# *OSMO-PROTECTANTS.*

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Due to significant overpopulation and poor water management the polders in The Netherlands and Belgium brackishness is becoming a serious issue. The same is valid for other overpopulated countries: 20% of the cultivated land worldwide is affected by salinity. As usual Nature tries to correct our obtuseness. Plants are able to reduce damage from saline stress by the accumulation of high intracellular levels of "osmo-protectants", molecular species that protect against undesired osmosis. These compounds include proline & hydroxyproline-based amphoterics, ectoine, betaine and trehalose, and have evolved in many different organisms.

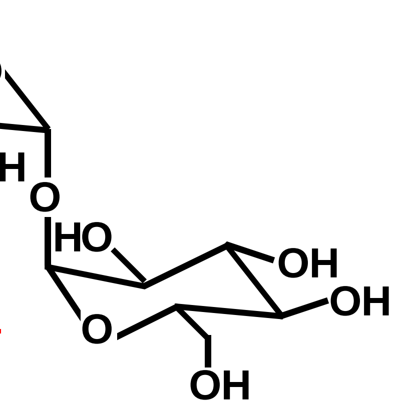
Proline-based osmo-protectants are found in Stachys officinalis (betony), also known as St. Bride's Comb. St. Bride is a Christian version of the goddess Bridget (the Church of St. Bride in London's Fleet Street was built over a temple to Bridget). A Gaelic prayer on St. Bride runs:

"Early on Bride's morn shall the serpent come from the hole.
I will not harm the serpent, nor will the serpent harm me."

Betony also has a rich history in traditional pharmacy, particularly on the British isles, but today it is almost completely forgotten. It was effectively used to treat high blood pressure and migraine, frequently in combination with rosemary. These proline and hydroxyproline derivatives are frequently identified as alkaloids, but we prefer to characterise them as quaternised amino acids. Stachydrine (N,N-dimethylpyrrolidine-2-carboxylate) is the major osmo-protectant in betony, and it is also found in bitter orange (Citrus aurantium), in germinating alfalfa seeds (Medicago sativa; ~0,8%), motherwort (Leonurus cardiaca) and yarrow (Achillea millefolium). Hydroxyproline may be converted to betonicine (4-hydroxy-N,N-dimethylpyrrolidine-2-carboxylate) or turicine, abundantly found in horehound (Marrubium vulgare). Horehound, already described in the 13$^{th}$ century by Jacob van Maerlant in "Der Naturen Bloeme", is traditionally used for the treatment of bronchitis where there is a non-productive cough. Topically horehound is used to promote the healing of wounds, with certainty due to its osmo-protecting properties.

Trehalose, a non-reducing disaccharide, is another osmo-protectant. It is composed of two glucose units combined via an $\alpha,\alpha$-1,1-glycosidic linkage. It is found in micro-organisms, insects and plants; significant sources for trehalose are honey and baker's yeast. In yeasts and plants it functions as a signalling molecule to direct/control particular metabolic pathways or to affect growth. It has also been demonstrated that trehalose protects proteins and cellular membranes from inactivation or denaturation caused by stress conditions, in particular desiccation, dehydration, heat, cold and oxidation. In mycobacteria and corynebacteria trehalose is an integral component of the glycolipids that are important to cell wall structures. Trehalose accumulates dramatically in micro-organisms during heat shock and osmotic stress and helps protect cells against thermal injury and oxygen radicals.

Trehalose has been shown active in cold-adaptation of bacteria. The genetic code Escherichia coli is easily changed disabling them to produce trehalose. When stored at 4°C they died significantly quicker than wild strains that do produce trehalose. Exposing modified strains and wild strains of E.coli to a cold shock (37°C→16°C) the trehalose concentration in the wild strains increased 8-fold while there was no increase in trehalose production in the mutants.




**TREHALOSE**

High concentrations of trehalose are found in many organisms that naturally survive dehydration, for example yeasts, some plants and bacteria. Mammals cannot produce trehalose, and are frequently dependant on E.coli to producing it on their behalf. Again a beautiful example of symbiosis.

The mechanism by which trehalose mediates tolerance to freezing or desiccation is not completely clear; but stabilization of certain cell proteins and/or lipid membranes in involved in the process. The exceptional stabilisation qualities of trehalose also enables to retain the activity of enzymes in solution as well as in the freeze-dried state. That is subject to highly significant cosmetic application: enzyme chemistry, where water is frequently a real bad guy as it deactivates many enzymes.
In food products protection and preservation of cell structures trehalose (which is heat stable) assists in the maintenance of the desired texture of the food. Trehalose has a GRAS status for food products.

The degree of cellular hydration is highly dynamic, and may change in seconds due to sudden changes in electrolyte concentration in the environment of the cell (Häussinger). This effect is named aniso-osmolarity. Cellular hydration is also affected by hormones and oxidative stress. All cells have build up powerful mechanisms to resist excessive loss or uptake of water to maintain the normal cell volume. By all means, excessive variation of cellular hydration will with certainty affect the functionality of proteins, enzymes and genetic material. Molecules that regulate the degree of hydration, osmolytes, must not influence the activity of proteins, even at high concentration. There are not to many of those, and to be named are inositol and sorbitol, but also particular amino acids such as taurine (2-aminoethanesulfonic acid; by-product from cysteine metabolism) and betaine (trimethylglycine). Betaine is commercially obtained from sugar beets and supplied by Danisco Cultor

$$(CH_3)_3N^+-CH_2-COO^-$$

**BETAINE**

Betaine, also named oxyneurine, is a white crystalline powder with a melting point of ~240°C. Chemically it is identified as a Zwitter-ion, i.e. it carries both positive and negative charge; in acidic conditions the carboxylate group is protonated and betaine will than behave as a cationic product.

$$(CH_3)_3N^+{-}CH_2{-}COO^- \underset{-H^+}{\overset{H^+}{\rightleftarrows}} (CH_3)_3N^+{-}CH_2{-}COOH$$

Betaine is easily soluble in water (64 g/100 ml) and is fully stable under ordinary conditions. In the body it is able to methylate homocysteine to form methionine while betaine is converted to dimethylglycine. Synthesis of betaine in body tissue may not be high enough to meet the betaine needs of animals, especially under stressful conditions, and it is therefore an essential supplement in the animal feed industry. Aqueous betaine solutions are excellent solvents: 5% salicylic acid solutions can be made using a 35% aqueous betaine solution. This can easily be explained on the basis of the above given equilibrium, but the intelligent formulation chemist will immediately recognise the advantages of this system. Far more interesting is the fact that such a highly concentrated salicylic acid solution is virtually non-irritant to the skin.

Based on the mechanism of osmo-protectancy betaine is a superior moisturiser, comparable in activity to ectoine, inositol and trehalose. We've been playing around ourselves with betaine in sun care products. In the presence of betaine the formation of an erythrema occurred only a long, long time after it should have occurred based on the *in vitro* SPF value, where the sun care product in absence of betaine behaved as expected. Obviously cell death was avoided due to the presence of betaine. Now "only" reversing the genetic damage caused by UV radiation and we're in the new age of sun care products. It's not to far away that you won't be using UV filters anymore, but than understand also that you're not doing cosmetics anymore. That is prophylactic pharmacy.