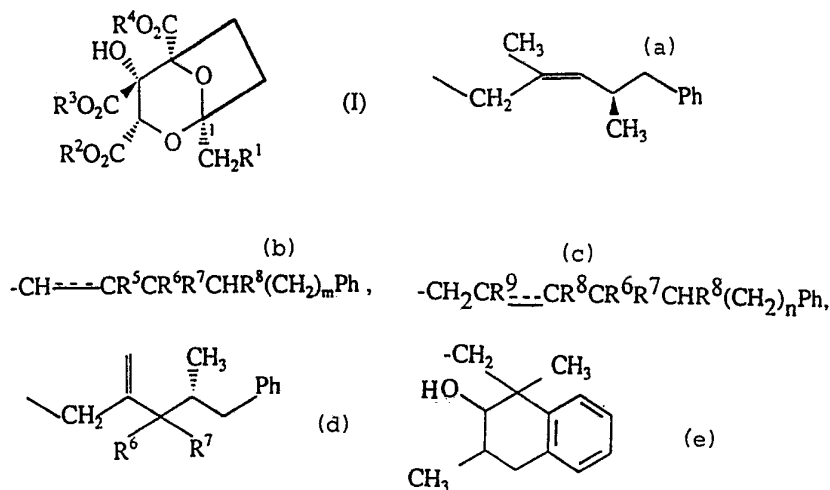




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(21) International Application Number: PCT/EP92/00015 (22) International Filing Date: 5 January 1992 (05.01.92) (30) Priority data: 9100431.7 9 January 1991 (09.01.91) GB (71) Applicant (for all designated States except US): GLAXO GROUP LIMITED [GB/GB]; Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : SIDEBOTTOM, Philip, James [GB/GB]; LESTER, Michael, George [GB/GB]; PROCOPIOU, Panayiotis, Alexandrou [GB/GB]; WATSON, Nigel, Stephen [GB/GB]; KIRK, Barrie, Edward [GB/GB]; ROSS, Barry, Clive [GB/GB]; Glaxo Group Research Limited, Berkeley Avenue, Greenford, Middlesex UB6 0HE (GB).		(74) Agents: BREWER, Christopher, Laurence et al.; Glaxo Holdings plc, Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB). (81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, RU, SD, SE, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent), US. Published <i>With international search report.</i>

(54) Title: BRIDGED CYCLIC KETAL DERIVATIVES**(57) Abstract**

Compounds are described of formula (I), wherein R^1 represents a group selected from (a), (b), (c), (d), $-\text{CH}_2\text{C}(\text{CH}_3)=\text{CHCH}(\text{CH}_2\text{OH})\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{C}(\text{CH}_2\text{OH})=\text{CHCH}(\text{CH}_3)\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{C}(=\text{CH}_2)\text{CH}(\text{OH})\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{C}(=\text{CH}_2)\text{CH}(\text{NHCOCH}_3)\text{CH}(\text{CH}_3)\text{CH}_2\text{Ph}$, and (e) (where the dotted line represents the absence or presence of a single bond, R^5 represents a hydrogen atom or a hydroxyl, acyloxy, C_{1-6} alkoxy or C_{1-4} alkyl group, R^6 represents a hydrogen atom and R^7 represents a hydrogen atom or a hydroxyl, C_{1-6} alkoxy or acyloxy group or CR^6R^7 forms a group $\text{C}=\text{O}$, R^8 represents a hydrogen atom or a C_{1-4} alkyl group, R^9 represents a hydrogen atom or a methyl group, m represents 1 or 2 and n represents zero or 1); R^2 , R^3 and R^4 may each independently represent a hydrogen atom or a methyl group; and salts thereof. These compounds inhibit the enzyme squalene synthase and/or are intermediates for the preparation of compounds which inhibit the enzyme squalene synthase. Compounds of the invention may be formulated for use in a variety of conditions where a lowering of the level of blood plasma cholesterol in animals would be beneficial and for use in combating fungal infections in animals.

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- 1 -

Bridged Cyclic Ketal Derivatives

This invention relates to novel compounds having hypocholesterolemic, hypolipidemic and/or antifungal activity, to processes for their preparation, to pharmaceutical compositions containing them and to their use in medicine, particularly in the treatment and/or prevention of atherosclerosis and associated cardiovascular diseases. The invention also relates to novel compounds which are useful as intermediates for the preparation of compounds having hypocholesterolemic, hypolipidemic and/or antifungal activity.

