A Survey of the Conjugated Fatty Acids of Seed Oils[1,2]

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Abstract

Fatty acids with conjugated unsaturation occur in many seed oils. Thirty of these acids are reviewed with emphasis on their detection, isolation, and structure determination. Their distribution among plant families is shown, and a botanical source of each acid is given. Some reactions, derivatives, and methods of determining configuration are described. Current theories of their biosynthesis in the seed, involving oxygenated precursors, are summarized.

Introduction

ABOUT 20 FATTY ACIDS with conjugated unsaturation have been discovered in seed oils within the past few years. Only five acids of this class were known in seeds up to 1947. By 1957 the number had risen to 10 and in 1967 to about 30. It is likely that more will be found because of the increasing interest in fat components and the availability of new techniques. This review will describe the various types of conjugated acids (C_{10} to C_{18}), their occurrence, methods of isolation, determination of structure, and some of the theories of biosynthesis of the acids in the seed.

Conjugation is defined here as the system of alternate single and double (or triple) bonds, that is, 1,3 unsaturation. Fatty acids of this type are mostly of chain-length C_{18}, but there are a few C_{17} acids and one each of C_{10} and C_{12}. As yet, conjugated acids of chain-length C_{16} or C_{20} have not been found in seed oils.

A typical conjugated triene acid has the structural formula $CH_3(CH_2)_3CH \underset{\text{trans}}{=} CHCH \underset{\text{trans}}{=} CHCH \underset{\text{cis}}{=} CH(CH_2)_7COOH$ and the systematic name, *cis*-9,*trans*-11,*trans*-13-octadecatrienoic acid. It is generally referred to by its common name, α-eleostearic acid. Most of the conjugated acids have been given short or trivial names for convenience. The abbreviated notation proposed by Holman (1) is informative and will be used in the tables which follow. It employs the symbols h (hydroxy), k (keto), y (yne, triple bond), ep (epoxy), c (*cis*), t (*trans*), e (ethylenic bond). Under this system α-eleostearic acid is written as c9,t11,t13–18:3; the final figures give the chain length and number of double bonds.

Occurrence in Various Plant Families

Conjugated fatty acids have been identified in the seed oils of 11 families of plants. This is a very small proportion of the 330 known plant families, but, of course, the oils of many of these families have not been studied at all. All of the 11 families are classed as dicotyledons. No conjugated acids have been found as yet in the monocotyledons.

Table I shows the families and the orders to which they belong, according to Engler's classification (2). It is evident that conjugated acids occur more or less at random. They are found in families scattered through nine orders of plants (out of a total of 48), ranging from the primitive Santalaceae to the relatively recent Compositae. The occurrence of the various classes of conjugated acids by plant families is shown in Table II. Again there seems to be little evidence of any pattern.

TABLE I
Plant Orders and Families Generating Conjugated Acids in Their Seed Oils

Orders	Families
Santalales:	Olacaceae, Santalaceae
Rosales:	Rosaceae
Geraniales:	Euphorbiaceae
Sapindales:	Balsaminaceae, Coriariaceae
Cucurbitales:	Cucurbitaceae
Myrtiflorae:	Punicaceae
Tubiflorae:	Bignoniaceae
Dipsacales:	Valerianaceae
Campanulatae:	Compositae

In most of the families listed in Table II, some species produce conjugated acids whereas others produce no appreciable amount of conjugated acids. Except for the Santalaceae and one or two others, most families appear to have more species with non-conjugated oils than with conjugated. It is also noted that more than one type of conjugated acid can occur in the same family, and most families listed in Table II produce more than one kind of conjugated acid.

Natural Conjugated Acids

Simple Dienes

The typical conjugated acid in seed oils has the unsaturation near the middle of the chain. However there are two examples with a double bond at the 2-position, viz., 2,4-decadienoic (3) and 2,4-dodecadienoic acid (4), both found in seed oils of the Euphorbiaceae family (Table III). The C_{10} acid is combined in an unusual way in the glycerides (5). These acids are easily recognized by the UV band near 260 $m\mu$, indicative of two double bonds in conjugation with the carbonyl group. The IR and NMR spectra are also distinctive.

The *trans*-10,*trans*-12-octadecadienoic acid occurs in the seed oil of *Chilopsis linearis* (Bignoniaceae) (6,7) and possibly in another species of the same family (8). It was the first isomer of linoleic acid to be isolated and identified as a seed oil component. It constituted 9–12% of the total fatty acids. Surprisingly the simple conjugated diene acids are apparently much less common in seeds than are the hydroxy dienes, the trienes, or the acetylenic acids. Work now in progress in several laboratories on the biosynthesis of fatty acids will doubtless provide an explanation for this anomaly.

