **25**, 218 (1960); R. G. Powell, C. R. Smith, Jr., I. A. Wolff, *J. Am. Oil Chemists' Soc.* **42**, 165 (1965).
75. F. D. Gunstone and L. J. Morris, *J. Chem. Soc.* **1959**, 2127 (1959).
76. M. J. Chisholm and C. Y. Hopkins, *Chem. Ind. London* **1959**, 1154 (1959).
77. C. R. Smith, Jr., T. L. Wilson, E. H. Melvin, I. A. Wolff, *J. Am. Chem. Soc.* **82**, 1417 (1960); C. R. Smith, Jr., M. C. Burnett, T. L. Wilson, R. L. Lohmar, I. A. Wolff, *J. Am. Oil Chemists' Soc.* **37**, 320 (1960).
78. M. J. Chisholm and C. Y. Hopkins, *Can. J. Chem.* **38**, 2500 (1960); C. Y. Hopkins and M. J. Chisholm, *ibid.* **43**, 3160 (1965).
79. R. G. Powell, C. R. Smith, Jr., C. A. Glass, I. A. Wolff, *J. Org. Chem.* **30**, 610 (1965).
80. S. P. Ligthelm, *Chem. Ind. London* **1954**, 249 (1954).
81. C. R. Smith, Jr., T. L. Wilson, R. B. Bates, C. R. Scholfield, *J. Org. Chem.* **27**, 3112 (1962).
82. K. L. Mikolajczak, F. R. Earle, I. A. Wolff, *J. Am. Oil Chemists' Soc.* **39**, 79 (1962).
83. F. D. Gunstone and L. J. Morris, *J. Sci. Food Agr.* **10**, 522 (1959).
84. C. R. Smith, Jr., T. L. Wilson, T. K. Miwa, H. Zobel, R. L. Lohmar, I. A. Wolff, *J. Org. Chem.* **26**, 2903 (1961).
85. K. L. Mikolajczak, C. R. Smith, Jr., I. A. Wolff, *J. Am. Oil Chemists' Soc.* **42**, 939 (1965).
86. C. R. Smith, Jr., and R. W. Miller, *Chem. Ind. London* **1965**, 1910 (1965). Also paper presented at national meeting, Am. Oil Chemists' Soc., Los Angeles, Calif., April 1966.
87. H. E. Carter, W. D. Celmer, D. S. Galanos, R. H. Gigg, W. E. M. Lands, J. H. Law, K. L. Mueller, T. Nakayama, H. H. Tomizawa, E. Weber, *J. Am. Oil Chemists' Soc.* **35**, 335 (1958).

88. I thank my colleagues F. L. Austin, F. R. Earle, and W. H. Tallent for constructive comments during the preparation of this manuscript, and especially C. R. Smith, Jr., who was also helpful in assembling recent references.