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NATURAL PRESERVATIVES**

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## **1. TITLE SLIDE**

Good afternoon, Mr. Chairman, ladies, gentlemen and fellow scientists.

Today I am going to speak on the subject of Natural Preservatives - a subject that probably has more academic interest than practical and economic virtue, because let's face it, the existing synthetic preservatives have a great deal going for them. They are safe, tried, tested, efficient, legal and available. However, having said that, the concept of a natural preservative does have a wonderful marketing angle which may justify the higher raw material costs.

I do not intend to concentrate on the highly technical aspects of this subject, since this, to a large extent, it has been covered in my preprint.

But, before I start on the botanical sources of natural preservative material, let me quickly review the most commonly known and already used preservation techniques.

I draw on the food and beverage industry for many of these examples:

## **2. EXISTING METHODS OF PRESERVATION**

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A review of the existing methods of preservation

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## **3. SUGARS AND GLYCOLS**

For those of us who count our calorific values let me first cite sugar. In the 'good old days' jam could be kept in the larder, but today, with the advent of artificial sweeteners, many must now be stored in the 'fridge. This is because the replacement of sugar by these synthetic sweeteners has meant that the natural level of preservation afforded by sugar has been compromised.

High levels of glycerine, up to 15-20%, will also have a preservative effect, similar to that effect obtained by the use of sugar just mentioned.

#### **4. HONEY**

Honey in its undiluted form is also a natural preservative and, indeed, there are many learned papers citing honey as a viscous barrier to bacteria and infection, whilst maintaining a moisture equalisation in skin tissue - especially in the treatment of burns (even those of a third degree severity).

#### **5. ALCOHOL**

Not all organisms are bad! The production of alcohol from sugar by yeast is a great favourite of mine! A wine carefully produced using sterilised equipment and fermented to 13% by volume will just about resist further infection from external organisms. It is the process in between where the ferment is vulnerable to infection. This naturally produced fermentation grade alcohol can be concentrated by distillation and used as a natural preservative in our toners, aftershaves and colognes.

#### **6. HEAT**

Heating, cooking and pasteurisation is another natural form of preservation that will sterilise our products, especially where that product is designed as a one-shot use product - for example, a phial or a sachet. Alternatively, once opened, the product can be stored in the fridge or freezer to prevent microbiological degradation.

#### **7. DESSICATION AND ANHYDROUS**

Removing water from a product or making it totally dehydrated will greatly reduce the possibility of spoilage; however, it must be recognised that the presence of spore-bearing organisms could become active once that water is reintroduced.

In a similar vein, we could make products with materials that never contained any traces of water, that is, to make an anhydrous product. However, creams that can be finished by the consumer, by introducing water to the blend of oils, fats and waxes will suffer similarly to the dessicated products.

#### **8. SALT**

The use of extreme levels of salt as used by the ancient mariners to preserve their meat is effective and I am sure that the preservation of the Egyptian mummies was, in part, achieved by the 40 day treatment in natron (a concentrated brine solution that osmotically drained the tissues of water).

#### **9. COLD**

Putting something in the cold merely 'stops the clock' on microbiological growth and is perfectly fine provided the product was sterile when it was placed in the cold and/or had sufficient preservative 'mass' to counter any new organisms subsequently introduced.

## 10. ACID pH

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The use of an acid environment to inhibit microbial growth

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We can boost our preservative activity by operating at as low a pH as possible. Many of the alpha hydroxy acids are obtained from citrus species where the major components derived are citric and malic acid.

Incidentally, I am surprised that suppliers of baobab oil (*Adansonia digitata*) have not exploited the extremely high levels of tartaric acid present in the outer pericarp. This is thrown away as a waste product when the oil is produced, so why are we fumbling around with expensive sources of natural alpha hydroxy acids, when there exists a source that is essentially free?

## 11. CHELATING AGENTS

### ACETATE 1

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The use of a chelating agent to enhance bactericidal activity

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In addition to formulating at low pH, we can add chelating agents such as phytic acid extracted from rice bran, to enhance the activity of our natural preservative.

I have not had the opportunity to fully investigate the immense chelating power that this material has at its disposal, but theoretically it has the potential to work as efficiently as our normally employed ethylene diamine tetra acetic acid or more simply EDTA.

## 12. ANTIOXIDANTS

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The use of antioxidants to enhance stability

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Antioxidants such as natural tocopherol and ascorbic acid will further aid in preservation. Though I accept that the stability of vitamin C is not very good, I have been assured that the stability of a new water soluble antioxidant "Antoxine" is not in question. It is derived by a fermentative process which consists in cultivating lactobacilli on a selected microalgae substrate, namely *Skeletonema costatum*, since I only heard of this material a couple of months ago, I cannot personally comment on its efficiency. However, having talked to Dr. Richard Schmidt at the Welsh School of Pharmacy, it sounds just the right idea to progress.

