Trehalose Glycolipids have Amazing Function

by J. C. Spencer

Trehalose is one of the sugar building blocks that creates great cellular communication. Here is evidence that trehalose is the sugar used to build unique glycolipids. Trehalose is the building block in a number of cell wall glycolipids. Glycoproteins are more on the cell surface while glycolipids do most of their work in the cell wall and within the cell itself.

Sugars are the OPERATING SYSTEM (OS) of the body, processing DNA data, thought, and all cellular communication. Glycomics, the science of sugars, may be thousands of times more complex than the genome project. The eight Royal Sugars used to construct glycoprotein receptor sites that coat every healthy human cell were described by Robert K. Murray, M.D., PhD, in Harper's Biochemistry first in 1996 and then in subsequent editions [and discussed in layman's language in my e-textbook *Expand Your Mind - Improve Your Brain*, foreword by Dr. Murray]. Our dear friend Dr. Rob, as we affectionately call him, is from Scotland and he will be glad to know some of the work you are about to read came out of the Lipid Analysis Unit of the Scottish Crop Research Institute in Dundee.

Ongoing glycoprotein and glycolipid research conducted around the world is getting us closer to understanding the different functions precipitated by the various natural designs. Change one sugar form or fatty acid and you have a whole new functionality and potential health benefit. Glycomics, the science of sugars, is indeed the future of medical science and healthcare.

One of the serendipitous facts about trehalose is not only that it has a hydration factor but that it is part of the construction of a surfactant that may help clean and extract waste from within the cells. That function is discussed in this report which is restricted to natural biosynthesis of trehalose and glycolipids.

Trehalose first esterifies to form monomycolate. Esterify is to form ester which is any of a class of organic compounds corresponding to the inorganic salts and is formed from an organic acid and an alcohol, usually with the elimination of water. Mycolic acids are long fatty acids found in some the cell walls. It is believed that monomycolate is the precursor to dimycolate. We have earlier reported on the Trehalose Dimycolate Project where progress has been made on a drug for treating tuberculosis. Trehalose 6,6'-dimycolate (TDM) is a primary immunostimulatory component of the cell wall of *Mycobacterium tuberculosis* (TB).

Extra-cellular trehalose lipids contain succinic acid. Succinic acid is a dicarboxylic acid occurring naturally in plant and animal tissues. It plays a significant role in intermediary metabolism (Krebs cycle) in the body. Krebs cycle (also called citric acid cycle or tricarboxylic acid cycle) is a sequence process of enzymatic reaction which a two-carbon acetyl unit is oxidized to carbon dioxide and water to provide energy in the form of high-energy phosphate bonds. The carboxylate anion, succinate, esters from succinic acid called alkyl succinates, powerful surfactants.

The citric acid cycle is the final common pathway for the oxidation to CO_2 of fuel molecules provides intermediates for biosynthetic reactions and generates ATP by providing electrons to the electron transport chain. The citric acid cycle results in a breakdown of glycose during glycolysis in the cytoplasm to fuel the mitochondrion. You may view an interactive graphic of the Krebs cycle designed by John Kyrk at http://www.johnkyrk.com/krebs.html

The Krebs cycle is also known as the citric acid cycle because citric acid is the first sequenced product generated by this chemical conversion. The foods you eat become the fuel supply for the citric acid cycle. How well it processes that food and gets the nutrients to your cells determines your health. The pH factor plays an important role. You can raise your pH by eating less acidic foods, less soft drinks, and less bad sugars while eating more alkaline foods and drinks. The alkaline effect on your body is based upon the mineral content of your food and the ash residue that remains after our foods are consumed. Some foods leave an acid ash and other foods leave an alkaline buffer. The foods that contain alkaline minerals leaving an alkaline buffer are all the foods that really are good for us including fresh vegetables and fruit and good sugars. The foods that leave an acid ash include the bad sugars, alcohol, saturated fats, meats, and dairy.

A higher pH, a more alkaline body, is conducive to better cell function, better cell absorption, cellular communication via better glycolipids and glycoprotein receptor sites, and better health.

More research is needed and much of the activity of trehalose within the human body is yet to be determined. The enzyme trehalase serves as a transporter where trehalose performs different functions. Also, it appears that some of the trehalose is split into two glucose molecules over an extended time which support sustained energy.

