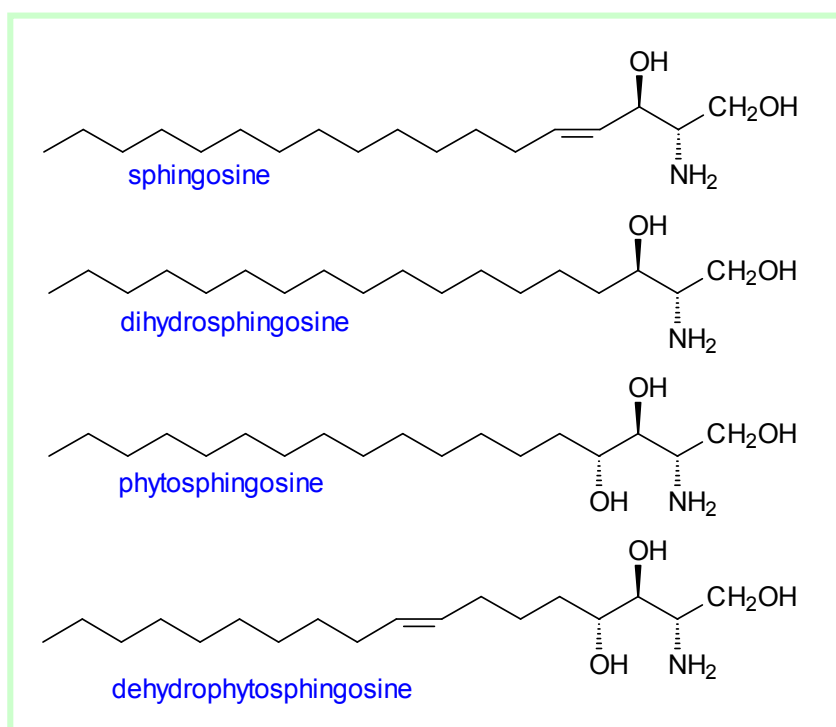


LONG-CHAIN OR SPHINGOID BASES

STRUCTURE, OCCURRENCE, BIOSYNTHESIS AND ANALYSIS

1. Structures and Occurrence

Long-chain bases (sphingoids or sphingoid bases) are the characteristic or defining structural unit of the sphingolipids. The bases are long-chain aliphatic amines, containing two or three hydroxyl groups, and often a distinctive *trans*-double bond in position 4. To be more precise, they are 2-amino-1,3-dihydroxy-alkanes or alkenes with (2*S*,3*R*)-*erythro* stereochemistry, with various further structural modifications.



The commonest or most abundant of these in animal tissues is **sphingosine** ((2*S*,3*R*,4*E*)-2-amino-4-octadecen-1,3-diol) or 4*E*-sphingenine, *i.e.* with a C₁₈ aliphatic chain, hydroxyl groups in positions 1 and 3 and an amine group in position 2; the double bond in position 4 has the *trans* (or *E*) configuration. This was first characterized in 1947 by Professor Herbert Carter, who was also the first to propose the term “sphingolipides” for those lipids containing sphingosine. It is usually accompanied by the saturated analogue, dihydrosphingosine or sphinganine.

For shorthand purposes, a nomenclature similar to that for fatty acids can be used; the chain length and number of double bonds are denoted in the same manner with the prefix ‘d’ or ‘t’ to designate di- and trihydroxy bases, respectively. Thus, sphingosine is denoted as d18:1 and phytosphingosine is t18:0. The position of the double bond may be indicated by a superscript, *i.e.* 4-sphingenine is d18:1^{Δ^{4t}} or 4*E*-d18:1. Alternative nomenclatures are sometimes seen in papers.

