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(54) Title: USE OF EPOXIDISED COMPOUNDS

(57) Abstract: Epoxidised compounds such as oils, esters and waxes, which are based on vernolic acid, have been found to be useful for treating lesions on mammalian skin. The lesions may be caused by disease or wounding. A particularly preferred epoxidised compound is oil extracted from Vernonia galamensis seed.

Use Of Epoxidised Compounds

The invention concerns uses of epoxidised compounds such as oils, esters and waxes. A particularly preferred epoxidised compound is vernonia oil derived from Vernonia galamensis seed.

Inedible oils of various compositions can be derived from Vernonia plants. There are many different varieties of Vernonia plants and oil may be obtained from many of these plants. Oils derived from Vernonia plants are often referred to as vernonia oil. Reference herein to vernonia oil means oil extracted from a Vernonia plant, whereas reference herein to Vernonia oil means oil extracted from Vernonia galamensis plants. Like other vegetable oils, vernonia oil comprises a mixture of glycerides, mainly triglycerides, a combination of three fatty acids esterified to a glycerol molecule. Like other vegetable oils, the composition of vernonia oil can vary, depending upon the plant part (root, leaf, stem, seed, etc) from which it is extracted. For example, Vernonia oil obtained from the seed of Vernonia galamensis seed is different from Vernonia oil obtained from the leaf of Vernonia galamensis plants. For example, Vernonia oil obtained from the seed of Vernonia galamensis has been found to have a vernolic acid content of about 50 to 80%, whereas Vernonia oil derived from Vernonia galamensis leaf has been found to have a vernolic acid content of 0.5% or less (Baye et al. "Vernonia galamensis, a natural source of epoxy oil: variation in fatty acid composition of seed and leaf lipids" J. Industrial Crops and Products, 21 (2005) 257-261). Vernolic acid is an epoxidised fatty acid also known as cis-12,13-epoxy-octadec-cis-9-enoic acid.

WO 02/055011 describes the use of amphiphilic derivatives having at least one fatty acid chain derived from vegetable oils, such as vernonia oil, in the production of vesicles, micelles and complexants. There is no disclosure in

this document of the vernonia oil being Vernonia oil i.e. oil derived from Vernonia galamensis.

WO 03/047499 describes the use of amphiphilic compounds to form vesicles or liposomes for use in drug targeting.

EP-A-1198237 discloses the use of extracts derived from the seeds of Centratherum anthelminticum (also known as Vernonia anthelmintica) for the treatment of skin disorders, such as impetigo and acne, and fungal infections of the skin and nails. US-A-5693327 discloses the use of compositions containing extracts of Centratherum anthelminticum, which compositions may be in a form suitable for topical application, for the treatment of psoriasis, eczema and lichen planus. Oil derived from Centratherum anthelminticum seed is known to contain significantly less vernolic acid than Vernonia Oil (Teymor et al. "Vernonia", Alternative Field Crops Manual, updated 26 Jan 2000, discloses the vernolic acid content of oil derived from Vernonia galamensis seed to be about 30% more than the best varieties of Vernonia anthelmintica).

DD91617 discloses an emulsifiable oil for cosmetics, dermatology and cleaning purposes, comprising a mixture of vegetable or animal oils with an oil-sol. emulsifier, especially a hydroxyethylated surface active compound and an epoxidised fatty acid triglyceride, such as epoxidised Soya oil.

The inventors have surprisingly found that Vernonia oil and other similar oils and waxes can be utilised in a number of new and unexpected ways.

The present invention, in its various aspects, is as set out in the accompanying claims.

In a first aspect, the present invention provides the use of an epoxidised compound obtainable by extraction from Vernonia galamensis seed in the preparation of a composition for the treatment of a lesion on the skin of a mammal. Preferably, the treatment is by topical administration of the composition onto the lesion.

In another aspect, the present invention provides an emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia galamensis seed. The emollient composition can be used for treating by topical administration a lesion on the skin of a mammal. Alternatively, the emollient composition can be used for the prophylactic treatment by topical administration to the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin.

In another aspect, the present invention provides a dosage form, which may be a botanical drug, comprising a botanical raw material, a botanical drug substance and/or a botanical ingredient from the seed of Vernonia galamensis. The terms "drug", "botanical drug", "botanical raw material", "botanical drug substance" and "botanical ingredient" are terms well know to those skilled in the art, but should the reader have any doubt they are referred to their definitions provided in WO-A-05079823, which definitions are incorporated herein by reference.

In another aspect, the present invention provides a method of treating a lesion on the skin of a mammal by administering to the mammal an epoxidised compound obtainable by extraction from Vernonia galamensis seed.

In another aspect, the present invention provides a method of treating the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin of a mammal by the prophylactic administration of an emollient composition

comprising an epoxidised compound obtainable by extraction from Vernonia galamensis seed.

In one embodiment of the above aspects, the skin lesion may be caused by a skin disease. The disease may be an infection, such as a bacterial infection, viral infection or fungal infection. Examples such a skin disease include psoriasis, dermatitis, ulceration or eczema.