TABLE II
Occurrence of Conjugated Acids by Classes

Conjugated acid class	Plant families
Dienes	Euphorbiaceae, Bignoniaceae
Oxygenated dienes	Santalaceae, Coriariaceae, Compositae
Trienes, *cis,trans,trans*	Rosaceae, Euphorbiaceae, Cucurbitaceae, Valerianaceae
Trienes, *cis,trans,cis*	Cucurbitaceae, Punicaceae, Bignoniaceae
Trienes, *trans,trans,cis*	Bignoniaceae, Compositae
Oxygenated trienes	Rosaceae, Euphorbiaceae
Tetraene	Rosaceae, Balsaminaceae
Acetylenic and hydroxy-acetylenic	Olacaceae, Santalaceae, Compositae

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[1] Issued as N.R.C. No. 9885.

[2] Presented at the AOCS Meeting, New Orleans, May 1967.

TABLE III

Simple Conjugated Diene Acids

No.	Abbreviated notation	Short name	Observer and reference
I	t2,c4–10:2	2,4-Decadienoic[a]	Devine (3)
II	2,4–12:2	2,4-Dodecadienoic	Holman and Hanks (9)
III	t10,t12–18:2	10,12-Octadecadienoic	Hopkins and Chisholm (6,7)

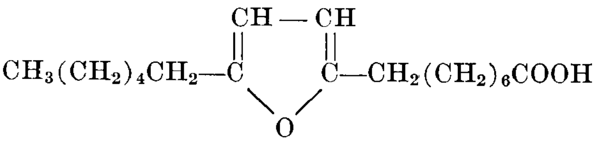
[a] $CH_3(CH_2)_4CH \overset{cis}{=} CHCH \overset{trans}{=} CHCOOH$.

Hydroxy Diene Acids

The monohydroxy conjugated diene acids were discovered in 1960. Smith et al. found and characterized 9-hydroxy-*trans*-10,*trans*-12-octadecadienoic acid, which they named dimorphecolic acid (10). In the same year Morris et al. (11), Chisholm and Hopkins (12) found independently the mixture of 9-hydroxy-10,12-octadecadienoic and 13-hydroxy-9,11-octadecadienoic in seed oils. These acids were shown to be either *cis,trans* or *trans,cis* in configuration and to occur in a number of species of Compositae.

Later the 13-hydroxy-9,11-acid was reported by Tallent et al. to occur by itself without the 9-hydroxy-10,12-isomer in a species of Coriariaceae (13). It was characterized as 13-hydroxy-*cis*-9,*trans*-11-octadecadienoic acid and named coriolic acid. Shortly afterwards Powell et al. examined a species of Compositae which contained both the 9-hydroxy- and 13-hydroxy-acids (14) and were able to separate them by countercurrent distribution. They were then characterized as 9-hydroxy-*trans*-10,cis-12- and 13-hydroxy-*cis*,9-*trans*-11-octadecadienoic acids (Table IV). This pair of hydroxy acids appears to be fairly common. Thus the hydroxy diene acids consist of three isomers with the groups *cis*-9,*trans*-11; *trans*-10,*cis*-12; and *trans*-10,*trans*-12 respectively. A *cis,cis* acid is unknown so far.

A dihydroxy diene acid was reported to occur in tung oil (*Aleurites fordii*) by Davis (15) and identified by him as 9,14-dihydroxy-10,12-octadecadienoic acid. The amount was less than 1%. It is not clear whether this acid is a natural component of the oil or an artifact. A new type of oxygenated acid, containing the furan ring, was found by Morris and coworkers (16), who showed that it had the following structure:



Triene and Tetraene Acids

The conjugated triene acids were discovered in the early days of fatty acid chemistry, and the history of α-eleostearic acid has been recorded (17). The 1947 edition of Markley's "Fatty Acids" (18) lists α-eleostearic, punicic, trichosanic, licanic, and parinaric acids as the conjugated acids known to occur in seed oils. Trichosanic acid has been shown to be identical with punicic acid, and the name "trichosanic" is no longer needed.

TABLE IV

Oxygenated Diene Acids

No.	Notation	Short name	Observer and reference
IV	9h,t10,t12–18:2	Dimorphecolic	C. R. Smith et al. (10)
V	9h,t10,c12–18:2		Morris et al. (11)
VI	13h,c9,t11–18:2	Coriolic	Tallent et al. (13)
VII	9,12ep,9e,11e–18:2		Morris (16)

The α-eleostearic and punicic acids are geometric isomers of 9,11,13-octadecatrienoic acid. Although there are eight possible isomers, these two were the only ones known to occur naturally until 1962 (19) when catalpic acid (t-9,t-11,c-13-) was found in the seed oil of *Catalpa ovata* (Table V). Meanwhile two positional isomers of octadecatrienoic acid were discovered. One of these is calendic acid (12), *trans*-8,-*trans*-10,*cis*-12-octadecatrienoic acid, which has the same configuration as catalpic acid. The other is the acid of *Jacaranda* seed oil, which is *cis*-8,*trans*-10,*cis*-12-octadecatrienoic acid (21) corresponding in configuration to punicic acid. The name jacaric acid is proposed for this. The analog of α-eleostearic acid, which would be *cis*-8,*trans*-10,*trans*-12, has not yet been found.