Here is the science paper on glycoproteins with references. Trehalose is discussed in #5 with Figures #7 and #8:

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RHAMNOLIPIDS, SOPHOROLIPIDS AND OTHER UNUSUAL GLYCOLIPIDS STRUCTURES, OCCURRENCE AND BIOLOGY

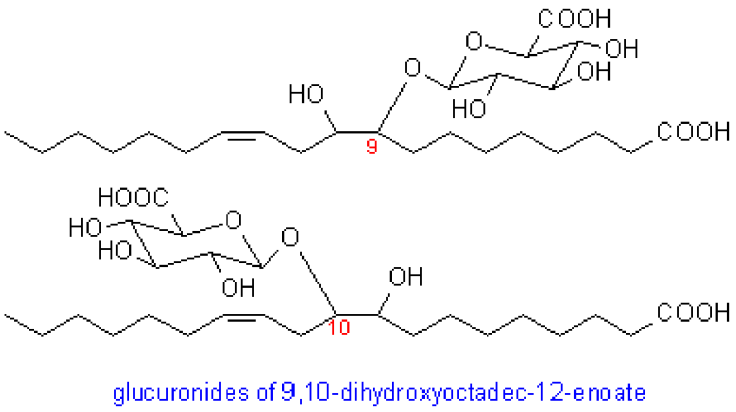
Innumerable simple glycolipids, comprising simply fatty acids esterified to a carbohydrate moiety have been described in nature, from animals, plants and microorganisms, and it is impossible to discuss more than a few representative examples here. They can vary in structure from monosaccharides with one or more fatty acyl substituents to complex carbohydrates, which can in turn be linked to terpenoids, aromatic compounds or nucleosides, as well as having multiple points of attachment to fatty acids via ester or glycosidic linkages. Some are integral components of tissues, while others produced by microorganisms are secreted into the growth medium. It is only possible to describe a few of the more important of these in this review. Because of their amphipathic nature, simple glycolipids are natural biodegradable detergents. In addition, some are reputed to have valuable pharmaceutical properties, for example as antibiotic, anti-fungal or even anticancer agents. A number of these lipids are major products of certain organisms, and have appreciable commercial importance. Substantial amounts of simple fatty acyl

derivatives of sugars, e.g. sucrose esters, are produced in industry by chemical synthesis, but the discussion here is restricted to natural glycolipids.

1. Simple Carbohydrate-Fatty Acid/Alcohol Conjugates

Simple conjugates of mono- and disaccharides with fatty acids via glycosidic or ester bonds (alkyl or acyl glycosides) are common in nature, but especially in marine organisms and in plants. Little or nothing is known of their biological functions or biosynthesis and the reviews by Dembitsky cited below cover the literature thoroughly. In contrast, a glucopyranosyl derivative of tuberonic acid is known to induce tuber formation in potatoes. Mycobacteria produce 6-*O*-acylglucosides of mycolic acids in addition to the more complex trehalose lipids described below.

Linoleic acid is oxidized in the human liver by a P450 mono-oxygenase to a mixture of 9,10 and 12,13 epoxides, which are converted to the corresponding diols, termed leukotoxin and isoleukotoxin, by an epoxide hydrolase. Specific enantiomers of each of the four possible hydroxyl groups can then be converted to glucuronides by the action of a UDP-glucuronosyltransferase. The products from 9,10-dihydroxyoctadec-12-enoate are illustrated.



glucuronides of 9,10-dihydroxyoctadec-12-enoate

Fig. 1: Structure - fattyacid glucuronide

A small proportion of the dihydroxy metabolites are also converted to glucuronide esters. As the precursor monoepoxides of linoleic acid are produced at high levels during acute inflammation, and in patients with adult respiratory distress syndrome or suffering from severe burns, it is believed that glucuronidation may be a detoxification mechanism, facilitating excretion. However, there are also suggestions that some fatty acid glucuronides, for example of phytanic and docosahexaenoic acids, may be ligands for hormone receptors in the nucleus or have signalling functions.

Many cyanobacterial species contain distinctive organelles termed heterocysts that are capable of fixing nitrogen. The cell walls of these maintain a micro-aerobic environment to enable the reaction to occur, and they consist of three extra layers external to the normal cell envelope, the innermost of which is comprised of unusual glycolipids, i.e. very-long-chain fatty alcohols linked to a carbohydrate moiety, such as the 1-(*O*-α-D-glucopyranosyl)-3*R*,25*R*-hexacosanediol illustrated.