The compositions of long-chain bases of sphingomyelins of some animal tissues are listed in Table 2 of our webpage on **sphingomyelins**. The main C₁₈ components are accompanied by small amounts of C₁₆ to C₁₉ dihydroxy bases, though the latter attain higher proportions in tissues of

ruminant animals. Eicosasphingosine (2*S*,3*R*,4*E*-d20:1) is found in gangliosides from human brain and intestinal tissues, for example. Shorter-chain bases are found in many insect species, and for example in the fruit fly, *Drosophila melanogaster*, widely used in genetic experiments, the main components are C₁₄ bases. 3-Keto-sphingoid bases, produced in the first step of sphingosine biosynthesis (see below), are only rarely detected in tissues.

A common long-chain base of mainly plant origin is a saturated C₁₈ trihydroxy compound **phytosphingosine** or 4*D*-hydroxy-sphinganine ((2*S*,3*R*,4*R*)-2-amino-octadecanetriol), although unsaturated analogues, for example with a *trans* (or occasionally a *cis*) double bond in position 8, i.e. dehydrophytosphingosine or 4*D*-hydroxy-8-sphingenine, tend to be much more abundant (see Table 2 of our webpage on **ceramide monohexosides** for tabulated data on two plant species). There are also lipid class preferences. In many plant species, dihydroxy long-chain bases are more enriched in glucosylceramides than in glycosylinositolphosphoceramide, but in the model plant *Arabidopsis thaliana* trihydroxy bases predominate in both classes and comprise nearly 90% of the total in the leaves (Table 1). Other plant long-chain bases have double bonds in position 4, which can be of either the *cis* (*Z*) or *trans* configuration, although *trans*-isomers are by far the more common.

Table 1. Sphingolipid long-chain base composition of leaves from *A. thaliana*.

Base	%	Base	%
t18:1 (8 <i>Z</i>)	20	d18:1 (8 <i>Z</i>)	1
t18:1 (8 <i>E</i>)	64	d18:1 (8 <i>E</i>)	8
t18:0	6	d18:0	1

Data from Chen *et al.*, *Plant Cell*, **18**, 3576-3593 (2006)

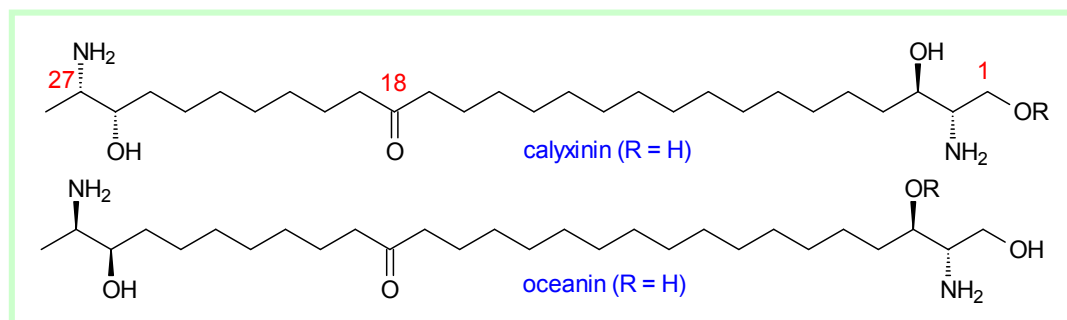
Phytosphingosine is found in significant amounts in intestinal cells of animals also, with much smaller relative proportions in kidney and skin.

The number of different long-chain bases that has been found in animals, plants and microorganisms must now number over one hundred, and many of these may occur in a single tissue, but almost always as part of a complex lipid as opposed to in the free form. The aliphatic chains can contain from 14 to as many as 27 carbon atoms, and they can be saturated, monounsaturated and diunsaturated, with double bonds of either the *cis* or *trans* configuration. Sphingoid bases with three double bonds, such as sphinga-4*E*,8*E*,10*E*-trienine, have been found in a dinoflagellate and some marine invertebrates. In addition, long-chain bases can have branched chains with methyl substituents (omega-1 (*iso*), omega-2 (*anteiso*) or other positions), hydroxyl groups in position 5 or 6, ethoxy groups in position 3, and even a cyclopropane ring. Similarly, saturated and monoenoic, straight- and branched-chain trihydroxy bases are found. A proportion of the phytosphingosine and related sphingoid bases found in animal tissues may enter via the food chain, although non-mammalian sphingoid bases in general tend to be poorly absorbed from the intestines.