### **13. PLANTS SELF-PRESERVATION**

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The examination of plant self-preservation

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Let us now turn our attention to plants, and especially to why they do not contract diseases or go mouldy in the wild. It has always struck me as being quite extraordinary, that a plant sits out in the rain all day and all night, endures all ranges of heat and humidity and does not support any noticeable microbiological growth, beyond what might be an accepted microbiological flora.

More to the point, it positively thrives when the contents of this fellow's rear end

### **14. HORSE**

or even this type of mobile composting unit deposits around it.

### **15. COW**

Indeed, if that was not enough, the Chinese farmer will actually empty the contents of the family piss-pot over his most treasured plants, in order to give them extra nutrients.

Plants have one object in life, and that is to survive and prosper, and to do this (and I will use just one method as an example) it must first flower (this is apple blossom).....

### **16. APPLE BLOSSOM**

.....and then fruit (surprise, surprise - this is an apple)

### **17. APPLE FRUIT**

In order to flower and fruit it must remain healthy, and it is during this period that the plant has its highest natural level of protection.

Once the plant has dropped its fruit, it tends to pass into decline having satisfied its main objective which is the survival of future generations. As the weather cools, the leaves fall off and are broken down by bacteria, moulds and other insects and micro-organisms. The plant withdraws the natural preservatives from its leaves, or else the chemical composition within the leaves change. Obviously, the time of harvest for natural preservatives is, therefore, critical.

Sometimes man in his pursuit of beauty upsets the natural plant resistance, and here the rose is a good example. In my garden I have two roses which live side by side. One is a highly refined cultivar rose, which has been selectively bred for its flowers - and a beautiful achievement it is.

## **18. CULTIVATED ROSE**

However, like all highly bred roses, it has lost much of its immunity and is prone to black spot, mould and mildew.

## **19. MILDEW ON CULTIVATED ROSE**

The older, original rose next to it is *Rosa sinensis*,

## **20. ROSA SINSENSIS**

which, unaltered and unbred appears totally oblivious to the blight and blemishes of its neighbour.

## **20. ROSA SINSENSIS FOLIAGE**

It is concluded, that the chemical constituents within each plant clearly differ in composition, even though the cultivated rose is a direct genetic relative of the Chinese rose.

## **22. STATEMENT**

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It is very probable that the highest concentration of chemical capable of preservation is to be found in the fresh plant.

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As many of the extracts that we use are produced from dried plants, it is unlikely that these will produce the desired preservation effects often intimated or promised in the literature.

Even when the extract is prepared from the fresh plant, it is quite usual for the activity of the preparation to decrease in potency with time. And just when you thought that things could not possibly get any worse, you then discover that the activity is also decreased by exposure to light and warmth.

This is not really surprising, when you think about it, because this is exactly what nature would have intended in the wild. If the protective potency did not wear off, then the natural break-down process to return the plant to the soil as humus would never be able to take place, and we would be up to our necks in leaves and other plant detritus.

## 23. NATURAL PRESERVATIVES AND DEFINITIONS

In this slide you will see that there are a large number of

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Words used to describe the killing of spoilage organisms:-

Antibiotic, antimicrobial, antifungal, antiseptic, bactericide, fungicide, preservative, prophylactic, germicidal, disinfectant.

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We can now search the data base using these words to find plants that will have the required effect. In the preprint I have concentrated on the specific challenge test organisms. In my paper today I shall be taking a slightly different approach and look at a different set of plants in order to try and identify the chemical entities which are responsible for the act of preservation.

## 24. LEGAL POSITION

Just before I start, I must remind you that :-

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Preservatives must appear in

Annex VI Part 1 or 2 of the EEC Cosmetic Directive 76/768/EEC - including all up to the 17th. amending Commission Directive 94/32/EC.

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However, there is no legislation for those natural materials, which, when used for their beneficial effect on the skin, may coincidentally have a positive effect on the total preservative requirement of the formulation. Of course, no material appearing in Annex II, the prohibited list may be considered.

Benzoic acid or benzyl alcohol from Siam Benzoin (*Styrax benzoin*), may be used quite legally though at a high price for the natural compared to the synthetic price.

## 25. *PASSIFLORA INCARNATA*

The passion flower is a very beautiful example, this is *Passiflora incarnata*.....

## 26. *PASSIFLORA EDULIS*

### ACETATE 2

.....while this is *Passiflora edulis*, which produces the passion fruits most commonly seen in your greengrocers.

