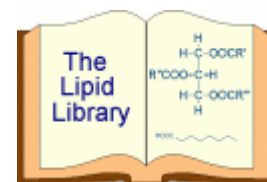# FATTY ACIDS: NATURAL ALICYCLIC

STRUCTURES, OCCURRENCE AND BIOCHEMISTRY

Alicyclic fatty acids occur naturally in plants, especially certain seed oils, and microorganisms, but only rarely in animal tissues, other than the **prostaglandins**, which are described elsewhere in these web pages, as are the plant **jasmonates**. Cyclopropane fatty acids are occasionally reported from marine animals, and some at least are produced by the animals themselves, although others may be synthesised by symbiotic bacteria. Cyclic fatty acids can also be formed as artefacts from conventional unsaturated fatty acids during food processing, and these are discussed on the frying oil pages of this website.

Cyclopropane Fatty Acids in Microorganisms

cis-11,12-Methylene-octadecanoic acid was first isolated from the phospholipids of *Lactobacillus arabinosus* and given the trivial name lactobacillic acid. It is a cyclopropane analogue of *cis*-vaccenic acid, which is the main unsaturated fatty acid in this organism and its biosynthetic precursor (see below).

lactobacillic acid
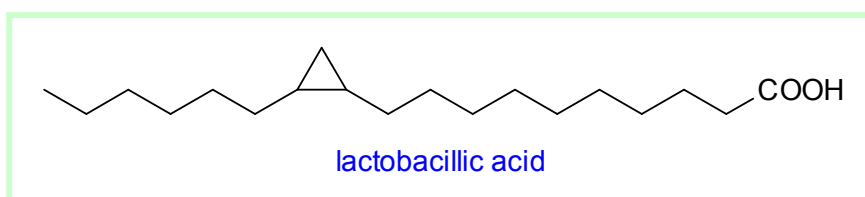
It has since been found in a wide range of bacterial species, both gram-negative and gram-positive, of many different types from strict anaerobes to obligate aerobes. It is often accompanied by *cis*-9,10-methylene-hexadecanoic acid, and other homologues (C_{14} to C_{20} in chain-length). Some organisms contain *cis*-9,10-methylene-octadecanoic acid (dihydrosterculic acid), the cyclopropane analogue of oleic acid, together with homologous fatty acids (C_{16} or C_{20} in chain-length) that are related biosynthetically. 3-Hydroxy-lactobacillic acid is the major amide-linked component of an ornithine-containing lipid of *Mesorhizobium ciceri*. Cyclopropane ring-containing fatty acids also occur among the branched-chain mycolic acids as discussed elsewhere (see the appropriate webpage).
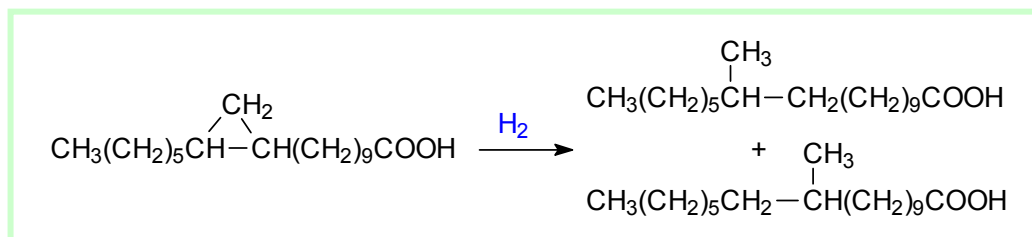
In addition, 17-methyl-*cis*-9,10-methylene-octadecanoic acid occurs in the parasitic protozoan, *Herpetomonas megaseliae*. While 9,10-methylene-5-hexadecenoic acid and 11,12-methylene-5-octadecenoic acid have been isolated from the cellular slime mould *Polysphondylium pallidum*, it is certain that they are metabolites of ingested microbial fatty acids. The same is presumably true of similar fatty acids characterized from the fresh-water invertebrate, *Acathogammarus grewingkii*, i.e. *cis*-11,12-methylene-5-eicosenoate, *cis,cis*-11,12-14,15-*bis*-methylene-5-eicosenoate, and their homologues.