In another embodiment of the above aspects, the lesion is a wound, such as a graze, cut, scratch or burn.
The lesion may be an inflammation of the skin, such as may be caused by an infection or by a wound such as a burn.

In another aspect, the present invention provides a protein having at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester bound to it. Preferably, the at least one epoxidised oil, wax or fatty acid ester is obtainable by extraction from Vernonia galamensis seed.

In other aspects, the present invention provides methods of changing i) the size, ii) the immunogenicity or iii) the biological half life of a protein. In each of these aspects, the method comprises bonding at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester to the protein.

In another aspect, the present invention provides a compound comprising a bio-available molecule bonded to at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester. Preferably, the at least one epoxidised oil, wax or fatty acid ester is obtainable by extraction from Vernonia galamensis seed.

In yet another aspect, the present invention provides a method of preparing a bio-available compound e.g. a pharmaceutically active compound in a

slow-release format (pro-drug), comprising bonding the bio-available compound to at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester.

In each of the above aspects, the epoxidised compound, oil, ester or wax is preferably obtained from Vernonia galamensis seed. For example, the epoxidised compound, oil, ester or wax is as found naturally in Vernonia oil obtained from Vernonia galamensis seed.

In another aspect, the present invention provides the use of an epoxidised compound obtainable by extraction from Vernonia plants in the preparation of a composition for the treatment of a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn. Preferably, the treatment is by topical administration of the composition onto the lesion.

In another aspect, the present invention provides an emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia plants. The emollient composition can be used for treating by topical administration a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn. Alternatively, the emollient composition can be used for the prophylactic treatment by topical administration to the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin originally caused by a wound, such as a graze, cut, scratch or burn.

In another aspect, the present invention provides a method of treating a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn, by administering to the mammal an epoxidised compound obtainable by extraction from Vernonia plants.

In another aspect, the present invention provides a method of treating the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin of a mammal originally caused by a wound by the prophylactic administration of an emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia plants.

In another aspect, the present invention provides a method of treating a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn, by administering to the mammal an oil obtainable by extraction from Vernonia plants.

In another aspect, the present invention provides a method of treating the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin of a mammal originally caused by a wound by the prophylactic administration of an emollient composition comprising an oil obtainable by extraction from Vernonia plants.

In another aspect, the present invention provides the use of an epoxidised compound, such as an epoxidised compound obtainable by extraction from Vernonia plants e.g. Vernonia galamensis seed, as an anti-inflammatory agent. For example, the present invention provides the use of an epoxidised compound, such as an epoxidised compound obtainable by extraction from Vernonia plants e.g. Vernonia galamensis seed in the preparation of a composition for the treatment by topical administration on a lesion on the skin of a mammal, wherein the lesion is an inflammation, e.g. a burn, and/or is caused by a bacterial, e.g. acne, viral or fungal infection e.g. eczema. The epoxidised compound may be vernolic acid or vernonia oil, such as Vernonia oil. The epoxidised compound is Vernonia oil.

In another aspect, the present invention provides a method of treating a lesion on the skin of a mammal wherein the lesion is caused by an

inflammation and/or infection of the skin by topical administration of a composition comprising an epoxidised compound. The epoxidised compound may be an epoxidised compound obtainable by extraction from Vernonia plants e.g. Vernonia galamensis seed. . The epoxidised compound may be vernolic acid or vernonia oil, such as or Vernonia oil. The epoxidised compound is preferably Vernonia oil.

When vernonia oil or Vernonia oil is used in the present invention, crude vernonia oil or crude Vernonia oil may be used, but purified vernonia oil or purified Vernonia oil may also be used. The crude or purified oil may be further processed e.g. by refining. Refining the crude or purified oil may provide an oil that contains less than 0.25% free fatty acid.

Where herein reference is made to a product being "obtainable" from a plant or other named source, the product is preferably "obtained" from that plant or other named source.

The term "oil" as used herein refers to a fixed oil. As such, an oil is a mixture of glycerides, including, for example, triglycerides, diglycerides and monoglycerides. Such an oil may also include a fraction of free fatty acids and glycerol. A glyceride molecule is at least one fatty acid attached to a glycerol molecule. As used herein the term "oil" also encompasses glycerides, that is non-mixed oils composed of a single type of glyceride.

The glycerides in an oil are frequently made up of a combination of fatty acids. An epoxidised oil is any oil which contains some glycerides that include a fatty acid containing an epoxy group. The oil may contain more than one type of fatty acid that includes an epoxy group. Preferably, at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75% and most preferably at least 80% of the fatty acid chains in the oil include an epoxy group. When the term "oil" applies to non-

mixed glycerides, the glyceride molecule contains at least one fatty acid containing at least one epoxy group.

The fatty acid including the epoxy group may naturally contain the epoxy group or may have been artificially epoxidised, by changing the fatty acid to make it include at least one epoxy group. The fatty acid may be epoxidised using standard techniques, known to those skilled in the art. Preferably the fatty acid naturally contains an epoxy group. Such fatty acids include, but are not limited to vernolic acid.