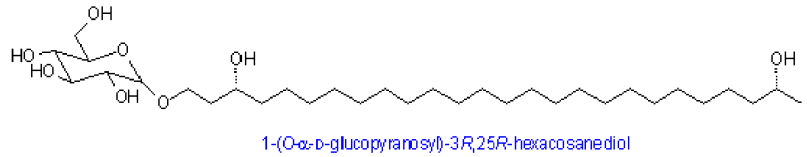


Fig. 2: formula of 1 - (O-alpha-D-glucopyranosyl) - 3R, 25R -hexacosanediol

Other forms exist differing in the number of carbon atoms, and the number and position of hydroxyl and/or keto groups.

Nematodes, including a number of human parasites, contain unusual glycolipids termed ascarosides especially in the eggs and ovaries. These consist of α-L-3,6-dideoxymannose or ascarylose, which occurs in few other organisms, linked glycosidically to the hydroxyl group of a 2-hydroxy alcohol or of an ω-1 hydroxy fatty acid. The free hydroxyl groups of the ascarylose moiety may be acetylated, and the chain-length of the alkyl component can vary from 6 to 29 and can contain further hydroxyl groups or double bonds. For example, the eggs of Ascaris sp. have a four-layer shell, the innermost layer of which consists of 75% of ascarosides and is responsible for the impermeability of the shell. It protect the contents from the harsh conditions in the intestines. Two representative examples are illustrated.

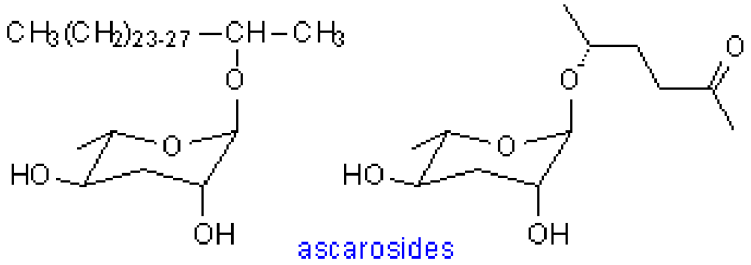


Fig. 3: formula of two representative ascarosides

In addition, certain ascarosides in the nematode *Caenorhabditis elegans* function as pheromones as well as regulating development and behaviour

2. Rhamnolipids

Pseudomonads are rod-shaped gram-negative bacteria found in soils that produce extracellular lipids known as rhamnolipids. The term is indicative of the fact that these lipids contain one or two rhamnose units, linked glycosidically to a 3-hydroxy acid, thence by an ester bond to a further 3-hydroxy acid as illustrated. Thus, the monorhamnolipid from *Pseudomonas aeruginosa* grown on hydrocarbons is 2-*O*-α-L-rhamnopyranosyl-α-L-3-hydroxydecanoyl-3-hydroxydecanoic acid.

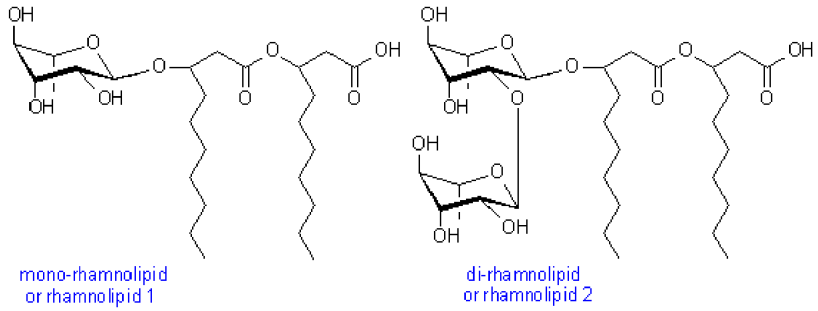


Fig. 4: formula of rhamnolipids

3- or β-Hydroxydecanoic acid is the most common fatty acid constituent, but other fatty acids may be found depending on the *Pseudomonas* species or growth conditions, including 12:0, 12:1, 12:2 and 8:2 (each with a 3-hydroxyl group), resulting in a number of distinct molecular species. All of these lipids have antifungal and antiviral properties, and they exhibit bactericidal properties to Gram-positive bacteria. On the other hand, they are considered as one of the virulence factors in *Pseudomonas sp*. Because of their potent detergent properties, they are produce commercially as soil remediation agents and to combat marine oil pollution. Although the exact mechanism is not clear, it is evident that rhamnolipids are able to bind to substrates with low degrees of aqueous solubility including hydrophobic pollutants. Rhamnolipids are also used as a source of L-rhamnose. Specific genetically modified *Pseudomonas* species can produce as much as 100g/L of culture medium under optimum conditions. While the wild organisms are pathogenic so must be cultured in a strictly regulated environment, the recombinant Pseudomonads appear to be safe.