Unusual cyclopropyl fatty acids with a ring of the *trans* configuration in the 4,5-position, not to mention conjugated double bonds, bromine
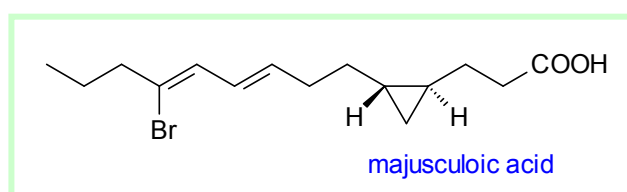

majusculoic acid

rings and methyl branches, have been found in various cyanobacteria of marine origin. For example, majusculoic acid from a marine cyanobacterial mat assemblage has the structure illustrated.

Although cyclopropyl fatty acids are fully saturated, they are chemically reactive because of the highly strained nature of the ring. For example, on catalytic hydrogenation, ring opening occurs with the formation of two methyl-branched fatty acids, a reaction that has proved useful for characterization purposes in conjunction with mass spectrometry (as illustrated below). However, picolinyl esters are now recognised as the most useful derivatives for mass spectrometric characterization. *Cis/trans* configurations of cyclopropane rings can be distinguished by ^{1}H NMR spectroscopy.

$$CH_3(CH_2)_5CH \overset{\overset{\displaystyle CH_2}{|}}{-} CH(CH_2)_9COOH \xrightarrow{\ H_2\ } CH_3(CH_2)_5\overset{\overset{\displaystyle CH_3}{|}}{CH} - CH_2(CH_2)_9COOH$$

$$+$$

$$CH_3(CH_2)_5CH_2 - \overset{\overset{\displaystyle CH_3}{|}}{CH}(CH_2)_9COOH$$

The cyclopropane ring is also opened under relatively mild conditions by reaction with bromine, mercuric acetate, or with acidic reagents, and especially boron trifluoride-methanol. However, cyclopropyl fatty acids are stable on silver nitrate chromatography, when they elute with saturated fatty acids, and they are unaffected by bases and by the oxidizing agents commonly used to locate double bonds.

The first step in the biosynthesis of the cyclopropane ring in a fatty acid is similar to that for certain methyl-branched fatty acids (see the appropriate web page), and involves addition of a methyl group from *S*-adenosylmethionine (which is converted to *S*-adenosylhomocysteine in the process) to the double bond. Experiments with double bonds labelled with deuterium confirm that the hydrogen atoms in the ring are retained, so the subsequent cyclization step involves a loss of hydrogen from the methyl group.

*S*-adenosylmethionine   *S*-adenosylhomocysteine
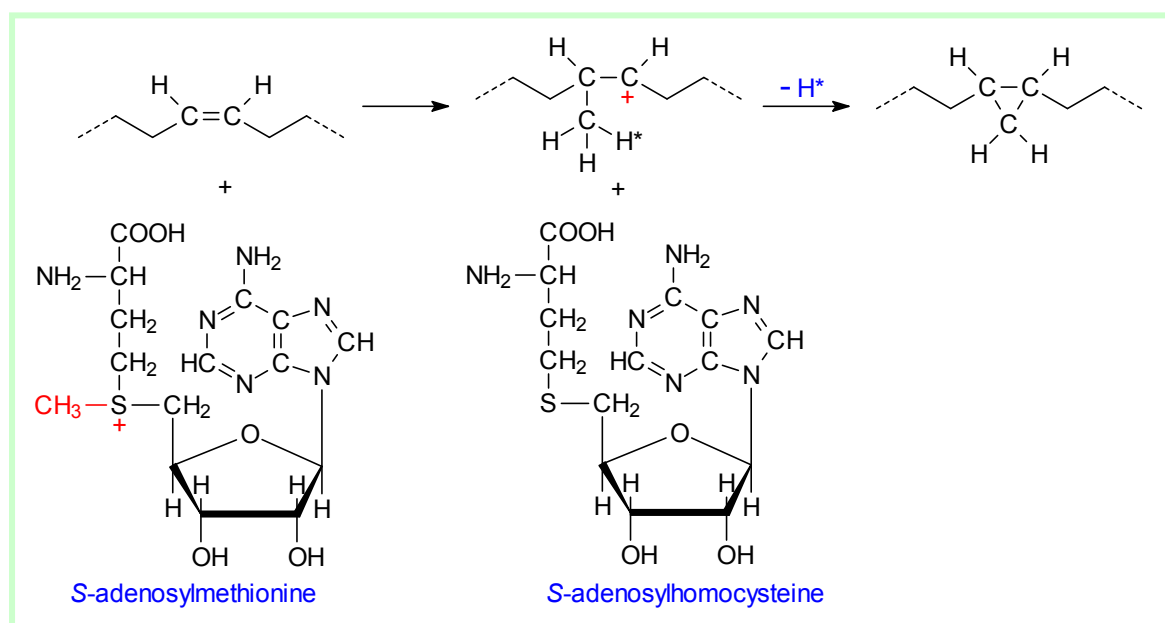
The reaction reaches its maximum during the stationary growth phase of the organism. A further important aspect of the process is that the substrate for the reaction is a monoenoic fatty acid in position *sn*-2 of phosphatidylethanolamine usually (or rarely position *sn*-1 depending on species),