Yeasts and fungi tend to have distinctive and characteristic long-chain base compositions; for example, fungi have 9-methyl-4*E*,8*E*-sphingadienine as the main sphingoid base in the glucosylceramides but not in the ceramide phosphoinositol glycosides. Yeasts contain mainly the saturated C₁₈ base sphinganine. In plants, the composition is dependent on species, but typically it is composed of up to eight different C₁₈-sphingoid bases, with variable geometry of the double bond in position 8, i.e. (*E/Z*)-sphing-8-enine (d18:1⁸), (4*E*,8*E/Z*)-sphinga-4,8-dienine (d18:2^{4,8}) and (8*E/Z*)-4-hydroxy-8-sphingenine (t18:1⁸); d18:1⁴, d18:0 and t18:0 are only present in small amounts.

In addition, many organisms produce sphingosine-like compounds that can interfere with sphingolipid metabolism, such as the mycotoxin fumonisins discussed below. *N*-Methyl, *N,N*-dimethyl and *N,N,N*-trimethyl derivatives of sphingoid bases have been detected in mouse brain. *N,N*-dimethylsphingosine is of particular interest in that it inhibits protein kinase C, sphingosine kinase and many other enzyme systems.

Some plants and marine organisms synthesise long-chain bases lacking the hydroxyl group in position 1, i.e. 1-deoxy-sphingoid bases. Among the more unusual of these are the C₂₈ α,ω- or two-headed- sphingoid base-like compounds, such as calyxinin and oceanin (and their β-glycosides) of sponges.



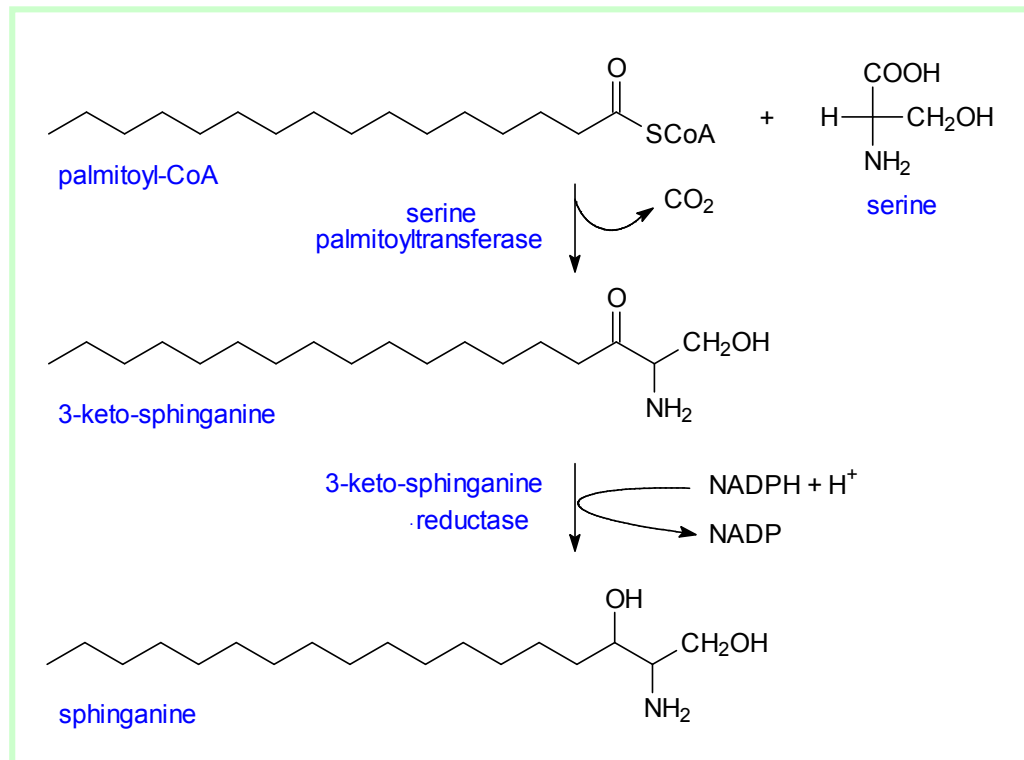
Sphingoid bases are surface-active amphiphiles, with critical micellar concentrations of about 20 μM. They are unusual amongst lipids in that they bear a small positive charge at neutral pH, though their p*K*_a (9.1) is lower than in simple amines as a consequence of intra-molecular hydrogen bonding. This together with their relatively high solubility (> 1 μM) enables them to cross membranes or move between membranes with relative ease. In so doing, they increase the permeability of membranes to small solutes.