Parinaric acid (XV), the only conjugated tetraene acid presently known in nature, was discovered in 1933 (27), but its configuration was not established until 1966, when Bagby et al. proved it to be *cis*-9,-*trans*-11,*trans*-13,*cis*-15 (28).

Two oxygenated triene acids are included in this group, both with 9,11,13-unsaturation. Licanic acid (XIII) has a ketonic oxygen at the 4-position, and kamlolenic acid (XIV) has a hydroxy group on the terminal carbon. Both acids are considered to have the same configuration as α-eleostearic acid, and this has been proven recently for kamlolenic acid (29).

Acetylenic Acids

A fatty acid with two acetylenic bonds in conjugation was found as early as 1896 by Hébert (30) in isano oil (*Ongokea gore*, Olacaceae). It was named isanic acid, and its structure (XXII) was finally determined in 1940 as a result of study by several groups of workers (Table VI).

Ximenynic acid, octadec-*trans*-11-en-9-ynoic acid, was first found in *Ximenia caffra* (Olacaceae) by Ligthelm and Schwartz (32) and later was observed to occur widely in the Santalaceae. It is also called santalbic acid. The *trans*-enyne group has strong ultraviolet absorption at 229 mμ whereas the diyne group, as in isanic acid, has weak absorption and is not easily detected by ultraviolet spectroscopy. Other acetylenic acids have been isolated or detected recently. Ongokea (isano) oil has yielded two additional acids (XVIII, XXIII) in work by Gunstone and Sealy (33) and by Morris (34). The XXIII may be the "bolekic" acid isolated by Meade but not fully characterized (38).

Exocarpic acid (XIX), which has the *trans*-endiyne grouping, has been obtained from a species of Santalaceae (35). Powell and Smith (36) isolated two *trans*-enyne acids from *Acanthosyris spinescens* (XX, XXI). Seeds of *Pyrularia pubera*, examined by Hopkins et al. (31), yielded pyrulic acid (XVI), which is a C_{17} analog of ximenynic acid, and other acetylenic acids (29). Oils from several of the less common genera of Santalaceae were observed to vary considerably in their fatty acid components, but all had one or more enyne conjugated acids (29). Thus the family may be unique since all of the species examined so far (about 15) have one or more conjugated acids.

Hydroxyacetylenic Acids

Hydroxyacetylenic acids tend to occur in fairly large proportions in some of the species that have acetylenic acids (Table VII). Ligthelm isolated 8-

TABLE V

Triene and Tetraene Acids

No.	Notation	Short name	Observer and reference
VIII	t8,t10,c12–18:3	Calendic	McLean and Clark (20)
IX	c8,t10,c12–18:3	Jacaric[a]	Chisholm and Hopkins (21)
X	c9,t11,t13–18:3	α-Eleostearic	Böeseken (22)
XI	c9,t11,c13–18:3	Punicic	Toyama and Tsuchiya (23)
XII	t9,t11,c13–18:3	Catalpic	Hopkins and Chisholm (19)
XIII	4k,c9,t11,t13–18:3	Licanic	Brown and Farmer (24)
XIV	18h,c9,t11,t13–18:3	Kamlolenic	Aggarwal et al. (25)
XV	c9,t11,t13,c15–18:4	Parinaric	Farmer and Sunderland (26)

[a] Proposed name.

hydroxyximenynic acid from *Ximenia* oil (39), and Riley (40) studied a hydroxy acid of *Ongokea* oil which was later shown to be 8-hydroxyisanic acid (41), i.e., isanolic acid (XXX).

An acid of unusual structure was found by Powell et al. in *Helichrysum* oil, Compositae (42,43). It has the grouping 9-hydroxy,*trans*-10-en,12-yne and was named helenynolic acid (XXVII). It is an acetylenic analog of dimorphecolic acid. Other hydroxyacetylenic acids have been identified in *Ongokea* oil by Gunstone and Sealy (33), also by Morris (34).

Powell et al. detected and identified several new hydroxyacetylenic acids in *Acanthosyris* oil, Santalaceae (44). One of these is the first C_{17} fatty acid (XXVIII) to be found in quantity in a seed oil (45). It appears that hydroxyacetylenic acids are nearly as numerous as hydroxyolefinic acids in seed oils but may not occur in as many plant families.

Detection of Conjugated Acids in Oils

A relatively high refractive index ($n_D^{25C} > 1.80$) is an indication of conjugated fatty acids in an oil. However practically all conjugated acids are readily detected by examining either the ultraviolet or infrared spectrum of the oil. (A possible exception is noted under acetylenic acids.) The absorption maxima are shown in Tables VIII and IX. The NMR spectra also show the presence of conjugated bonds by a widely split signal in the region of 4τ. Some interference can result from α,β unsaturation, a terminal double bond, or aromatic rings, which give signals in about the same region (46).

If the content of conjugated acid is small or doubtful, as shown by the spectra, positive detection can usually be made by concentrating the conjugated component by crystallization of the mixed acids from acetone at low temperature (21). The saturated acids are first removed at about 0C, and the filtrates are then cooled to about −40C. At this temperature most conjugated acids crystallize, but the nonconjugated polyenes remain in solution. The conjugated fraction is re-examined by ultraviolet or infrared. Since the entire procedure is conducted at room temperature or below, the conjugated acids are essentially unchanged.