Both contain a number of alkaloids. One substance with antifungal activity was designated passicol. This substance exhibited the characteristics of polyacetylenic compounds. In vitro experiments have demonstrated that passicol kills a wide variety of moulds, yeasts, and bacteria. Group A haemolytic *Streptococci* are much more susceptible than *Staphylococcus aureus*, with *Candida albicans* being intermediate in susceptibility.

The antimicrobial activity of passicol disappears rapidly from dried plant residues and fades gradually in aqueous extracts. Surprisingly the addition of dextran, milk, or milk products has a stabilising effect on dry passicol.

## **27. LANTANA CAMARA**

### **LANTANA**

*Lantana camara* L. Is also known as Wild Sage, a member of the Verbenaceae family.

Traditional use: The leaves are used to relieve itching, either by making a powder from the dried leaves and applying it directly to the affected area or by bathing in a leaf infusion. While there are numerous traditional uses that involve ingestion of the leaf, this plant is toxic and should not be taken internally (Robineau 1991).

Research Results: This is a widely used and well-studied plant. It is a troublesome weed from the standpoint of livestock farmers, inducing photosensitivity in sheep, cattle, and other animals and proving toxic when ingested. The primary substance responsible is a polycyclic triterpenoid named lantadene A. Children eating the berries have been poisoned, exhibiting muscular weakness, circulatory collapse and, in lesser cases, gastrointestinal irritation. In an *in vitro* study, the essential oil has shown antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ross et al. 1980). An extract of dried leaves has shown antifungal activity against *Aspergillus fumigatus* and *Aspergillus niger* (Saksena and Tripathi 1985). In humans, a leaf paste exhibited antihemorrhagic activity, effective on wounds in 80% cases studied (Wanjari 1983). As the pharmacological literature is abundant with cases of toxicity in animals (e.g. hepatotoxic activity, nephrotoxic activity, etc.), caution is advised when using this plant.

The essential oil is rich in caryophyllene, aldehydes and alcohol which together may account for the antiseptic action of the plant.

## **28. JUNIPERUS COMMUNIS**

### **JUNIPER**

### **ACETATE 3**

Contains terpene hydrocarbons  $\alpha$ -pinene,  $\beta$ -pinene, and limonene, it also contains a bitter alkaloid-like substance called juniperine, as well as sesquiterpenes such as  $\alpha$ -caryophyllene, cardinene and elemene. There is also junione, 10% resin, 30-33% sugars and organic acids.

The Council of Europe, in Plant Preparations used as ingredients of cosmetic products, refer to juniper as an antiseptic.

The infusion of the berries is used as a diuretic and as a disinfectant of the urinary tracts (cystitis). Used externally, a tincture of the branches is used as a rub for some skin conditions and to combat

alopecia. In homoeopathy, a tincture of the berries is used for a large number of diseases. It is also used in the preparation of various liqueurs and spirits.

It is likely that the alkaloid type materials are responsible for its antiseptic properties. The oil from the berries is known as Cade oil, and this is a very powerful antiseptic, but is aggressive to the point of being quite irritant. Care should be taken when used internally, as this material is a possible abortifacient.

### **29. *ARBUTUS UNEDO* STRAWBERRY TREE**

It contains tannins, arbutoside and ethyl gallate (which is ethyl 3,4,5-trihydroxy benzoate). It is the ethyl gallate that possesses strong antibiotic activity against *Mycobacterium* bacteria. The bark, root, leaves and fruit are all used as an antiseptic, anti-inflammatory, astringent and diuretic.

We all know that Propyl gallate is used in Embanox 3 as an antioxidant, and I wonder whether all gallates have this property?

### **30. *SOLIDAGO VIRGAUREA* GOLDEN ROD**

It contains volatile oil, tannin, saponins. It is mildly diuretic, antidiarrhoeal and anti-inflammatory. It contains saponins, flavonoids, and an astringent principle. It is diaphoretic, antiseptic, carminative, diuretic, and the value of the plant was known by the Arabs in the Middle Ages. It was used principally used as a wound herb. It is the flavonoids that are most likely responsible for the antiseptic properties.

### **31. *CITRUS LIMONUM* LEMON ACETATE 4**

Lemon peel yields its virtues to alcohol, water or wine. It contains an essential oil and a bitter principle. Crystals of the flavonoid glycoside hesperidin are deposited by evaporation of the white pulpy portion boiled in water.