The complex sphingolipids are discussed elsewhere in these web pages, but in most the sphingoid base is linked via the amine group to a fatty acid, including very-long-chain saturated and 2-hydroxy components, i.e. to form a **ceramide**, while a polar head group is attached to the primary hydroxyl moiety to produce more complex sphingolipids. An important exception is **sphingosine-1-phosphate**, which has signalling functions in cells akin to those of lysophospholipids.

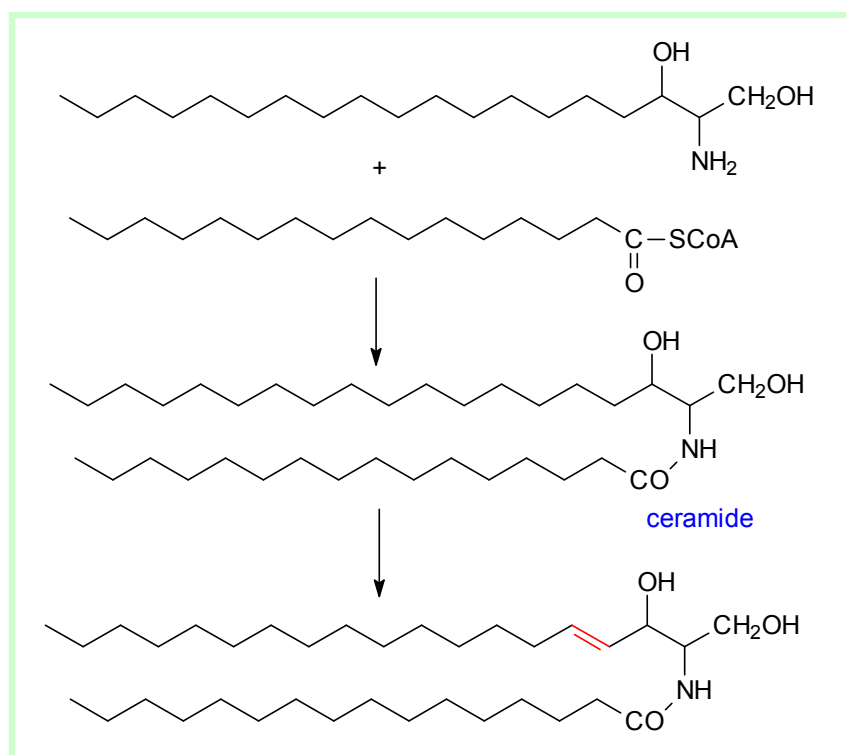
2. Biosynthesis and Metabolism

The basic mechanism for the biosynthesis of sphinganine involves condensation of palmitoyl-coenzyme A with serine, catalysed by a membrane-bound enzyme *serine palmitoyltransferase* on the cytosolic side of the endoplasmic reticulum in animal cells as illustrated, to form 3-keto-sphinganine. This is believed to be the key regulatory step in ceramide biosynthesis.