Free acids, esters, or glycerides may be examined by ultraviolet absorption. Examination by infrared is best done with methyl esters or with neutral glycerides. In the infrared, free acids produce a broad band at about 925 cm^{-1} (in CS_2), which encroaches on the region of conjugated unsaturation.

TABLE VI

Acetylenic Acids

No.	Notation	Short name	Observer and reference
XVI	8y,t10–17	Pyrulic[a]	Hopkins et al. (31)
XVII	9y,t11–18	Ximenynic	Ligthelm and Schwartz (32)
XVIII	9y,11y–18		Gunstone (33), Morris (34)
XIX	9y,11y,t13–18	Exocarpic	Hopkins and Chisholm (35)
XX	8y,t10,16e–17		Powell and Smith (36)
XXI	9y,t11,17e–18		Powell and Smith (36)
XXII	9y,11y,17e–18	Isanic	Boekenoogen (37)
XXIII	9y,11y,c13,17e–18		Gunstone (33), Morris (34)

[a] Proposed name.

Quantitative Determination

The content of conjugated acid in an oil or other mixture is ordinarily determined by ultraviolet absorption analysis, provided the identity of the acid is known. The absorptivities for some conjugated acids are given in Table VIII. The values depend to some extent on the solvent, as indicated. The absorptivity depends also on the geometric configuration of the conjugated linkages. Thus all triene acids do not have the same absorptivity (*cf.* punicic, α- and β-eleostearic). Similarly the calculation will depend on chain length although most of the presently known acids in the class have a C-18 chain.

Determination of diene acids in the presence of triene acids must take into account the absorption by triene acids in the region of 233 $m\mu$. A correction is made on the basis of the content of triene acid (7).

Isolation of Conjugated Acids

Crystallization at room temperature or below is a useful method of isolating conjugated acids (19). The acids are obtained by saponifying the oil, either at room temperature or by a short period of reflux with alkali, e.g., 30 minutes, under nitrogen. Most acids are unaffected by this treatment. The free acids are collected in petroleum ether.

Recovery of the acids from solution must be done with great care to avoid deterioration. It is convenient to evaporate the solvent at room temperature with slightly reduced pressure (water pump) and to bubble nitrogen slowly through the solution and over the residue until dry. The container should then be stoppered and stored in a Dewar flask containing solid CO_2.

Acetone is the solvent of choice for crystallizations down to −60C. Pentane is useful for crystallizing the partly purified material. Methyl esters may be crystallized, but they are more soluble than the free acids. The progress of purification is followed by measuring the ultraviolet absorption of the product. The crystallized material is collected on a Büchner funnel, either in a cold chamber or by a filter flask in a cold bath up to the rim of the funnel. The crystals are then transferred quickly to a small flask, and dry nitrogen is passed through the flask to remove the rest of the solvent. The flask is stoppered and stored in a Dewar flask containing solid CO_2. Before a sample of the acid is removed from storage, the container is allowed to warm to room temperature while a current of nitrogen is passed through; otherwise moisture will condense on the sample.

The crystallization method has been applied to all types of conjugated acids, including dienes (7), enynes (35), and hydroxy acids (47). Ordinary column chromatography has been used to some extent. Countercurrent distribution methods are valuable, especially

TABLE VII
Hydroxy Acetylenic Acids[a]

No.	Notation	Short name	Observer and reference
XXIV	7h,8y,t10–17		Powell et al. (44)
XXV	8h,9y,t11–18	8-Hydroxyximenynic	Ligthelm (39)
XXVI	8h,9y,11y–18		Morris (34), Gunstone (33)
XXVII	9h,t10,12y–18	Helenynolic	Powell et al. (42,43)
XXVIII	7h,8y,t10,16e–17		Powell et al. (45)
XXIX	8h,9y,t11,17e–18		Powell et al. (44)
XXX	8h,9y,11y,17e–18	Isanolic	Riley (40)

[a] Other acids of this class occur in small proportions, viz.,8h,9y,11y,13e–18 and 8h,9y,11y,13e,17e–18 (33,34).

when only small amounts of material are available. Good separations yielding pure conjugated acids are possible (36). Thin-layer chromatography and GLC are of doubtful utility because of the greater hazard of oxidation, isomerization, or polymerization. Most conjugated acids are unstable at room temperature. Exposure of conjugated triene acids to air for more than a few minutes at room temperature causes appreciable deterioration. The esters are slightly more stable.

Identification and Characterization

Identification of an acid obtained from a seed oil or other source is comparatively simple if it can be shown to be identical with a known acid. The first step is to obtain the acid in a pure state. Its melting point, ultraviolet and infrared spectra give clues to the probable identity (*cf.* Tables VIII, IX). A mixed melting-point of the acid with a pure reference sample of a known acid shows whether they are identical or not. If they appear to be identical, confirmation should be obtained by preparing a derivative of each, e.g., the 4-phenylphenacyl ester, and carrying out a mixed melting-point. If there is no depression of melting point, the identity of the acid is established.