The oil is preferably Vernonia oil. Vernonia oil from Vernonia galamensis is well known in the art and contains vernolic acid as its principal fatty acid. Vernolic acid makes up about 75 to 80 % of the total fatty acids in Vernonia oil extracted from Vernonia galamensis seed, the remainder being e.g. linoleic, oleic and steric acids.

A wax is a fatty acid ester of a long chain monohydroxyl alcohol. An epoxidised wax is a wax in which one or both of the fatty acid and alcohol chains contains at least one epoxy group. Preferably the fatty acid chain contains at least one epoxy group.

A wax usually contains a mixture of fatty acid esters of alcohols. It is preferred that all the fatty acid esters in the wax contain an epoxide group.

The term "wax" as used also herein encompasses waxes containing only a single fatty acid ester of a single monohydroxyl alcohol.

An epoxidised wax may be naturally or artificially epoxidised. A naturally epoxidised wax is a wax in which one or both of the fatty acid and alcohol chains naturally contains at least one epoxy group. An artificially epoxidised wax is a wax in which one or both of the fatty acid and alcohol chains has

been artificially changed to introduce at least one epoxy group. The ester may be epoxidised using standard techniques, known to those skilled in the art.

The term fatty acid ester means any ester that includes a fatty acid. Examples include methyl, ethyl and propylate esters of a fatty acid. An epoxidised fatty acid ester is one in which the fatty acid contains at least one epoxy group.

The fatty acid including the epoxy group may naturally contain the epoxy group or may have been artificially epoxidised, by changing the fatty acid to make it include at least one epoxy group. The fatty acid may be epoxidised using standard techniques, known to those skilled in the art. Preferably the fatty acid naturally contains an epoxy group. Such fatty acids include, but are not limited to vernolic acid.

An epoxy group is a carbon-carbon-oxygen ring, and has a structure well known to those skilled in the art. The term is well known in the art, and would be easily understood by a person skilled in the art.

The inventors have discovered that the epoxy group provides epoxidised oils, esters and waxes with very useful properties, such as the ability to bind to proteins, resulting in the compounds being useful for medical uses including the treatment of skin diseases.

Skin diseases are disorders that affect the skin, and include, but are not limited to, psoriasis, dermatitis, ulcers and eczema.

The invention also provides a method of treating a skin disease, comprising administering an epoxidised oil, epoxidised wax or epoxidised fatty acid ester to a subject in need thereof.

Preferably the epoxidised oil, epoxidised wax or epoxidised fatty acid ester is in a pharmaceutically acceptable condition, possibly having been combined with a carrier or vehicle.

Preferably the epoxidised oil, epoxidised wax or epoxidised fatty acid ester is suitable for topical administration.

The invention also provides the use of an epoxidised oil, epoxidised wax or epoxidised fatty acid ester in the preparation of a medicament for the treatment of a wound.

In particular, the medicament is preferably for augmenting the wound healing process. A wound is a break in the normal structure of an organ or tissue caused by an external agent, including, for example, a graze, a cut, a scratch, a puncture and a burn. The medicament is preferably for the treatment of a wound to the skin.

The invention also provides a method of treating a wound comprising administering an epoxidised oil, epoxidised wax or epoxidised fatty acid ester to a subject in need thereof.

Preferably the epoxidised oil, epoxidised wax or epoxidised fatty acid ester is in a pharmaceutically acceptable condition, possibly having been combined with a carrier or vehicle.

Preferably the epoxidised oil, epoxidised wax or epoxidised fatty acid ester is for topical administration.

According to one aspect of the invention, there is provided a protein having at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester molecule bound to it.

Preferably the epoxidised oil, epoxidised wax or epoxidised fatty acid ester is as defined in relation to the first aspect of the invention.

Preferably the epoxidised oil, epoxidised wax or epoxidised fatty acid ester is bound to the protein via the epoxy group. Preferably the epoxy group allows bonding to the proteins via a hydroxyl group, a sulphydryl group or an amino group on the protein.

By linking the epoxidised oil, epoxidised wax or epoxidised fatty acid ester to the protein, the protein size may be increased, the immunogenicity of the protein changed, especially lowered, and the potential half-life of the protein, when injected in to the blood stream for example, modified, particularly increased.

Preferably the protein has more than 1 epoxidised oil, epoxidised wax or epoxidised fatty acid ester molecule bound to it, preferably more than 2, preferably more than 3, more preferably more than 4, even more preferably more than 5 and most preferably more than 6.
A protein is, as is well known in the art, a chain of amino acids. The term protein as used herein includes any length of amino acid chain and encompasses molecules that might otherwise be known as peptides or oligopeptides. The protein according to the invention may be a naturally occurring protein or may be an artificial protein. The protein may be glycosylated.

It is particularly useful if the protein is immunogenic.

The invention also provides a protein having at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester molecule bound to it for use in therapy.

Further provided by the invention is a method of changing the size, immunogenicity or biological half life of a protein comprising bonding one or more epoxidised oil, epoxidised wax or epoxidised fatty acid ester molecules to the protein under basic catalysis.