The specificity of this enzyme controls the chain-length of the base. The keto group is then reduced to a hydroxyl by a specific *reductase*, also on the cytosolic side of the endoplasmic reticulum, a step that must occur rapidly as these intermediates are rarely encountered in tissues. The enzymes are presumed to be in a similar location in plant cells.



The free sphinganine is rapidly *N*-acylated by acyl-coA to form dihydroceramides by dihydroceramide synthases, which in animals are located on the cytosolic face of the endoplasmic reticulum. Animals and plants have multiple isoforms of this enzyme, each with characteristic and partially distinct specificities for the chain-length of the base and fatty acyl-CoA moieties, suggesting that ceramides containing different fatty acids have differing roles in cellular physiology. For example, animals have six ceramide synthases of which ceramide synthase 2 is most abundant and is specific for coA esters of very-long-chain fatty acids (C₂₀ to C₂₆).



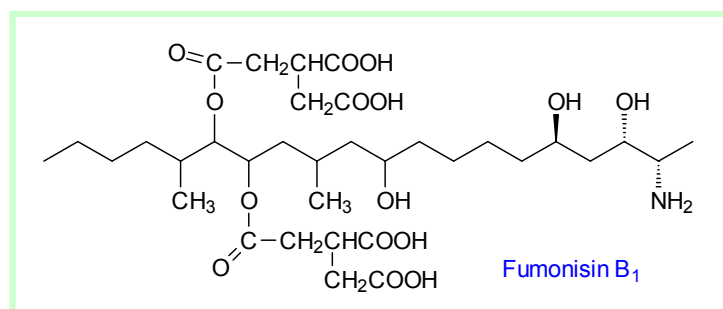
Insertion of the *trans*-double bond in position 4 to produce sphingosine occurs only after the sphinganine has been esterified in this way to form a **ceramide**, as illustrated (see also our web pages on **ceramides**). The desaturases were first characterized in plants, and this subsequently simplified the isolation of the appropriate enzymes in humans and other organisms.

A considerable family of Δ^4 -sphingolipid desaturases has now been identified, and an early study by Stoffel and colleagues demonstrated that Δ^4 -desaturation involves first *syn*-removal of the C(4)-H_R and then the C(5)-H_S hydrogens. This appears to have been the first evidence that desaturases in general operate in this stepwise fashion. Two distinct types of sphingoid Δ^8 desaturase have been characterized in plants that catalyse the introduction of a double bond at position 8,9 of phytosphinganine to form both *trans* and *cis* isomers in the ratio of 7:1. It appears that the *trans* isomer is formed when the hydrogen on carbon 8 is removed first, and the *cis* when carbon 9 is the point of attack. The main group of Δ^8 desaturases requires a 4-hydroxysphinganine moiety as substrate, but the second does not. Fungi only produce *trans* Δ^8 isomers. Δ^4 and Δ^8 desaturases do not occur in yeasts such as *S. cerevisiae*.

Phytosphingosine is formed by hydroxylation of sphinganine in position 4, possibly via the free base in plants although it can also be formed from a ceramide substrate in yeasts. Sphinganine linked to ceramide is the substrate for 4-hydroxylation in intestinal cells. Much remains to be learned of the processes involved, but it is known that the enzyme responsible is closely related to a Δ^4 desaturase. Indeed, it has been shown that there are bifunctional Δ^4 -desaturase/4-hydroxylases in *Candida albicans* and mammals with which both 4-hydroxylation and Δ^4 -desaturation are initiated by removal of the proR C-4 hydrogen. In plants, fatty acid desaturases and hydroxylases are also closely related. However, the substrates for desaturation in plants (free bases or ceramides) are still uncertain. In the biosynthesis of sphingoid bases in fungi, the double bonds in positions 4 and 8 and the methyl group in position 9 are inserted sequentially into the sphinganine portion of a ceramide, the last by means of an S-adenosylmethionine-dependent methyltransferase similar to plant and bacterial cyclopropane fatty acid synthases.