If it is not feasible to prepare a completely pure sample of the acid, it may be possible to prepare one or more pure derivatives from a concentrate. Thus it is relatively easy to prepare a maleic anhydride adduct of an impure *trans,trans* acid and to remove the unreacted impurities by petroleum ether (47).

Characterization of an unknown conjugated acid usually begins with the isolation of a pure sample. Its purity is assessed by melting point and absorptivity at specified wavelengths, which should not change on further crystallization. The characteristics to be determined then are chain length, number of double bonds in conjugation, position of the conjugated group in the chain, and geometric configuration of each double bond.

Chain length is determined by complete hydrogenation with platinum catalyst. The resulting saturated acid is identified by mixed melting-point with a reference standard (e.g., stearic acid). The number of double bonds in conjugation is shown by the ultraviolet maxima (Table VIII) and can be confirmed by infrared or by quantitative hydrogenation.

The position of the conjugated group in the chain is determined by oxidative splitting and identification of the fission products. Splitting is done by von Rudloff's method (periodate-permanganate) (48). Although it was developed for locating isolated double bonds, the procedure has been found satisfactory for conjugated groupings (19). Some anomalous substances may be formed during the oxidation of conjugated acids, but the main products show the location of the double bonds. The acidic products are converted to methyl esters, avoiding loss by evaporation (19), and are identified by gas-liquid chromatography (GLC). Thus if an octadecadienoic acid (A) gives nonanedioic (azelaic) acid and heptanoic acid, it is clear that the first double bond is at carbon 9 and the second at carbon 11 (Fig. 2). Octadec-11-en-9-ynoic acid and octadec-9-en-11-ynoic acids would give the same pair of acids on splitting. Oxidative splitting by ozone distinguishes between these two isomers, pro-

TABLE VIII
Ultraviolet Spectra of Typical Conjugated Acids

No.	Acid	Chromophore	Absorption max.		
			Solvent	mμ	E(1% 1cm)
Dienes					
I	2,4-Decadienoic (methyl ester)	tc	EtOH	265	1210
IV	Dimorphecolic	tt	MeOH	231	1170
VI	Coriolic (methyl ester)	ct	Hexane	233	873
Trienes					
X	α-Eleostearic[a]	ctt	Cyclohexane	262 272 283	1766
XI	Punicic	ctc	Cyclohexane	265 275 287	1694
Tetraene					
XV	Parinaric (methyl ester)	cttc	Isooctane	278.5	870
				289.5	1690
				302.5	2560
				317	2240
Acetylenic					
XVII	Ximenynic	t-enyne	Cyclohexane	229	583
XX	Isanic	diyne	MeOH	227	13
				239	12
XIX	Exocarpic	t-endiyne	Hexane	215	2135
				229,240,253	
				267	850
				282	
XXVII	Helenynolic (methyl ester)	OH-t-enyne	Isooctane	228	565
				238	464

[a] β-Eleostearic acid (ttt) has $\lambda_{max.}$ 259, 268, 279 and E(1% 1 cm) 2190 at 268 mμ (in EtOH).

TABLE IX

Infrared Spectra of Typical Conjugated Acids

No.	Acid	Solvent	$\nu_{max.}$ cm^{-1}	Assignment
Dienes				
III	t10,t12-Octadecadienoic	CS_2	985	tt-diene
VI	Coriolic	Neat	952,985	ct-diene
Trienes and tetraene				
X	α-Eleostearic[a]	CS_2	958,987	ctt-triene
XI	Punicic	CS_2	931,981	ctc-triene
XV	Parinaric	CS_2	950,993	cttc-tetraene
Acetylenic				
XVII	Ximenynic (methyl ester)	CS_2	947	t-enyne
XIX	Exocarpic (methyl ester)	CS_2	947	t-endiyne
XX	Isanic	Neat	2220	diyne

[a] β-Eleostearic acid (ttt) has $\nu_{max.}$ 990 cm^{-1} in CS_2.

vided that the ozonides are reduced by alkaline sodium borohydride. Under these conditions the 11-en-9-ynoic acid would give azelaic acid but the 9-en-11-ynoic acid (B) would give 9-hydroxynonanoic acid (36).

The configuration at the double bonds is determined as follows. The number of *trans* bonds is found by infrared absorption, preferably with the methyl ester, by comparison with methyl elaidate. Thus an all-*cis* or all-*trans* grouping can be identified at once. If a diene has one *cis* and one *trans* bond, these are located by partial hydrogenation of the acid or ester by hydrazine (49). The resulting monoenoic acids are separated from the saturated and polyene acids by crystallization and are further separated into *cis* and *trans* monoene acids by column chromatography on silicic acid: silver nitrate (50). The *trans* acid is split by von Rudloff's method, and the identity of the fission products shows the position of the *trans* bond. Splitting by ozonolysis is also recommended (51). The *cis* acid can be split in the same way if desired.