The term "basic catalysis" means that the method is carried out at an alkaline pH, that is a pH of greater than 7. Preferably the pH is between 8 and 12, more preferably 8 and 11, most preferably between 8.5 and 10.0.

The method is preferably carried out in a non-reactive buffer such as sodium bicarbonate/sodium hydroxide or carbonate

Preferably more than one epoxidised oil, epoxidised wax or epoxidised fatty acid ester molecule is bonded to the protein. More preferably 3 or more molecules are bonded to the protein.

Also provided is the use of an epoxidised oil, epoxidised wax or epoxidised fatty acid ester to modify the size, immunogenicity or half life of a protein.

Immunogenicity means the propensity of a protein, or other molecule to generate an immune reaction when it is put into a subject's body. The term is well known in the art. It is particularly useful to be able to modify a protein's immunogenicity, particularly to reduce it, such that useful proteins may be supplied to an individual without a large or deleterious immune reaction being generated.

Biological half-life is a measure of the time a protein, or other molecule remains available to provide its biological effect, for example, when injected into a subject's blood stream. Biological half-life means the time taken for half of the protein to be broken down. The term is well known in the art.

In one aspect of the invention, there is provided an epoxidised oil, epoxidised wax or epoxidised fatty acid ester bound to a small molecule. By the term "small molecule", we mean a molecule which when introduced into the body is instantly bio-available, for example a pharmaceutically active compound. The small molecule is preferably a molecule useful in therapy. Such small molecules include, but are not limited to, methotrexate, aminopterin, aminosalicylate, doxirubicin.

The small, bio-available molecule is bonded to the epoxidised oil, wax or ester via the epoxy group.

By linking the small molecule to the epoxidised oil, wax or ester, the in use, e.g. *in vivo* release characteristics of the molecule can be modified. In particular, it is possible to obtain sustained or prolonged release i.e. slow release.

Also provided is an epoxidised oil, epoxidised wax or epoxidised fatty acid ester bound to a small molecule for use in therapy.
The invention also provides a method of altering the in use release characteristics of a molecule by bonding the molecule to an epoxidised oil, epoxidised wax or epoxidised fatty acid ester under basic catalysis.

The term "basic catalysis" means that the method is carried out at an alkaline pH, that is a pH of greater than 7. Preferably the pH is between 8 and 12, more preferably 8 and 11, most preferably between 8.5 and 10.0.

Also provided is the use of an epoxidised oil, epoxidised wax or epoxidised fatty acid ester to modify the release characteristics of a small molecule.

Any small molecule introduced into the body is instantly bio available. Covalently bonding it to another molecule such as an epoxidised oil, epoxidised wax or epoxidised fatty acid ester means that the drug is not immediately available but comes available as body enzymes hydrolyse the linkage between the oil and the drug i.e. it forms a pro-drug.

Without wishing to be bound to any extent by this explanation, the inventors believe that the performance of the epoxidised compounds is attributable to the presence of the epoxy group on the vernolic acid per se and/or on epoxidised derivatives of vernolic acid.

Vernonia oil may be extracted from Vernonia galamensis seed by a process involving harvesting the seed and compressing the seed in a press. The oil so obtained may by purified e.g. by filtering to remove solid contaminants, and may be further purified so as to render the oil substantially colourless e.g. by passing through charcoal. The seed may be pressed immediately after harvesting or it may be subjected to one or more treatment steps before pressing. For example, before pressing, the harvested seed may be subjected to a heat treatment, such as by contacting the seed with water at a temperature above 50°C, preferably at boiling, for a period of time sufficient to heat the seed contents to a temperature of more than 50°C, preferably more than 80°C; and/or the harvested seed may be dried to a water content of 10% by wt or less, based on the weight of seed. In a preferred process, the seed is harvested, contacted with boiling water for a period of at least 15 minutes, e.g. for about 1 to about 1.5 hours, dried to a water content of 6-8 wt%, and then pressed to release the oil. It is believed that the heat treatment process causes deactivation of lipase enzyme(s). Lipase enzyme(s) is(are) present in Vernonia seed and is(are) known to

readily break down triacylglycerols to give free fatty acid(s). Preferably, the vernonia oil used in the present invention contains no more than 5 wt% free acid, preferably less than 2 wt% free acid.

The invention will now be described in detail by way of example only with reference to the figures, in which

Figures 1A and 1B are photos of a subject with psoriasis, taken 1 and 8 days after beginning treatment with Vernonia oil.

Figures 2A and 2B are photos of a subject with wounds, taken on the day the wounds were made and 9 days after beginning treatment with Vernonia oil.

Figures 3A and 3B are photos of a subject with an ulcer, taken on the day of beginning treatment with Vernonia oil and 11 days later.

Examples

In the following examples, reference to Vernonia oil means Vernonia oil extracted from Vernonia galamensis seed.