The lemon oil extract is obtained from pressing the outer portion of the pericarp. To obtain one kilo, more than three thousand lemons are needed. The extract contains terpenes (such as camphene, limonene, phellandrene, pinene, sesquiterpenes), citral, citronellal, linalool, linalyl and geranyl acetates, aldehydes, camphor etc. It is either these sesquiterpenes or the flavonoid glycosides that are most likely responsible for the antiseptic properties. It also contains apigenin, which is a flavone.

Incidentally, some references mention hesperidin while others mention diosmin as being the flavonoid glycoside in lemon, Merck makes no distinction in their drawn structures, though there are minor differences in the IUPAC names.

The compound hesperidin is also found in citron, mandarin, lemon, orange, bilberry, buchu and butcher's broom. Though there is none of this specific flavonoid in grapefruit, there is the flavonoid naringenin, which is half the molecular structure of hesperidin. Perhaps this explains why Citricidal has a biocide action.



### **32. HYPERICUM PERFORATUM**

#### **ST. JOHN'S WORT**

#### **ACETATE 5**

It is a sedative, anti-inflammatory, anti-depressant, antiviral, antibiotic, antispetic and astringent plant. It is one of the most effective agents for assisting in the healing of wounds or burns when applied externally, especially where nervous tissue has been damaged.

According to Stuart the plant contains an antibiotic, which has been patented as a food preservative. Extracts of the plant have been shown to be active against Gram-negative and Gram-positive bacteria in vitro.

The flowered plant contains traces of an essential oil; a glycoside (hypericine) which is a red pigment; and the o-dihydroxyphenol substituted flavonoid hyperoside or quercetin-3-D-galactoside, which is most likely responsible for the antiseptic action.

### **33. PRUNELLA VULGARIS**

#### **SELF-HEAL**

The flowering stems are used medicinally on wounds, for cases of bleeding, infected wounds and sores, also as a cleansing wash.

Their constituents include tannins, bitter compounds, an essential oil, saponins and a glycoside (aucubin). These materials give selfheal astringent, anti-inflammatory, haemostatic and antiseptic properties.

Aucubin is also present in Globe flower (*Globularia vulgaris*), Eyebright (*Euphrasia officinalis*), Plantain (*Plantago major*) and Chaste Tree (*Vitex agnus castus*)

### **34. TRILLIUM GRANDIFLORUM**

#### **BIRTHROOT OR BETHROOT**

It contains steroidal saponins (diosgenin), essential oil. Little is known about the chemistry. *Trillium* species have been reported to contain a fixed and volatile oil, a saponin (trillarin, which is a diglycoside of diosgenin), a glycoside resembling convallamarin, tannic acid and considerable starch. There is evidence to support the use of trillium for snoring. The saponin glycosides have been shown to have significant antifungal activity.

### **35. PLUMBAGO EUROPAEA, PLUMBAGO ZEYLANICA LEADWORT**

The root has been shown to contain plumbagin or plumbagol, 2-methyl-5-hydroxy-1,4-naphthoquinone, a yellow naphthoquinone, which is responsible for its antimicrobial and antibiotic activity.

A very dilute solution, this is at a concentration of 1:50,000 of plumbagin is lethal to a wide spectrum of bacteria and to pathogenic fungi, i.e. *Coccidioides immitis*, *Histoplasma capsulatum*, *Trichophyton ferrugenum*, *Candida albicans*, *Aspergillus niger* and *A.flavus*.

It has a marked antibiotic action towards pathogenic bacteria and in vitro, the growth of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pneumococcus* was completely inhibited at 1:100,000, *Mycobacterium tuberculosis* at 1:50,000 and *Escherichia coli* and *Salmonella* at 1:10,000.

### **36. DIOSPYROS LOTUS CHINESE PERSIMMON**

There are many other plants that contain plumbagin and these include the sundews, such as *Drosera anglica*, *Drosera rotundifolia* and *Drosera ramatacea* to name but a few. and also the bark of *Diospyros mespiliformis* or Swamp ebony, or monkey Guava, as it is sometimes known. The slide shows *Diospyros lotus*, which also contains plumbagin and is antiseptic.

### **37. CARLINA ACAULIS CARLINE THISTLE ACETATE 6**

It is a wild plant containing essential oil; resin; tannins; inulin; antibiotic substances, carlinoxide and carlinene. The dried root is used as a cholagogue, diuretic, antibacterial, vulnerary and stomachic. The root is used, which contains an antibiotic (carline oxide). It is also, carminative, diaphoretic and antibiotic. It is a protected plant.