Configuration of Triene Acids

All-*cis* or all-*trans* groupings can be recognized by observing the intensity of the infrared band at 965 cm^{-1}. The two most common groupings, ctt and ctc, have different IR and UV maxima (Tables VIII and IX). The IR spectra of these two groupings are quite distinct. However there is no appreciable difference between a ctt and a ttc group in either spectrum. To distinguish between these two, as in α-eleostearic and catalpic acids (19), the first step is to form the adduct with maleic anhydride (Fig. 3). If the adduct has no IR peak at 965 cm^{-1}, the remaining double bond is confirmed as *cis*. The adduct is hydrogenated, and the product is compared (by mixed mp) with the corresponding adduct, which is prepared from *trans*-9,-*trans*-11-octadecadienoic acid. If they are identical, the two *trans* bonds of the triene acid must be at positions 9 and 11. Alternatively the adduct can be split by oxidation with permanganate-periodate to give acid fragments that are identifiable by GLC. In this procedure α-eleostearic acid gives azelaic acid and catalpic acid gives valeric acid (Fig. 3).

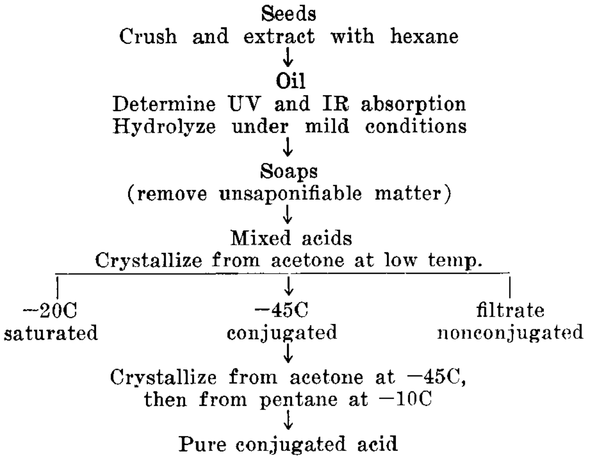


FIG. 1. Example of the isolation of a conjugated acid.

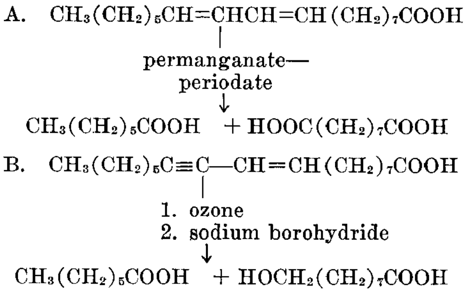


FIG. 2. Determining position of unsaturated linkages.

Acids without a *trans,trans*-diene grouping do not ordinarily form a Diels-Alder adduct. Thus triene acids with the groupings tct, tcc, ctc, or cct must be examined by other means, e.g., by comparison with the known acids (as such or as derivatives) or by the technique of partial hydrogenation by hydrazine.

A new conjugated triene acid was characterized by a study of its properties which made possible the elimination of all but one of the eight possible configurations (21). The acid did not form an adduct with maleic anhydride hence three isomers were eliminated, ctt, ttc, and ttt. The infrared spectrum was similar to that of punicic acid, indicating one *trans* bond, thus the forms ccc and tct were excluded. The UV and IR spectra were quite different from the spectra of a synthetic cct acid. On this basis, both the cct and tcc forms were excluded since their infrared spectra would be almost identical. Only one possible configuration remained, viz., *cis,trans,cis*, and all of the properties were consistent with this.

Positional Isomerism

The α,β-unsaturation seems to be rare in long-chain acids. Except for the C_{10} and C_{12} 2,4-dienes, the conjugated grouping tends to occur near the middle of the chain, e.g., at 9,11 or 10,12 in dienes and at 8,10,12 or 9,11,13 in trienes. In the C_{17} enyne acids the unsaturation is at 8,10. Thus the occurrence of positional isomers is quite limited as far as is known at present.

Reactions and Derivatives

The acids can be esterified in the usual way with an alcohol and an acid catalyst. Isolation of the product is carried out with the precautions described above. Solid esters, such as 4-bromo-phenacyl and 4-phenyl-phenacyl, are useful as derivatives for identification

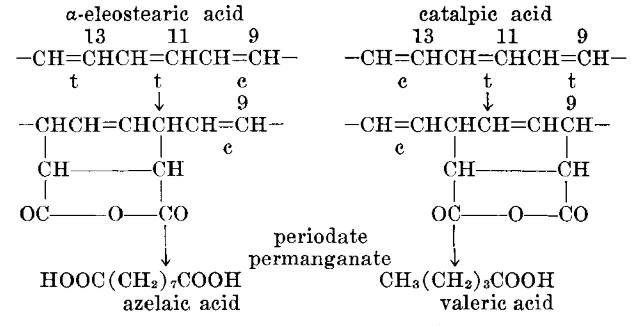


FIG. 3. Locating adjacent *trans* bonds (adducts with maleic anhydride).