Example 1: Purification of Vernonia Oil

0.5 litres of crude (black) Vernonia oil can be purified by 2 passages through activated charcoal. The charcoal (200g) is placed in a glass (or plastic) column and the oil allowed to flow through at about 0.5l/hr. After passing through the column, yellow Vernonia oil can be collected, complete decolourisation of the Vernonia oil can be achieved by re-passage through the same column at the same flow rate. The charcoal may be Eheim charcoal particles for fish filters.

Example 2: Wound Healing

In February 2005 a 14 mm sharp wound occurred on the left hand of a subject across the base of the thumb as a result of an accident with a kitchen knife. The wound was clean and open but despite using plasters had failed to heal after 48 hours and still bled. Approximately 0.25 ml of purified Vernonia oil was rubbed into the still-angry wound and the wound re-plastered for 24 hours. After removal of the plaster it was noted that the wound had begun to "knit" and that the size of the open section had decreased to 8.5 mm. A further application of 0.25 ml Vernonia oil was made and rubbed in as before. The wound was not re-plastered. After a further 48 hours it was noted that the wound had closed completely and that healing was now proceeding normally. A further 0.25 ml Vernonia oil was then made and rubbed in. Healing was complete, with only a thin silver scar left, within a further 60 hrs.

Example 3: Wound Healing

In May 2005 a torn thumb nail (right hand and from right to left) caused a 4 mm wound between the nail and the flesh of a subject. Such wounds have, in the past, been difficult to heal and have taken several weeks. Blood from the wound was removed with cotton pads and the area "patted dry" as much as possible. An application of 0.15 ml of Vernonia oil was made and a plaster placed across the area. After 77 hrs the plaster was removed. The wound had effectively healed. All bleeding had stopped and normal scar lines could be observed. The wound was left open and no problems occurred.

Example 4: Wound Healing

As shown in figure 2A, a subject had wounds on two fingers. The subject applied a small amount of Vernonia oil once or twice daily. After 9 days the wounds had healed, as shown in figure 2B.

Example 5: Healing an ulcer

As shown in figure 3A, a subject had an ulcer on his leg. The subject applied a small amount of Vernonia oil once or twice daily. After 11 days the ulcer had healed, as shown in figure 3B.

Example 6: Treatment of a non-specific skin irritation

After a non-specific viral infection in March 2005 itching occurred on both the arms and legs of a subject. Application of known topical medicines such as E45®, Eurax® and use of antihistamine based drugs such as Benadryl® and Zirtek® had failed to alleviate the problem. As a result areas of the arms and legs had been scratched and were raw and angry. Vernonia oil was poured into the palms of the hand and applied in two ways:

(i) Vernonia oil was applied to the broken scratched skin once daily for 3 days. Itching was significantly reduced (from 7 to 3 on a 10 mark scale) and open skin lesions healed rapidly.

(ii) Vernonia oil was applied to unbroken but itching skin. Itching was significantly reduced (from 8 to 2 on a 10 mark scale). Application continued for 4 days. When treatment was stopped no further itching occurred in the affected area.

Example 7: Treatment of Psoriasis

A subject had a mild outbreak of psoriasis on his chest. The subject applied neat Vernonia oil to the area daily. Itching of the area stopped after two days and the psoriasis had virtually gone after 8 days. Figures 1A and 1B show the psoriasis 1 day and 8 days after the initial application of vernonia oil.

Example 8: Protein Binding

The natural epoxide linkage in Vernonia oil and other epoxidised oils and waxes allows base-catalysed (pH 9.0) bonding of proteins through hydroxyl,

sulphydryl or amino groups of proteins such as bovine or human serum albumin (BSA or HSA) HSA, cytochrome C etc.

In this way the protein size can be increased, immunogenicity decreased (through "cloaking" and subsequent reduction in opsonisation) and the potential half life of a protein injected into the blood stream can be modified. Steric calculations indicate that about 2 to 4 triglyceride molecules could be bonded onto an average of 35,000 dalton protein chain.

Base-catalysed reaction of Vernonia oil with BSA indicates that the protein molecule size increases (as determined by HPLC) from about 67,000 dalton to about 75,000 dalton indicating some 7 to 8 triglyceride molecules per 67,000 dalton original protein size.

Protein binding was achieved as follows:
5ml of 5mg/ml albumin in 0.1M NaHCO3 – NaOH, pH9.2 was mixed with 2.0mls of Vernonia oil at 25°C for 24hrs, with constant stirring. The mixture was then chromatographed on a column of sephacryl 5200 equilibrated in 50mM potassium phosphate, pH6.9, 100mM NaCl. The protein peak was detected by UV adsorption, collected and rechromatographed to remove all traces of free Vernonia oil.

Changing a protein by altering its size and surface characteristics by binding a molecule to it is known to affect the protein's immunogenicity and half life.

Example 9: Small Molecule Binding

It is often desirable to modify the release characteristics of small drugs in the body to obtain sustained or prolonged release. Again through the epoxide of Vernonia oil it is possible to obtain base-catalysed (pH 8.0 to 10.0) bonding of small molecules containing amino, sulphydryl or hydroxyl (or

other suitable nucleophilic) functions. A whole range of small molecules are feasible. Experiments with methotrexate indicate that bonding through the relatively inactive amino groups of that molecule occurs at pH 9.5. The covalent bonding appears to be one methotrexate molecule per triglyceride unit based on analysis.