Two unusual rhamnolipids, designated myxotyrosides A and B, have been isolated from a *Myxococcus sp* (Myxobacteria are gliding bacteria). These have a rhamnose unit linked to tyrosine and thence to a fatty acid such as (*Z*)-15-methyl-2-hexadecenoic and (*Z*)-2-hexadecenoic acid.

The biosynthesis of monorhamnolipid in *Pseudomonas* species involves two sequential glycosyl-transfer reactions catalysed by specific rhamnosyltransferases, in which 3-hydroxydecanoyl-3-hydroxydecanoate is linked to an activated rhamnose moiety (thymidine diphospho-rhamnose). The lipid intermediate in rhamnolipid biosynthesis has a separate function in the swarming motility of the organisms.

3. Sophorolipids

Some yeast species, and in particular *Candida (Torulopsis) bombicola*, secrete extracellular glycolipids known as sophorolipids (or sophorosides), as they contain the sugar sophorose (β-D-Glc-(1→2)-D-Glc). This is linked glycosidically to the hydroxyl group of a 17-hydroxy-C_{18} saturated or monoenoic (*cis*-9) fatty acid, the carboxyl group of which is usually linked to the 4'-hydroxyl group of the second glucose unit to form a lactone, though it can also remain in free form and then have more powerful detergency properties. One or both of the 6-hydroxyl groups on the glucose units are acetylated. With the organism *C. bognoriensis*, the fatty acid is 13-hydroxydocosanoate, while in *C.*

batistae it is 18-hydroxy-stearic acid (and the acidic form of the lipid predominates).

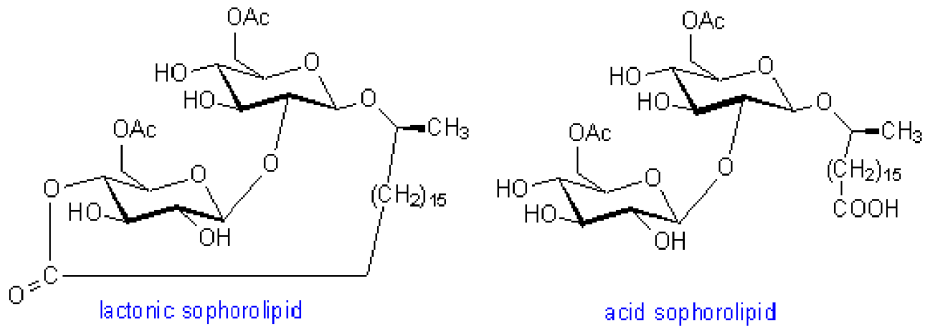


Fig. 5: Formula of sophorolipids

Biosynthesis involves sequential transfer of activated glucose molecules, UDP-glucose (see our webpage on glycosyldiacylglycerols), to a hydroxy acid in processes catalysed by two different glycosyltransferases. Finally, the molecule is acetylated by an acetyltransferase. The fatty acid constituents can be synthesised *de novo* from acetate or by modifying alkanes in the growth medium.

While the physiological role of sophorolipids in yeast species is uncertain, it seems likely that they serve for extracellular carbon storage (reducing the cellular sugar content) and as a defense against competing microorganisms.

These lipids are produced on a commercial scale when the organism is cultured on substrates containing glucose and a source of alkyl moieties, such as alkanes or seed oils, which influence the nature of the fatty acid constituent. Yields can be as much as 300g/L from organisms in the stationary phase. Sophorolipids are used in commerce in cosmetics as deodorant, anti-dandruff and bacteriostatic agents, and they are also known to possess antifungal, antiviral and spermicidal properties. The hydroxy acid constituents are in demand for lactonization for use in perfumes.

4. Mannosylerythritol and Cellobiose Lipids

The yeast *Candida (Pseudozyma) antarctica* secretes an extracellular mannosylerythritol lipid (4-*O*-(2',6'-di-*O*-acyl-β-D-mannopyranosyl)-D-erythritol), with biosurfactant properties, when grown on a vegetable oil substrate. When grown on glucose, the same lipid accumulates intra-cellularly as an energy store until it amounts to 10% or more of the dry weight of the cell.