TABLE X

Typical Derivatives of Conjugated Acids

Compound	Melting point, °C
Calendic acid, t8,t10,c12–18:3	40.0–40.5
All-*trans* isomer	77–78
Maleic anhydride adduct	73–74
Hydrogenated adduct	105.5–106.5
Tetracyanoethylene adduct	112.5–113.5
Catalpic acid, t9,t11,c13–18:3	31.5–32
All-*trans* isomer[a]	72
Maleic anhydride adduct	73–73.5
Adduct of methyl catalpate	38–39
Tetracyanoethylene adduct	91–92

[a] β-Eleostearic acid.

by melting point (21,52). Reduction by lithium aluminum hydride converts the acid group to a primary alcohol without affecting the unsaturated grouping (10). The resulting product has somewhat greater stability than the acid.

Catalytic hydrogenation at the double bonds proceeds to completion without difficulty. Addition of halogens seems to be of limited value either for identification or for analytical purposes since the addition is not stoichiometric.

Addition of Diels-Alder reagents at the conjugated linkage is an important reaction. The solid product is stable and valuable for identification purposes (53). Maleic anhydride is the usual dienophile although others have been tried. Tetracyanoethylene gives a satisfactory product (19). Derivatives of some conjugated acids are listed in Table X.

Theories of Biosynthesis

In 1965 Gunstone suggested that many of the known conjugated acids might be formed in the seed via a reactive intermediate, 11-hydroxy-9,12-octadecadienoic acid (54). The process would involve dehydration to give triene acids or rearrangement to give hydroxydiene acids. However there was no direct evidence for the existence of the 11-hydroxy acid, and a new theory was brought forward in 1966 which was based on epoxy acids as intermediates (55). It was postulated that the key intermediates are the known epoxy acids and that they are converted by enzymic reactions to the known conjugated diene and triene acids (Fig. 4). The hydroxy diene, 9h,t10,c12–18:2, considered to be the precursor of calendic acid, has been shown to occur in *Calendula* seed oil along with calendic acid (56). Most of the common conjugated acids can be accounted for by the application of this theory, e.g., parinaric acid (c9,t11,t13,c15–18:4), which could be formed from linolenic acid.

Morris considers that the conjugated acids are formed from linoleic acid and from the geometric isomers of linoleic acid although not via the epoxy acids (57). He has shown that *cis,trans*-linoleic acid occurs in *Dimorphotheca* oil (57), and it is known that *trans,trans*-linoleic acid occurs in *Chilopsis* oil. Occurrence of these isomers makes it easier to account for the formation of dimorphecolic (*trans,trans*) acid and catalpic acid (9-*trans*,11-*trans*-13-*cis*). This theory indicates a probable pathway of biosynthesis. Most of the required intermediates are known to occur and, in some cases, are in the same oils as the end-products. This is not final proof of the process but is very good supporting evidence. It is postulated that the initial step is enzymic peroxidation of a linoleic acid, followed by reduction and rearrangement to produce a hydroxy conjugated diene acid (Fig. 5).

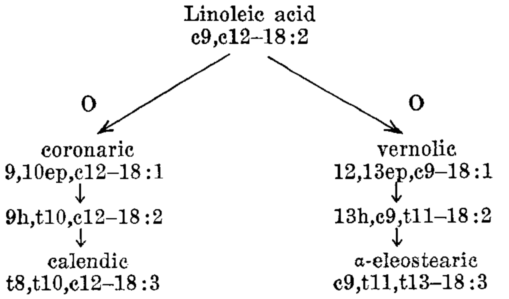


FIG. 4. Gunstone hypothesis.

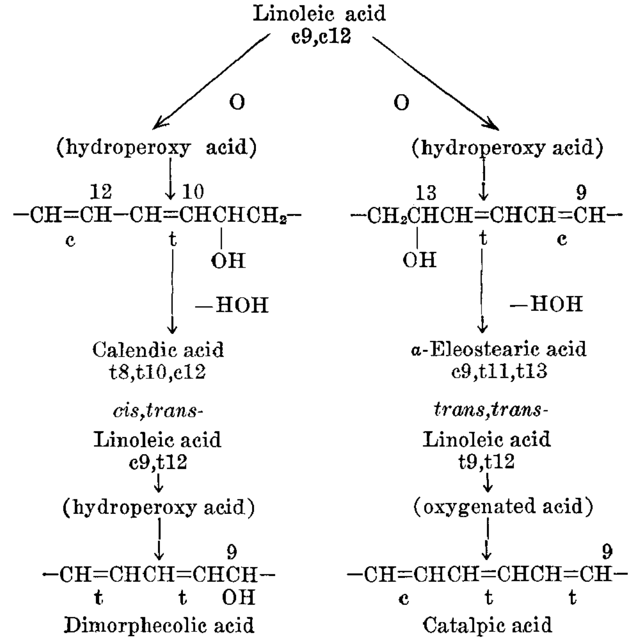
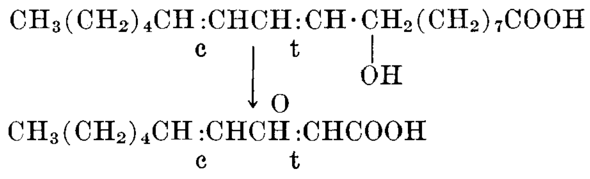


FIG. 5. Morris hypothesis.