High levels of blood cholesterol and blood lipids are conditions which are implicated in the onset of vessel wall disease. Methods for effective reduction of plasma cholesterol levels are therefore of high interest. Cholesterol concentrations can be reduced, for example, by lowering the dietary intake of the sterol, by enhancing its metabolism and elimination or by decreasing its rate of biosynthesis. The most effective approaches to lowering physiological cholesterol levels are likely to include inhibition of cholesterol biosynthesis as a component since cholesterol synthesis is subject to feedback regulation, so that decreases in cholesterol levels tend to be compensated for by increased biosynthesis.

One rate-controlling step in the biosynthesis of cholesterol is the formation of mevalonic acid from 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) and clinical successes have been achieved with the mevinic acid family of HMG CoA reductase inhibitors in the treatment of hypercholesterolemia. Mevalonic acid, however, is a common precursor of all isoprenyl derivatives, including in animals coenzyme Q, heme A and the dolichols.

The first biosynthetic step which leads exclusively to sterols, the condensation of two farnesyl pyrophosphates to give squalene, is a second site of regulation. The synthesis of squalene from farnesyl pyrophosphate involves an isolable intermediate, presqualene pyrophosphate, and the entire synthetic sequence is catalysed by squalene synthase (farnesyl diphosphate: farnesyl diphosphate farnesyltransferase, EC 2.5.1.21), a membrane-bound enzyme. Agents which act to inhibit the enzyme

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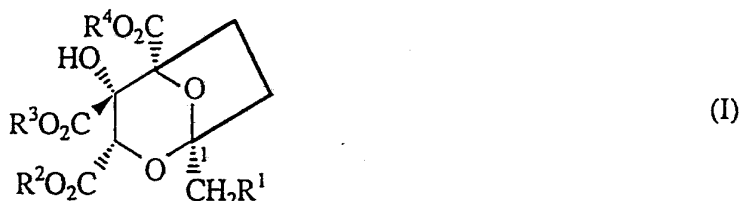
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squalene synthase are therefore potential drugs for the regulation of cholesterologenesis. The use of such agents is attractive as non-steroidal pathways should be minimally affected.

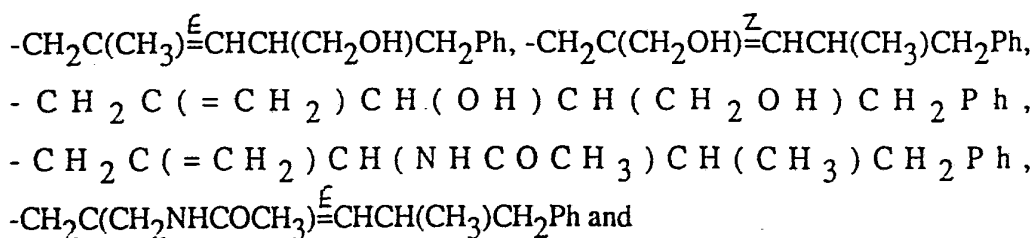
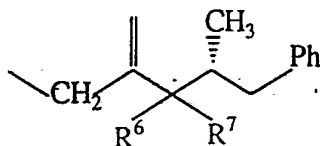
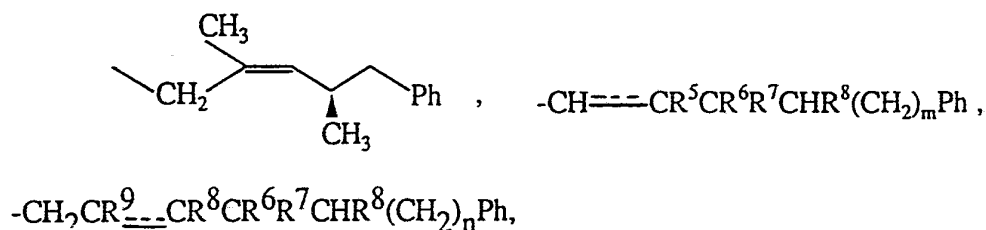
The biosynthesis of ergosterol, the major sterol component of fungal cell membranes, is analogous to that of cholesterol in mammals, including humans, and is thus mediated by the enzyme squalene synthase. Agents which act to inhibit the enzyme squalene synthase in fungal cells are therefore potential drugs for antifungal chemotherapy.

We have now found a group of novel compounds which act as inhibitors of the enzyme squalene synthase and/or are intermediates for the preparation of compounds which act as inhibitors of the enzyme squalene synthase.