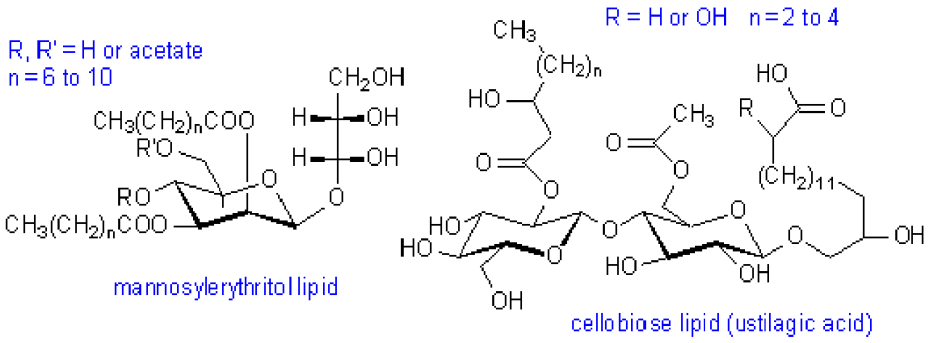


Fig. 6: formula mannosylerythritol and cellobioselipids

One or two of the hydroxyls on the mannose residue are acetylated, and there are two esterified fatty acids, which are both are odd- and even-numbered from C_8 to C_{12} in chain-length (longer in related species). While this organism gives the greatest yields of these lipids, they were first found in the fungus *Ustilago maydis* and termed 'ustilipids'. In this instance, the 2-hydroxyl group of the mannose residue is esterified with a C_2 to C_8 fatty acid, while the 3-hydroxyl group is esterified by a C_{12} to C_{20} fatty acid. Several other species of the genus *Pseudozyma* are now known to produce similar lipids in which the nature, number and positions of the acyl groups vary. As with other biosurfactants, these compounds are believed to facilitate dissolution of organic hydrophobic compounds so that they can be consumed by the organism. Mannosylerythritol lipids have been shown to have a number of profound biological effects in animals, but especially to induce the differentiation of certain cancer cells.

Ustilago maydis also contains distinctive cellobiose lipids (or 'ustilagic acid'), consisting of the disaccharide cellobiose linked *O*-glycosidically to the ω-hydroxyl group of the unusual long-chain fatty acid 15,16-dihydroxyhexadecanoic acid or 2,15,16-trihydroxyhexadecanoic acid. Others of the hydroxyl groups are esterified either to acetate or a medium-chain 3-hydroxy fatty acid. A further unusual cellobiose lipid is produced by the fungal biocontrol agent, *Pseudozyma flocculosa*, and has been show to be 2-(2',4'-diacetoxy-5'-carboxy-pentanoyl)octadecyl cellobioside (flocculosin), the compound responsible for the antifungal activities of the organism.

5. Trehalose Lipids

Trehalose is a non-reducing disaccharide in which the two glucose units are linked in an α,α-1,1-glycosidic linkage. It is the basic component of a number of cell wall glycolipids in Mycobacteria and Corynebacteria. Of these trehalose lipids, cord factor is the best known. It is a component of the cell wall lipid of *M. tuberculosis* and comprises a distinctive branched-chain mycolic acid esterified to the 6-hydroxyl group of each glucose to give trehalose 6,6'-dimycolate. In addition to being one of the major toxic components of the cell wall, it is believed to be responsible for the low permeability of the membranes conferring appreciable drug resistance to the organisms.

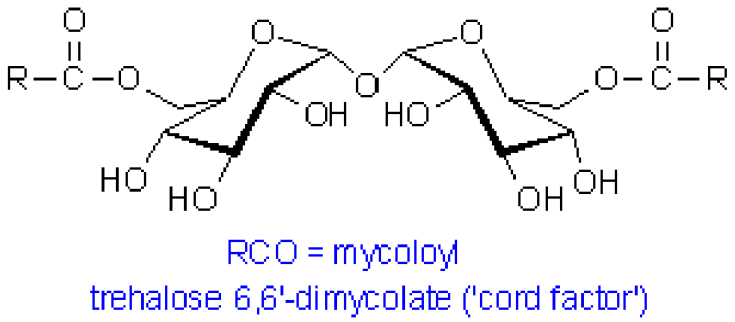


Fig. 7: structure-cord factor

During biosynthesis, trehalose is first esterified to form the monomycolate, which is believed to be the precursor to the dimycolate, although via the action of a mycolyl transferase it also may be the donor of mycolic acid residues to the cell wall arabinogalactan to produce the mycolyl-arabinogalactan-peptidoglycan complex.