Small molecule binding was achieved as follows: 10ml of 3mg/ml methotrexate buffered with 0.1M NaOH to pH 9.6 was mixed with 2.5mls of Vernonia oil at 25°C for 24hrs with constant stirring. The mixture was then rotary evaporated to 3.5mls and chromatographed on a column of Sephadox G-25 equilibrated in 0.1M NaHCO3 pH 8.5. The second running and peak yellow representing Vernonia oil coupled to methotrexate was then collected and rechromatographed to ensure purity. Mass spectral analysis indicated one methotrexate per triglyceride unit as an average linkage.

Claims

1. Use of an epoxidised compound obtainable by extraction from Vernonia galamensis seed in the preparation of a composition for the treatment of a lesion on the skin of a mammal.

2. Use as claimed in claim 1, wherein the treatment is by topical administration of the composition onto the lesion

3. Use as claimed in claim 1 or claim 2, wherein the composition is a medicament for the treatment of a lesion caused by a skin disease.

4. Use as claimed in claim 3, wherein the skin disease is psoriasis, dermatitis, ulceration or eczema.

5. Use as claimed in claim 1 or claim 2, wherein the composition is a medicament and the lesion is a wound.

6. Use as claimed in claim 5, wherein the wound is a graze, cut, scratch or burn.

7. Use as claimed in any one of the preceding claims, wherein the epoxidised compound is an oil, ester or wax obtained from Vernonia galamensis seed, or a component of said oil, ester or wax.

8. Use as claimed in claim 7, wherein the epoxidised compound is vernonia oil obtained from Vernonia galamensis seed or a component of said oil.

9. Use as claimed in claim 8, wherein the vernonia oil is purified vernonia oil.

10. Use of an epoxidised compound obtainable by extraction from Vernonia galamensis seed in the preparation of an emollient composition for treatment by topical administration to a lesion on the skin of a mammal, as claimed in any one of claims 1 to 9, or for the prophylactic treatment by topical administration to the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin.

11. A dosage form comprising a botanical raw material, botanical drug substance and/or botanical ingredient from the seed of Vernonia galamensis.

12. A dosage form as claimed in claim 11, wherein the dosage form is suitable for topical administration for the treatment of a lesion on the skin of a mammal.

13. A dosage form as claimed in claim 12, wherein the dosage form is for the treatment of a lesion caused by a skin disease.

14. A dosage form as claimed in claim 13, wherein the skin disease is psoriasis, dermatitis, ulceration or eczema.

15. A dosage form as claimed in claim 12, wherein the dosage form is for the treatment of a lesion that is a wound.

16. A dosage form as claimed in claim 15, wherein the wound is a graze, cut, scratch or burn.

17. A dosage form as claimed in any one of claims 11 to 16, wherein the botanical raw material, botanical drug substance or botanical ingredient

comprises an epoxidised oil, ester or wax obtained from Vernonia galamensis seed, or an epoxidised component of said oil, ester or wax.

18. A dosage form as claimed in claim 17, wherein the epoxidised compound is vernonia oil obtained from Vernonia galamensis seed or an epoxidised component of said oil.

19. A dosage form as claimed in claim 18, wherein the vernonia oil is purified vernonia oil.

20. A dosage form as claimed in claim 11, wherein the dosage form is suitable for topical administration for the prophylactic treatment of the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin.

21. A method of treating a lesion on the skin of a mammal by administering to the mammal an epoxidised compound obtainable by extraction from Vernonia galamensis seed.

22. The method as claimed in claim 21, wherein the treatment is by topical administration of the compound onto the lesion.

23. The method as claimed in claim 21, wherein the lesion is caused by a skin disease.

24. The method as claimed in claim 23, wherein the skin disease is selected from psoriasis, dermatitis, ulceration and eczema.

25. The method as claimed in claim 21, wherein the lesion is a wound.

26. The method as claimed in claim 25, wherein the wound is selected from a graze, a cut, a scratch and a burn.

27. The method as claimed in claim 21, wherein the epoxidised compound is selected from an oil, ester and wax, components of said oil, ester and wax, and mixtures thereof, all obtained from Vernonia galamensis seed.

28. The method as claimed in claim 27, wherein the epoxidised compound is vernonia oil or a component thereof obtained from Vernonia galamensis seed.

29. The method as claimed in claim 28, wherein the vernonia oil is purified vernonia oil.

30. A method of treating the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin of a mammal by the prophylactic administration of an emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia galamensis seed.

31. An emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia galamensis seed.

32. An emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia galamensis seed for treatment by topical administration to a lesion on the skin of a mammal or for the prophylactic treatment by topical administration to the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin.

33. A protein having at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester bound to it.

34. The protein as claimed in claim 33, wherein the at least one epoxidised oil, wax or fatty acid ester is obtainable by extraction from Vernonia galamensis seed.

35. A method of changing the size, immunogenicity or biological half life of a protein comprising bonding at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester to the protein.