It has been established that long-chain bases with 4-hydroxyl groups are necessary for the viability of the filamentous fungus *Aspergillus nidulans* and for growth in plants such as *Arabidopsis thaliana*. The presence of an 8*E* double bond confers aluminum tolerance to yeasts and plants. However, a *trans*-4 double bond in the sphingoid base does not appear to be essential for growth and development in *Arabidopsis*.

Synthesis of sphingoid bases *de novo* appears to be essential in most organisms, and indeed in animals dietary sphingoids are largely degraded in the intestines. Inhibition of these pathways affects growth and viability. For example, certain fungal toxins that have structural similarities to sphingoid bases (e.g. fumonisin B₁ illustrated) are found in maize and other crop plants and can cause a number of disease states in humans (including oesophageal cancer) and other animals (as well as in plants) by inhibiting the dihydroceramide synthase, leading to an accumulation of sphinganine and sphinganine-1-phosphate together with a reduction in the amounts of complex sphingolipids.



1-Deoxysphingosine (2-amino-,3-hydroxy-octadecane) and its *N*-acyl derivatives (ceramide analogues) also accumulate in cells treated with fumonisin B₁, as a result of condensation of palmitoyl-CoA with L-alanine catalysed by the serine palmitoyl transferase. Such compounds are present in normal tissues at low levels, especially the liver, but they are not usually noticed because they are swamped by the much larger amounts of conventional ceramides.

Although free sphingoid bases are rarely found at greater than trace levels in tissues (typically 1-10 nmoles/g wet tissue), they may have important functions as mediators of many cellular events. In animal cells, they inhibit protein kinase C indirectly, for example, by a mechanism involving inhibition of diacylglycerol synthesis. In addition, sphingoid bases are known to be potent inhibitors of cell growth, although they stimulate cell proliferation and DNA synthesis. It has been suggested that they may be involved in the process of apoptosis in a manner distinct from that of ceramides. They may also have a protective role against cancer of the colon in humans. Similarly, *N,N*-dimethylsphingosine and dihydrosphingosine are known to induce cell death in a variety of different types of malignant cells. In consequence, synthetic analogues of long-chain bases are being tested for their pharmaceutical properties. While sphingosine does not appear to participate in raft formation in membranes, it may rigidify pre-existing gel domains in mixed bilayers.

Free sphingosine is believed to have a signalling role in plants by controlling pH gradients across membranes. In addition, free long chain bases (and the balance with the 1-phosphate derivatives) are essential for the regulation of apoptosis in plants.

A cycle of reactions occurs in tissues by which sphingoid bases are incorporated via ceramide intermediates into sphingolipids (see the web pages on individual sphingolipids), which are utilized for innumerable functions, before being broken down again to their component parts. All the free sphingosine *per se* in tissues must arise by this route, in particular by the action of ceramidases (see the webpage on **ceramides**). The levels of free sphingoids and their capacities to function as lipid mediators are controlled mainly by re-acylation. Catabolism of sphingosine and long-chain bases occurs after conversion to **sphingosine-1-phosphate** and analogues as discussed in our web pages on this metabolite.

3. Analysis

The first step in the analysis of the sphingoid bases of sphingolipids is hydrolysis of any glycosidic linkage or phosphate bonds as well as the amide bond to the fatty acyl group. This should be accomplished by a procedure in which the minimum degradation or rearrangement of the bases occurs, such as *O*- or *N*-methylation. While many analysts claim that base-catalysed hydrolysis causes least disruption, rapid acid-catalysed methods are often preferred for convenience. Subsequently, the bases are best analysed by gas chromatography after derivatization to reduce their polarity.

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© *W.W. Christie*

Scottish Crop Research Institute (and Mylnfield Lipid Analysis), Invergowrie,
Dundee (DD2 5DA), Scotland

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