not the free acid or coenzyme A ester. However, in the symbiotic soil bacterium *Sinorhizobium meliloti*, cyclopropanation occurs in many different lipids independently of the polar head group and in precursors in both positions of the glycerol moiety.

In *Escherichia. coli*, it is believed that the function of cyclopropane fatty acids is to protect against acid shock, as can occur during passage of ingested organisms through the stomach for example, possibly by reducing the proton permeability of the membranes. Cyclopropanation is believed to play a role in the pathogenesis of *Mycobacterium tuberculosis.*

Fatty Acids with Terminal Ring Structures in Microorganisms

11-Cyclohexylundecanoic acid was first isolated as a minor component of butter fat, but it is almost certainly produced by bacteria in the rumen, which are later digested and the fatty acids released for uptake into the tissues of the host animals.

11-cyclohexylundecanoic acid
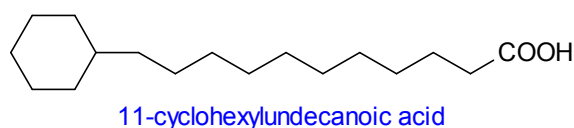
It has now been found together with homologous fatty acids in a number of bacterial species, especially some that are tolerant of extreme environmental conditions. For example, certain *Bacillus* species produce 11-cyclohexylundecanoic and 13-cyclohexyltridecanoic acids normally, but can synthesise fatty acids with terminal C4 to C7 rings, when provided with appropriate exogenous precursors. The normal endogenous mechanism involves conversion of sugars to shikimic acid and thence to cyclohexanecarboxylic acid, which serves as the primer for the fatty acid synthase.


shikimic acid   cyclohexanecarboxylic acid   11-cyclohexylundecanoic acid
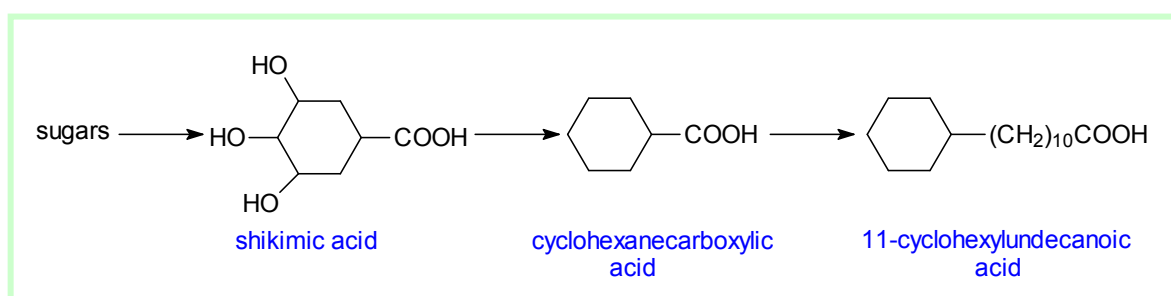
Perhaps more surprising is the finding of a variety of *omega*-cycloheptyl fatty acids in bacteria of the genus A*licyclobacillus.* 11-Cycloheptylundecanoic (and the 2-hydroxy analogue) and 13-cycloheptyltridecanoic acids, together with three minor homologues, comprise nearly 80% of the fatty acids of A*licyclobacillus cycloheptanicus* , for example.