36. A method as claimed in claim 35, wherein the protein is bonded to the epoxidised oil, wax or fatty acid ester by a process carried out a pH>7.

37. A method as claimed in claim 35 or claim 36, wherein the at least one epoxidised oil, wax or fatty acid ester is obtainable by extraction from Vernonia galamensis seed.

38. A compound comprising a bio-available molecule bonded to at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester.

39. A compound as claimed in claim 38, wherein the at least one epoxidised oil, wax or fatty acid ester is obtainable by extraction from Vernonia galamensis seed.

40. A method of preparing a bio-available compound in a slow-release format, comprising bonding the bio-available compound to at least one epoxidised oil, epoxidised wax or epoxidised fatty acid ester.

41. A method as claimed in claim 40, wherein the bio-available compound is bonded to at least one epoxidised oil, wax or fatty acid ester obtainable by extraction from Vernonia galamensis seed.

42. Use of an epoxidised oil, wax or fatty acid ester obtainable by extraction from Vernonia galamensis seed as a slow-release means for bio-available molecules.

43. The use of an epoxidised compound obtainable by extraction from Vernonia plants in the preparation of a composition for the treatment of a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn.

44. The use of claim 43, wherein the treatment is by topical administration of the composition onto the lesion.

45. An emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia plants.

46. Use of the composition of claim 45 for the prophylactic treatment by topical administration to the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin originally caused by a wound, such as a graze, cut, scratch or burn.

47. A method of treating a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn, by administering to the mammal an epoxidised compound obtainable by extraction from Vernonia plants.

48. A method of treating the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin of a mammal, wherein the lesion was originally caused by a wound, by the prophylactic administration of an emollient composition comprising an epoxidised compound obtainable by extraction from Vernonia plants.

49. A method of treating a lesion on the skin of a mammal, wherein the lesion is a wound, such as a graze, cut, scratch or burn, by administering to the mammal an oil obtainable by extraction from Vernonia plants, preferably an oil from Vernonia galamensis seeds.

50. A method of treating the skin of a mammal to prevent or reduce the recurrence of a lesion on the skin of a mammal originally caused by a wound by the prophylactic administration of an emollient composition comprising an oil obtainable by extraction from Vernonia plants, preferably oil from Vernonia galamensis seeds.

51. The use of an epoxidised compound in the preparation of a composition for the treatment by topical administration of a lesion on the skin of a mammal, wherein the lesion is an inflammation and/or an infection.

52. A method of treating a lesion of the skin of a mammal wherein the lesion is caused by an inflammation and/or infection of the skin by topical administration of a composition comprising an epoxidised compound.

53. The use, method, composition, dosage form or biological drug claimed in any one of the preceding claims, wherein the epoxidized compound is vernolic acid or vernonia oil.

54. The use, method, composition, dosage form or biological drug claimed in claim 53, wherein the epoxidized compound is oil extracted from Vernonia galamensis seed.