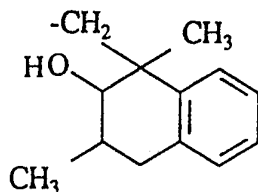
Thus, in a first aspect of the present invention, we provide compounds of the general formula (I)



wherein R^1 represents a group selected from



- 3 -



(where the dotted line represents the absence or presence of a single bond, R^5 represents a hydrogen atom or a hydroxyl, acyloxy, C_{1-6} alkoxy or C_{1-4} alkyl group, R^6 represents a hydrogen atom and R^7 represents a hydrogen atom or a hydroxyl, C_{1-6} alkoxy or acyloxy group or CR^6R^7 forms a group $C=O$, R^8 represents a hydrogen atom or a C_{1-4} alkyl group, R^9 represents a hydrogen atom or a methyl group, m represents 1 or 2 and n represents zero or 1);

R^2 , R^3 and R^4 may each independently represent a hydrogen atom or a methyl group; and salts thereof.

It will be appreciated that R^1 in formula (I) may contain a double bond and/or one or more chiral centres. It is to be understood that where the configuration in R^1 is not defined the present invention is intended to cover all geometrical and optical isomers, including diastereoisomers, of such compounds of formula (I).

It will also be appreciated that compounds of the invention containing a keto group may exist in the corresponding enolic form.

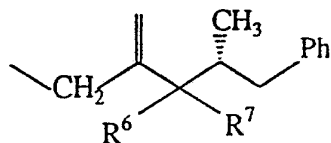
It is to be understood that when the dotted line in the relevant R^1 groupings represents the absence of a single bond

$-CH=CR^5CR^6R^7CHR^8(CH_2)_mPh$ and $-CH_2CR^9=CR^8CR^6R^7CHR^8(CH_2)_nPh$ will represent $-CH_2CHR^5CR^6R^7CHR^8(CH_2)_mPh$ and $-CH_2CHR^9CHR^8CR^6R^7CHR^8(CH_2)_nPh$ respectively, and when the dotted line represents the presence of a single bond $-CH=CR^5CR^6R^7CHR^8(CH_2)_mPh$ and $-CH_2CR^9=CR^8CR^6R^7CHR^8(CH_2)_nPh$ will represent $-CH=CR^5CR^6R^7CHR^8(CH_2)_mPh$ and $-CH_2CR^9=CR^8CR^6R^7CHR^8(CH_2)_nPh$ respectively.

Compounds of formula (I) in which R^2 , R^3 and R^4 represent hydrogen atoms and physiologically acceptable cations are generally preferred.

R^1 preferably represents a group selected from $-CH=CR^5CR^6R^7CHR^8CH_2Ph$ and

- 4 -



The group $-\text{CH}=\text{CR}^5-$ within R^1 may preferably represent a group selected from $-\text{CH}=\text{C}(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{OH})-$, $-\text{CH}_2\text{C}(=\text{O})-$, $-\text{CH}_2\text{CH}_2-$ and $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)-$.

The group $-\text{CR}^6\text{R}^7-$ within R^1 may preferably represent a group selected from $-\text{CH}(\text{OH})-$, $-\text{C}(=\text{O})-$, $-\text{CH}_2-$ and $-\text{CH}(\text{OCOCH}_3)-$.

It is to be understood that this invention covers any combination of the abovementioned particular and preferred groupings.

Compounds of the present invention may form salts with inorganic and organic bases. Physiologically acceptable salts include inorganic base salts such as alkali metal salts (e.g. sodium and potassium salts including the trisodium, dipotassium and tripotassium salts), alkaline earth metal salts (e.g. calcium salts), ammonium salts and amino acid salts (e.g. lysine and arginine salts including the tri-L-lysine salts). Suitable organic base salts include amine salts such as trialkylamine (e.g. triethylamine), dialkylamine (e.g. dicyclohexylamine), optionally substituted benzylamine (e.g. p-bromobenzylamine) and tris(hydroxymethyl)methylamine salts.

Compounds of the invention have been found to inhibit the enzyme squalene synthase and cholesterol biosynthesis and are therefore of use in medicine, particularly in a variety of conditions where a lowering of the level of blood plasma cholesterol in animals (especially humans) would be beneficial. Examples of such conditions include diseases associated with hypercholesterolemia and hyperlipoproteinemia, especially atherosclerosis and cardiovascular diseases (such as cardiac ischaemic diseases, cerebral ischaemic diseases and peripheral arterial disease).