2-hydroxy-11-cycloheptylundecanoic acid
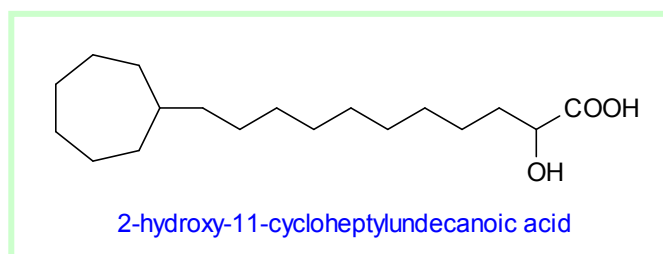
Many aspects of the biosynthesis of such fatty acids are still unclear, but it appears that the expected precursor, cycloheptanecarboxylic acid, is produced in the organisms from phenyl acetic acid by a complex sequence of reactions. In addition, four *omega*-phenyl fatty acids (with C10, 12, 13 and 14 saturated chains) are minor components of the lipids of a halophilic *Bacillus* species from Bulgaria.

Arguably the most unusual fatty acids of all are present in anammox bacteria, which derive their energy by oxidizing ammonia via the anaerobic combination of the substrates ammonia and nitrite into nitrogen gas. The structures are composed of three to five linearly concatenated cyclobutanes attached to the terminal carbon of a short- to medium-chain fatty acid, and occurring in the organisms as the free acid or glycerol esters; related structures are found as alcohols or as alkylglycerols of various kinds, or with 5, 6 or 7-membered rings attached to the fused cyclobutane rings. Studies of the NMR spectra showed that all the rings are fused by *cis*-ring junctions, resulting in a staircase-like arrangement of the fused cyclobutane rings, defined in the example illustrated as [5]-ladderane.

ladderane fatty acids
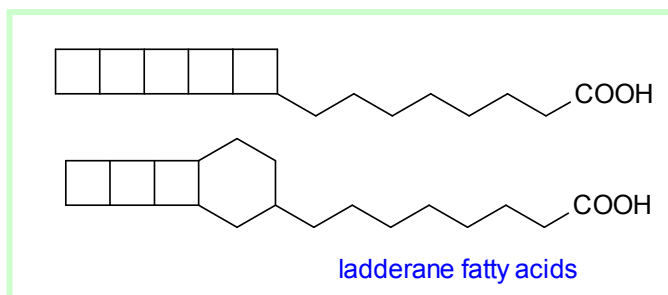
Identification of most of these terminal cyclic fatty acids is straightforward by gas chromatography-mass spectrometry, especially as the picolinyl ester and pyrrolidide derivatives, although 4,4-dimethyloxazolines (DMOX) have also been used. However, the ladderane lipids present special problems, as they have a tendency to isomerize with formation of 6-membered rings when heated, as during analysis by gas chromatography-mass spectrometry. LC-MS with APCI detection presents fewer problems.

Cyclopropane and Cyclopropene Fatty Acids from Plants

A fatty acid containing a cyclopropene ring was first isolated from the seed oil of *Sterculia foetida*, and was characterized as 8-(2-octyl-cyclopropen-1-yl)-octanoic acid (9,10-methylene-octadec-9-enoate or 'sterculic acid'). Shortly afterwards, a related fatty acid with one fewer carbon atom was characterized from a related seed oil, i.e. 7-(2-octyl-cyclopropen-1-yl)-heptanoic acid (9,10-methylene-heptadec-9-enoate or 'malvalic acid').