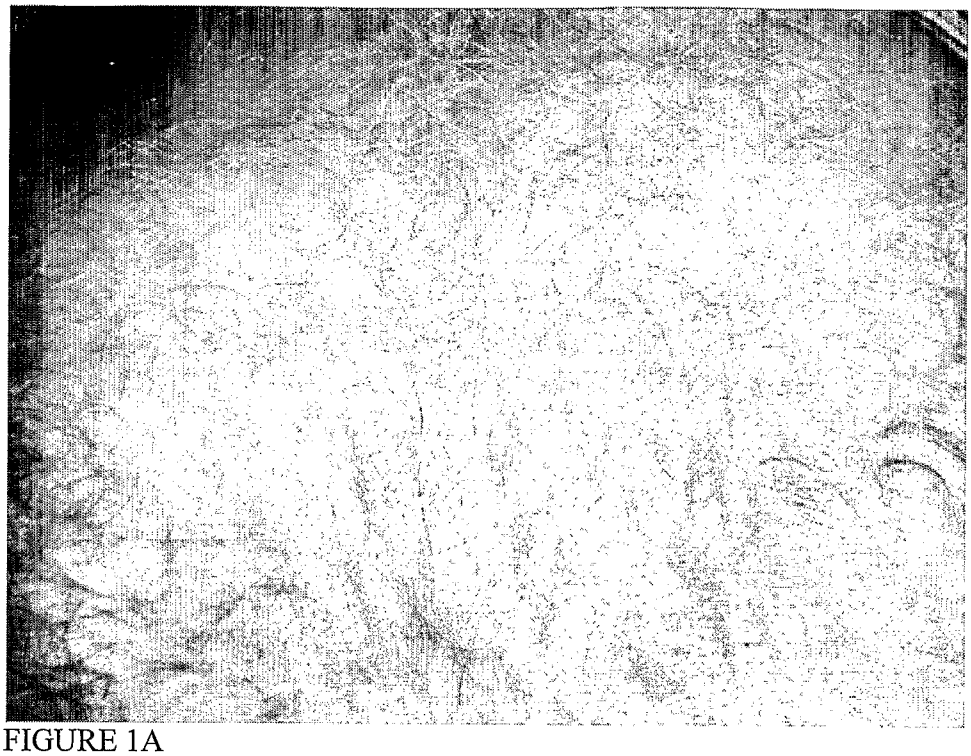

FIGURE 1A

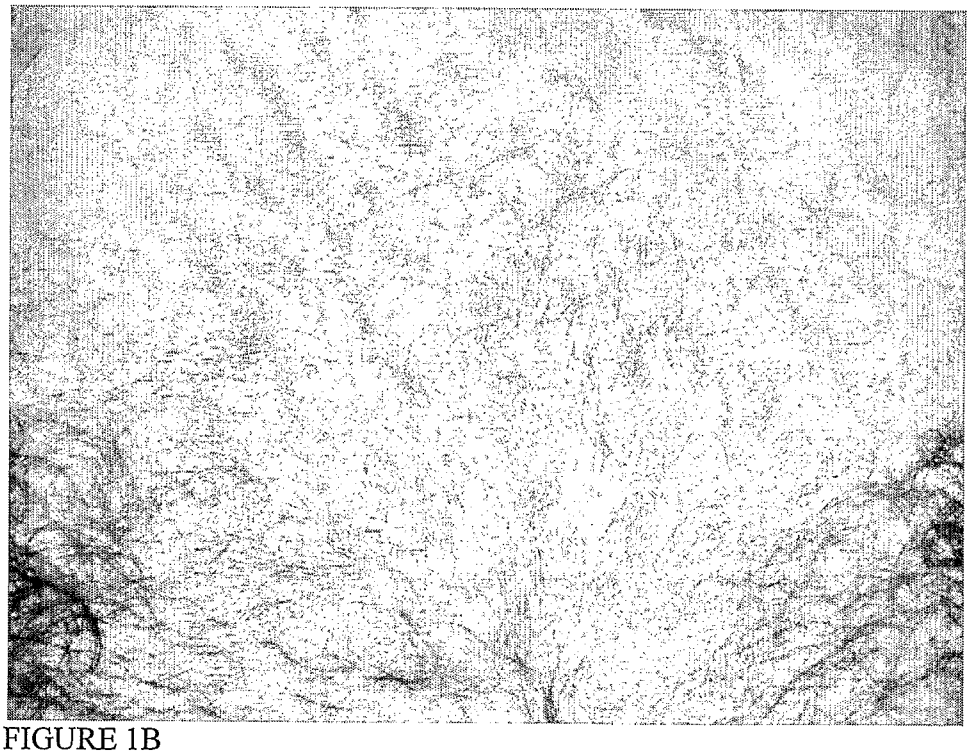

FIGURE 1B



FIGURE 2A

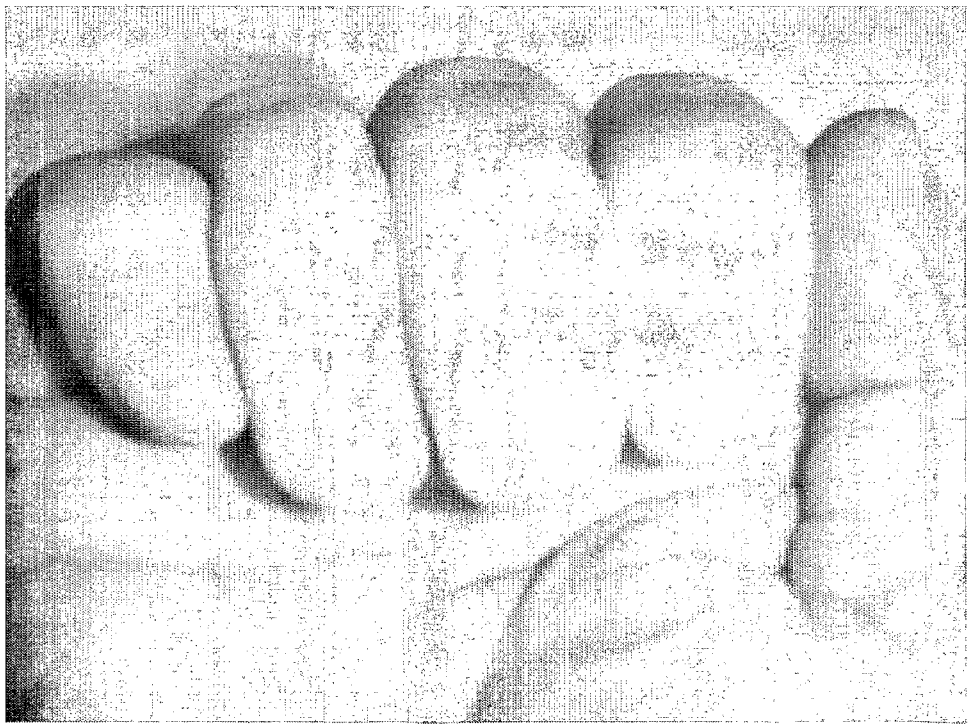

FIGURE 2B



FIGURE 3A



FIGURE 3B