Compounds of the invention which inhibit squalene synthase may also be of use in combating fungal infections in animals, including humans. For example, they may be useful in the treatment of systemic infections caused by, for example Candida (e.g. Candida albicans, Candida glabrata, Candida parapsilosis and Candida

- 5 -

pseudotrop), Cryptococcus neoformans, Aspergillus Sp (e.g. Aspergillus flavus and Aspergillus fumigatus), Coccidioides (e.g. Coccidioides immitis), Paracoccidioides (e.g. Paracoccidioides brasiliensis), Histoplasma (e.g. Histoplasma capsulatum) or Blastomyces (e.g. Blastomyces dermatitidis). They may also be useful in treating topical infections caused by species of Trichophyton, Microsporum or Epidermophyton (e.g. Trichophyton mentographytes, Microsporum canis or Epidermophyton floccosum). They may also be of use in treating fungal diseases caused by Torulopsis glabrata and Pityrosporum ovale.

The in vitro evaluation of the anti-fungal activity of compounds of the invention can be performed by determining the minimum inhibitory concentration (MIC) which is the concentration of the test compound in a suitable medium at which growth of a particular microorganism fails to occur.

In view of their potential in antifungal therapy, compounds of the invention which inhibit squalene synthase may recommend themselves for the treatment of a variety of fungal infections in human beings and animals. Such infections include mycotic infections such as candidiasis and chronic mucocandidiasis (e.g. thrush and vaginal candidiasis) and skin infections caused by fungi, cutaneous and mucocutaneous candidiasis, dermatophytoses including ringworm and tinea infections, athletes foot, paronychia, pityriasis versicolor, erythrasma, intertrigo, fungal nappy rash, candida vulvitis, candida balanitis and otitis externa. They may also be useful as prophylactic agents to prevent systemic and topical fungal infections. Use as prophylactic agents may, for example, be appropriate as part of a selective gut decontamination regimen in the prevention of infection in immunocompromised patients. Prevention of fungal overgrowth during antibiotic treatment may also be desirable in some disease syndromes or iatrogenic states.

The ability of compounds of the invention to inhibit the enzyme squalene synthase in mammals and fungi may be demonstrated in vitro using [2-¹⁴C]farnesylpyrophosphate as a substrate under assay conditions similar to those described by S. A. Biller et al. in J. Medicinal Chemistry 31(10), 1869-1871 (1988); [¹⁴C]squalene is separated from unreacted substrate on thin layer chromatography plates and determined by liquid scintillation counting. The ability of compounds of

- 6 -

the invention to inhibit cholesterol biosynthesis may be demonstrated by measuring inhibition from [^{14}C]-acetate in liver slices from male Wistar rats using a method similar to that described by Y. Tsujita *et al.* in *Biochem. Biophys. Acta*, Volume 877, 50-60 (1986) and modified to include measurement of cholesterol by high performance liquid chromatography (h.p.l.c.).

While it is possible that, for use in therapy, compounds of the invention which inhibit squalene synthase may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation. The invention thus further provides a pharmaceutical formulation comprising compounds of the invention which inhibits squalene synthase together with one or more pharmaceutically acceptable carriers thereof and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The compositions of the invention include those in a form especially formulated for oral, buccal, parenteral, implant, rectal, topical, ophthalmic or genito-urinary administration or in a form suitable for administration by inhalation or insufflation.

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch or polyvinylpyrrolidone; fillers, for example, lactose, sugar, microcrystalline cellulose, maize-starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch or sodium starch glycollate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose,

- 7 -

aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example, almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; and preservatives, for example, methyl or propyl *p*-hydroxybenzoates or sorbic acid. The compositions may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

The composition according to the invention may be formulated for parenteral administration by injection or continuous infusion. Formulations for injection may be presented in unit dose form in ampoules, or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For administration by inhalation the compositions according to the invention are conveniently delivered in the form of an aerosol spray presentation from pressurised packs with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas, or from a nebuliser. In the case of a pressurised aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation the compositions according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges of e.g. gelatin, or blister packs from which the powder may be administered with the aid of an inhaler or insufflator.

- 8 -

The compositions may take the form of a suppository, e.g. containing a conventional suppository base, or a pessary, e.g. containing a conventional pessary base.

The compositions may also be formulated for topical administration in the form of ointments, creams, gels, lotions, shampoos, powders (including spray powders), pessaries, tampons, sprays, dips, aerosols, drops (e.g. eye, ear or nose drops) or pour-ons. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Ointments for administration to the eye may be manufactured in a sterile manner using sterilised components. Pour-ons may, for example, be formulated for veterinary use in oils containing organic solvents, optionally with formulatory agents, e.g. stabilising and solubilising agents. Pessaries and tampons for vaginal insertion may be formulated using conventional techniques and, where appropriate, may contain an effervescent vehicle. Such compositions may also contain other active ingredients such as corticosteroids, antibiotics or antiparasitics as appropriate.