sterculic acid

malvalic acid
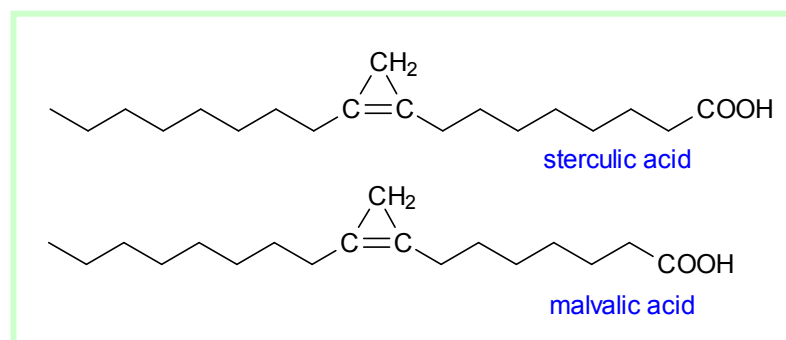
These two fatty acids have now been found in a large number of seed oils from plant families of the order Malvales (Sterculiaceae, Malvaceae, Bombaceae and Tiliaceae). Generally, both fatty acids are present together in concentrations that vary up to 60%, depending on species, and they are usually accompanied by small amounts of the cyclopropanoid analogues, i.e. dihydrosterculic and dihydromalvalic acids. They are also found in leaves, roots and shoots. Dihydrosterculic acid is the major carbocyclic fatty acid in the seed oils of *Litchi chinensis* and *Euphorbia longana*, while lactobacillic acid is a substantial component of the seed oil of *Byrsocarpus coccineus* (accompanied by small amounts of two branched-monoenes that may be intermediates in its biosynthesis). Cyclopropene fatty acids are troublesome impurities in commercial cottonseed oil,

Gossypium hirsutum, which contains 1.1% malvalic and 0.4% sterculic acids, and they must be removed by vigorous refining processes (see below).

Two further cyclopropenoid fatty acids have been characterized from seed oils, i.e. 2-hydroxysterculic acid and 9,10-methylene-octadec-9-en-17-ynoate or 'sterculynic acid'. The former may be an intermediate in the bio-conversion of sterculic to malvalic acid by an *alpha*-oxidation mechanism.

The cyclopropene ring is highly strained and is therefore very reactive. In particular, it reacts readily with thiol groups and other sulfur compounds. For example, one of the first tests for cyclopropene-containing oils was a pink coloration formed on solution in carbon disulfide (the Halphen test). The facile addition reaction with methanethiol has been used as an aid to mass spectrometric characterization.
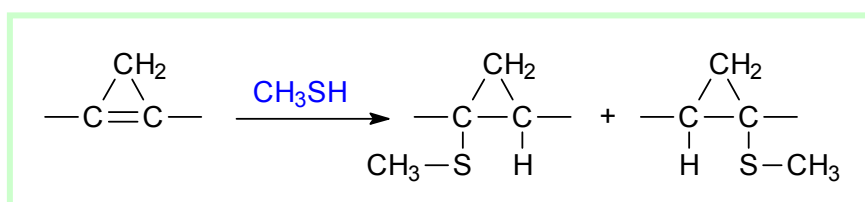
Acid-catalysed transesterification to prepare methyl ester derivatives brings about rapid destruction of the cyclopropene ring, but base-catalysed transesterification can be used safely. Silver nitrate reacts rapidly with cyclopropene fatty acids, so silver ion chromatography cannot be used for isolation purposes, although the reaction has been used as an aid to structural analysis by mass spectrometry.

In the early days of gas chromatography when packed columns were standard, cyclopropenoid fatty acids decomposed on the columns and could not be analysed directly. With modern wall-coated open-tubular columns, this does not appear to be a problem and quantitative analysis as the methyl esters can be accomplished by GC. Reversed-phase HPLC has also been employed. Similarly, identification of cyclopropenoid fatty acids is nowadays straightforward by gas chromatography-mass spectrometry, especially in the form of the picolinyl ester, DMOX or pyrrolidide derivatives.