### **38. ARCTIUM LAPPA BURDOCK ROOT**

The British Herbal Pharmacopoeia says that it is also known as Bardane Herb which consists of the dried aerial parts of *Arctium lappa* gathered in the first year of growth, which contain flavonoids. It contains antibacterial substances and the leaves are used as a poultice for boils and abscesses. The dried underground parts of Burdock contains a bitter principle and 45% inulin (a polysaccharide), and is useful for cutaneous eruptions and is specific for eczema, especially in dry or desquamatory phase of psoriasis. The seeds contain a glycoside called arctiine. The lignans lappaol A and B have also been isolated from the plant.

Recent studies suggest that the plant may contain compounds that play a role in suppressing cell mutation and chromosomal aberrations.

Acetylenic compounds are also present and bacteriostatic action has been attributed these polyacetylenes, which is interesting, because the bacterial activity of *Passiflora* was attributed to a similar substance.

### **39. *ACHILLEA MILLEFOLIUM***

**YARROW** or **MILFOIL**

**ACETATE 7**

It contains essential oil, achillein, stachydrine, choline, glyco-collbetain, poltines, apigenin (as seen in chamomile), achilline, matricine, proazulenes, inuline, ascorbic acid, antibiotic substances, tannins, aconitic acid, asparagine, waxy oil, an enzyme, gum, benzaldehyde cyanhydrin-glycoside, flavones. The azulene content can be up to 40% or more. Now look at the structure of apigenin, it is nearly identical to that of naringenine found in grapefruit, except for the double bond.

It is antibiotic and antiseptic. In Germany in the 16th century the seeds were put in wine barrels as a preservative.

Azulene is also found in the essential oil of *Matricaria* and *Anthemis* (the chamomiles) and *Artemisia* species, and is believed to contribute to the anti-inflammatory activities of those oils.

But the antibacterial properties might be down to a number of interesting sources, since the plant contains a wealth of flavonoids, polyacetylenes and phenolic acids.

### **40. *ARMERIA MARITIMUM***

**THRIFT** or **SEA PINK**

It contains a naphthaquinone, plumbagone, mineral salts, comprising mainly iodine, bromine, fluorine, mucilage. The antibiotic action is due to plumbagone. Used for its antibiotic action, antiobesic.

### **41. *CALLUNA VULGARIS***

**HEATHER**

It contains flavone glucosides, quercitrin, myricitrin, arbutin. 7% tannin, silicic acid, and an alkaloid ericodin. It is a diuretic, urinary antimicrobial, cholagogue, antirheumatic, vulnerary. The combined effect of its constituents is predominantly antibacterial and antiseptic.

### **42. *SALIX VITELLINA***

**WILLOW**

*Salix alba* which is the White Willow or European Willow contains salicin (itself salicylic aldehyde) and tannin - these are substances with feeble antibiotic and antiseptic action. This plant was the precursor to aspirin and 50g per half litre is used as an application to wounds.

According to Merck, salicylic acid is used as a preservative of food products, but its use for this purpose is forbidden in some countries. In veterinary practice it is used externally as an antiseptic and antifungal agent, and for various skin conditions.

### **43. *AQUILEGIA VULGARIS***

## **COLUMBINE**

It contains cyanogenic glycoside; emulsin; tannin; aquilegine; vitamin C; lipid; an uncharacterised alkaloid. The root, flowers and leaves are used, antiseptic; astringent; weakly sedative.

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## **44. CONCLUSIONS**

The following must be considered:-

1. The difficulty of preserving naturally
2. The processing requirements
3. Time of harvest of raw material
4. Storage of the raw material
5. Protection of both phases
6. The legality of your formulation

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So in conclusion, I would like to make a few comments relating to the formulation of products containing natural preservatives, and that is these....

You have to be very canny and patient. You will have to think about the position that the material is going to take up in your product, for example, in terms of phase residence.

At the same time you will need to consider the temperature and method of addition, which will more than likely affect your preservative efficacy and stability.

As I have mentioned, you will have to think carefully about the way in which the plant has been harvested and the time of year in which that material has been gathered.

The freshness and storage of that material will be of prime importance.

There are numerous oil based materials available, which have bactericidal properties, however, it is the water soluble materials that are in short supply. How many of us would rely solely on, say, Propyl paraben or Nipazol M and totally ignore the water phase, which in the most part is the external phase most likely exposed to direct contamination in our emulsions.

Finally, I would draw your attention again to the legality of using plant preservatives. If the plant is being used for its claims can it act as a preservative, or alternatively, if the plant is being used as a preservative can it be claimed for its cosmetic benefits on the pack copy? Bearing in mind that the 6th Amendment will require you to prove it. As we say in the naturals business, its a right old chestnut! I will leave you with these thoughts and now thank you for listening to me this afternoon.

**FINAL SLIDE**