Among the other antigenic glycolipids in the mycobacterial cell wall based upon trehalose, there are acylated trehaloses with various fatty acids attached to the 2 and 3 hydroxyl groups of the same glucose. These fatty acids include n-C_{16-19} saturated fatty acids, C_{21-25} α-methyl branched fatty acids, and C_{24-28} α-methyl-branched, β-hydroxy fatty acids. Trehalose lipids produced by Corynebacteria and Nocardia are similar in structure but contain the corynomycolic or nocardomycolic acids, respectively, which are related in structure to the mycolic acids.

A strain of *Rhodococcus erythropolis* produces extra-cellular trehalose lipids containing succinic acid, i.e. 2,3,4,2''-di-*O*-succinoyl-di-*O*-alkanoyl-α,α-trehalose and 2,3,4-mono-*O*-succinoyl-di-*O*-alkanoyl-α,α-trehalose, while 3,4-di-*O*-alkanoyl-2-*O*-succinoyl-α-D-glucopyranosyl-2'-*O*-succinoyl-α-D-glucopyranoside produced by *Rhodococcus* sp. SD-74 is illustrated. They are powerful surfactants. More complex sulfated trehalose lipids are also known.

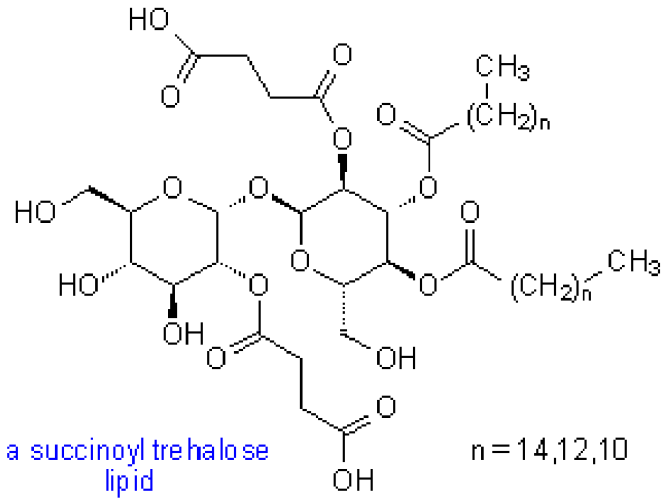


Fig. 8: a succinoyl trehalose lipid

Suggested Reading

Arutchelvi, J.I., Bhaduri, S., Uppara, P.V. and Doble, M. Mannosylerythritol lipids: a review. *J. Ind. Microbiol. Biotechnol*., **35**, 1559-1570 (2008).

Boulton, C.A. Extracellular microbial Lipids. In: *Microbial Lipids. Volume 2*. pp. 669-694 (Ed. C. Ratledge & S.G. Wilkinson, Academic Press, London) (1989).

Brennan, P.J. Mycobacterium and other actinomycetes. In: *Microbial Lipids. Volume 1*. pp. 203-298 (Ed. C. Ratledge & S.G. Wilkinson, Academic Press, London) (1988).

Dembitsky, V.M. Astonishing diversity of natural surfactants: 1. Glycosides of fatty acids and alcohols. *Lipids*, **39**, 933-953 (2004) (there are six further reviews by this author in Lipids that are also relevant).

Jude, A.R., Little, J.M., Freeman, J.P., Evans, J.E., Radominska-Pandya, A. and Grant, D.F. Linoleic acid diols are novel substrates for human UDP-glucuronosyltransferases. *Arch. Biochem. Biophys*., **380**, 294-302 (2000).

Soberón-Chávez, G., Lépine, F. and Déziel, E. Production of rhamnolipids by *Pseudomonas aeruginosa. Appl. Microbiol. Biotechn.*, **68**, 718-725 (2005).

Van Bogaert, I.N.A., Saerens, K., De Muynck, C., Develter, D., Soetaert, W. and Vandamme, E.J. Microbial production and application of sophorolipids. *Appl. Microbiol. Biotechn*., **76**, 23-34 (2007).

http://lipidlibrary.aocs.org/Lipids/rhamno/index.htm

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http://www.cyberlipid.org/cyberlip/home0001.htm

http://www.cyberlipid.org/glycolip/glyl0612.htm

http://www.vet.uga.edu/vpp/Sakamoto/Lab/Index.php

www.endowmentmed.org