Cyclopropene fatty acids produce unwanted biological effects when ingested by animals by inhibiting desaturases, leading to an accumulation of saturated fatty acids. It is possible that there is an irreversible reaction between the ring structures and thiol moieties on the enzymes. The effect has been noted in many animal models, but is most troublesome when poorly refined cottonseed oil is fed to laying hens; the resultant eggs have a rubbery texture and the white of the yolk can have a pink coloration. However, cyclopropene fatty acids also inhibit desaturation during the synthesis of fatty acids and pheromones in insects and so may protect plants against insect attack. It is possible that they have evolved for this purpose.

Much remains to be learned of the biosynthesis of cyclopropenoid fatty acids in plants, but it appears that the mechanism involves conversion of oleate to dihydrosterculate and thence to sterculate. The cyclopropane synthase in *Sterculia foetida* has been studied, and has much in common with the bacterial enzyme described above, catalysing the addition of a methylene group from *S*-adenosylmethionine across the double bond. However, the primary substrate for the enzyme is oleic acid in position *sn*-1 of phosphatidylethanolamine (rather than position *sn*-2).

Fatty Acids with Terminal Ring Structures from Plants

Fatty acids with a terminal cyclopent-2-enyl ring are found in seed oils from many species of the plant family Flacourtiaceae. 'Chaulmoogra' oil is extracted from seeds of *Hydnocarpus*

(Taraktogenus) kurzii and is used in folk medicine as a treatment for leprosy. The three most common fatty acids of this type are 11-cyclopent-2-enyl-undecanoic (hydnocarpic), 13-cyclopent-2-enyl-tridecanoic (chaulmoogric) and 13-cyclopent-2-enyltridec-6-enoic (gorlic) acids. All have the (*R*)-(+) stereochemistry at carbon 1 of the cyclopent-2-enyl ring.

chaulmoogric acid

gorlic acid
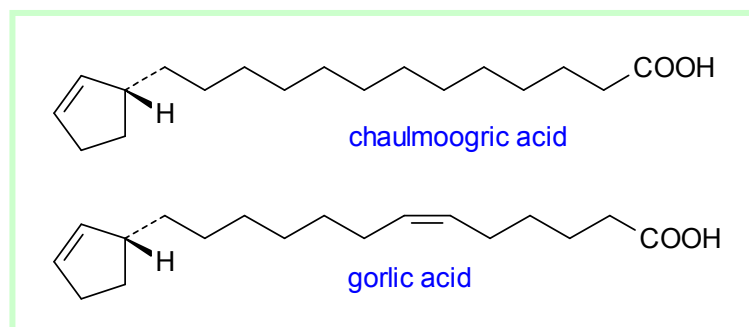
In addition, 15-cyclopent-2-enylpentadec-9-enoic (hormelic) acid is a minor component of most oils of this type, while 13-cyclopent-2-enyltridec-4-enoic acid is the main component of the seed oil of *Hydnocarpus anthelmintica*, which also contains trace levels of 11-cyclopentylundecanoic acid. These fatty acids are found in all lipid classes in the seeds, but especially in the triacylglycerols.

Little is known of the biosynthetic pathway for the biosynthesis of these fatty acids, but the evidence points to cyclopentenylglycine as the precursor of cyclopent-2-enylcarboxylic (aleprolic) acid, which is then subjected to chain-elongated and desaturation by conventional enzyme systems.

Seed oils of the Araceae (Aroid) family, such as *Arum maculatum*, contain fatty acids with ω-phenyl moieties. 13-Phenyltridecanoic acid is the major component, accompanied by C_{17} and C_{21} homologues, and analogous fatty acids with double bonds in position 9.

13-phenyltridecanoic acid
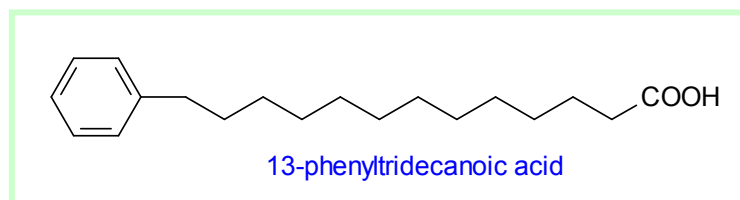
All of these fatty acids are readily characterized by mass spectrometry and nuclear magnetic resonance spectroscopy.