The unusual C_{10} conjugated acid, *trans*-2,*cis*,-4-decadienoic acid, could be formed from ordinary linoleic acid by the same mechanism, followed by a final step in which the chain is split between carbons 8 and 9 by oxidation.



$$\underset{}{CH_3(CH_2)_4\underset{c}{CH}:\underset{t}{CHCH}:CH\cdot \underset{OH}{CH_2}(CH_2)_7COOH} \xrightarrow{O} CH_3(CH_2)_4\underset{c}{CH}:\underset{t}{CHCH}:CHCOOH$$

This process was postulated by Heinz (58) to account for the presence of the C_{10} acid, which he identified as a metabolite of certain fruits.

Biosynthesis of the acetylenic acids has been given considerable study, coincident with the recent discovery of stearolic (59) and other acetylenic acids (33,34) not previously known in seed oils. Bu'Lock et al. (60) obtained evidence for the synthesis of polyunsaturated acids by successive desaturation in a direction away from the carboxyl group, followed by

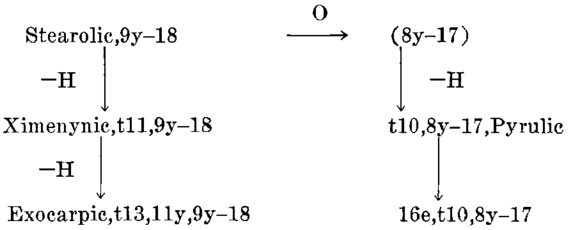


FIG. 6. Acetylenic acids.

TABLE XI
Seed Oil Sources of Conjugated Acids

No.	Acid	Species	Family
I	2,4-Decadienoic	Sapium sebiferum	Euphorbiaceae
II	2,4-Dodecadienoic	Sebastiania lingustrina	Euphorbiaceae
III	10,12-Octadecadienoic	Chilopsis linearis	Bignoniaceae
IV	Dimorphecolic	Dimorphotheca sinuata	Compositae
V	9-Hydroxy-*trans*,10-*cis*,12-octadecadienoic	Xeranthemum annuum	Compositae
VI	Coriolic	Coriaria nepalensis	Coriariaceae
VII	8(5-Hexylfuryl-2)-octanoic	Exocarpus cupressiformis	Santalaceae
VIII	Calendic	Calendula officinalis	Compositae
IX	Jacaric	Jacaranda mimosifolia	Bignoniaceae
X	α-Eleostearic	Aleurites fordii	Euphorbiaceae
XI	Punicic	Punica granatum	Punicaceae
XII	Catalpic	Catalpa ovata	Bignoniaceae
XIII	Licanic	Licania rigida	Rosaceae
XIV	Kamlolenic	Mallotus philippinensis	Euphorbiaceae
XV	Parinaric	Impatiens balsamina	Balsaminaceae
XVI	Pyrulic	Pyrularia pubera	Santalaceae
XVII	Ximenynic	Santalum album	Santalaceae
XVIII	Octadec-9,11-diynoic	Ongokea gore	Olacaceae
XIX	Exocarpic	Buckleya distichophylla	Santalaceae
XX	Heptadec-*trans*,10,16-dien-8-ynoic	Acanthosyris spinescens	Santalaceae
XXI	Octadec-*trans*,11,17,diene-9-ynoic	Acanthosyris spinescens	Santalaceae
XXII	Isanic	Ongokea gore	Olacaceae
XXIII	Octadec-*cis*,13,17-dien-9,11-diynoic	Ongokea gore	Olacaceae
XXIV	7-Hydroxy-heptadec-*trans*,10-en-8-ynoic	Acanthosyris spinescens	Santalaceae
XXV	8-Hydroxyximenynic	Ximenia caffra	Olacaceae
XXVI	8-Hydroxy-octadec-9,11-diynoic	Ongokea gore	Olacaceae
XXVII	Helenynolic	Helichrysum bracteatum	Compositae
XXVIII	7-Hydroxy-heptadec-*trans*,10,16-dien-8-ynoic	Acanthosyris spinescens	Santalaceae
XXIX	8-Hydroxy-octadec-*trans*,11,17-dien-9-ynoic	Acanthosyris spinescens	Santalaceae
XXX	Isanolic	Ongokea gore	Olacaceae

desaturation of the olefinic to acetylenic linkages. It was suggested that stearolic acid is probably the parent acid of the acetylenic acids of Santalaceae (61). This mechanism would account for such acids as those shown in Fig. 6, assuming that chain-shortening by loss of one carbon atom occurs at the carboxyl end to produce the C_{17} acids. These processes are under study in the laboratories of Bu'Lock (62) and James (63). Other mechanisms are not excluded (57,64).

Sources of the acids described in this review are listed in Table XI.

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