Cyclic Fatty Acids in Animals

19,20-Methylene-hexacosanoic acid is a minor component of the sponge Calix niceaensis. The marine sponge *Plakortis simplex* contains unusual prenylated galactosylceramides (plakosides) in which both the fatty acid and long-chain base components contain a *cis*-cyclopropane ring in position 11 of the chain. The fatty acid is 2-hydroxy-11,12-methylene-docos-5-enoate.

2-hydroxy-11,12-methylene-docos-5-enoic acid
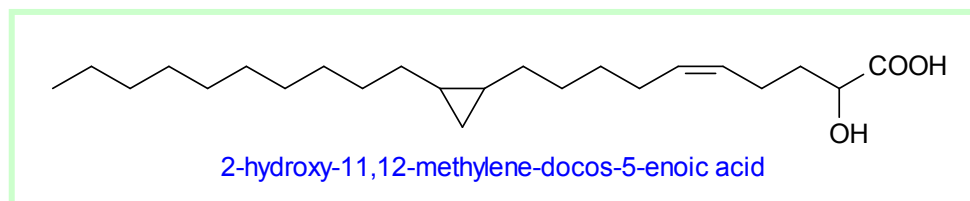
Several unusual C_{27} to C_{30} unsaturated fatty acids have been isolated from an Australian sponge, *Amphimedon sp.*, which are distinctive in possessing a cyclopropylidene group and have been termed 'amphimic acids', two of which are illustrated below. Apart from the ring structure they are

obviously closely related to the demospongic acids (see the appropriate web page). The biosynthetic origin of many sponge fatty acids is obscure, and it is known that some can arise from further metabolism of ingested microbial lipids. However, these particular fatty acids bear no obvious relationship to bacterial fatty acids.

amphimic acids
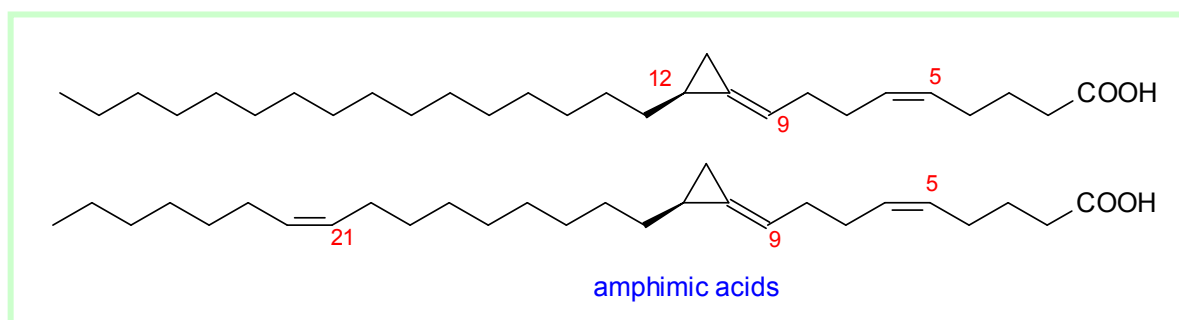
A number of eicosanoids (lactones) with cyclopropane rings adjacent to an oxygenated functional group have been isolated from corals, other marine invertebrates and red algae. In these organisms, the cyclopropane ring is of the *trans* configuration, and the biosynthetic mechanism appears to differ from that for more conventional cyclopropyl fatty acids. Similarly, unusual cyclopropyl fatty acids with *trans* ring structures in the 4,5-position (and with *iso-* and *anteiso-* methyl branches) have been isolated from the sponge *Pseudospongosorites suberitoides*. However, in this instance, it is possible that they originate in cyanobacteria that are symbiotic with the sponge.

Other natural fatty acids with ring structures are occasionally reported from animal tissues (see the cyclohexyl fatty acids above, for example), but these come from the food chain.

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