Liquid preparations for intranasal delivery may take the form of solutions or suspensions and may contain conventional excipients such as tonicity adjusting agents, for example, sodium chloride, dextrose or mannitol; preservatives, for example benzalkonium chloride, thiomersal, phenylethyl alcohol; and other formulating agents such as suspending, buffering, stabilising and/or dispersing agents.

Transdermal administration may be affected by the design of a suitable system which promotes adsorption of the active compound through the skin and would typically consist of a base formulation enclosed within an adhesive stick-on patch comprising backing films, membranes and release liners.

The composition according to the invention may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, a compound of the invention may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable

- 9 -

oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

When the compositions comprise dosage units, each unit will preferably contain 0.001mg to 1000mg, advantageously 0.01mg to 400mg, of active ingredient where a compound of the invention is to be administered orally. The daily dosage as employed for adult human treatment will preferably range from 0.001mg to 5000mg of active ingredient, most preferably from 0.01mg to 2000mg which may be administered in 1 to 4 daily doses, for example, depending on the route of administration and on the condition of the patient and the disease to be treated.

The compound may be administered by intravenous infusion using, for example, up to 50mg/kg/day of the active ingredient. The duration of treatment will be dictated by the rate of response rather than by arbitrary numbers of days.

Compounds of the invention which inhibit squalene synthase may also be used in combination with other therapeutic agents, and the invention thus provides, in a further aspect, a combination comprising a compound of the invention which inhibits squalene synthase together with another therapeutically active agent, such as an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase or another agent which reduces serum cholesterol and/or inhibits cholesterol biosynthesis, for example a bile acid sequestrant or an antihyperlipoproteinemic or antihyperlipemic agent such as probucol, gemfibrozil, clofibrate, dextrothyroxine or its sodium salt, colestipol or its hydrochloride salt, cholestyramine, nicotinic acid, neomycin, p-aminosalicylic acid, aspirin, DEAE-Sephadex, a poly(diallylmethylamine) derivative, an ionene or poly(diallyldimethylammonium) chloride.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier thereof comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

- 10 -

When a compound of the invention is used in combination with a second therapeutic agent against the same condition the dose of each compound may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

According to another aspect of the present invention, we provide a compound of formula (I) or a physiologically acceptable salt thereof or a pharmaceutical composition comprising a compound of formula (I) or a physiologically acceptable salt thereof as defined above for use in therapy, particularly for the treatment of conditions where a lowering of the level of blood plasma cholesterol in animals (especially humans) would be beneficial, or for the treatment of fungal infections in animals (especially humans).

In a particular aspect of the present invention, we provide a compound of formula (I) or a physiologically acceptable salt thereof or a pharmaceutical composition comprising a compound of formula (I) or a physiologically acceptable salt thereof as defined above for use in the treatment of diseases associated with hypercholesterolemia and/or hyperlipoproteinemia, especially atherosclerosis and cardiovascular diseases (such as cardiac ischaemic diseases, cerebral ischaemic diseases and peripheral arterial disease).

According to a further aspect of the present invention, we provide the use of a compound of formula (I) or a physiologically acceptable salt thereof in the manufacture of a medicament for the treatment of diseases associated with hypercholesterolemia and/or hyperlipoproteinemia, especially atherosclerosis and cardiovascular diseases (such as cardiac ischaemic diseases, cerebral ischaemic diseases and peripheral arterial disease).

According to another aspect of the present invention, we provide the use of a compound of formula (I) or a physiologically acceptable salt thereof in the manufacture of a medicament for the treatment of fungal infections in a human or non-human animal patient.

According to a further aspect of the present invention, we provide a method of treatment of the human or non-human animal body to combat diseases associated with hypercholesterolemia and/or hyperlipoproteinemia, especially atherosclerosis

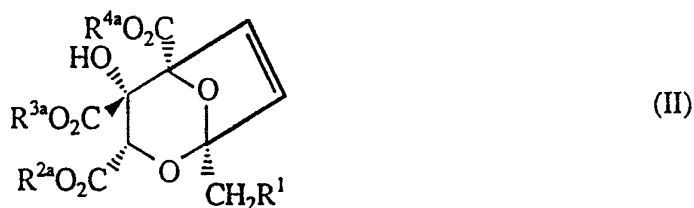
- 11 -

and cardiovascular diseases (such as cardiac ischaemic diseases, cerebral ischaemic diseases and peripheral arterial disease) or to combat fungal diseases, which method comprises administering to said body an effective amount of a compound of formula (I) or a physiologically acceptable salt thereof.

It will be appreciated by those skilled in the art that references herein to treatment extend to prophylaxis as well as the treatment of established conditions or infections.

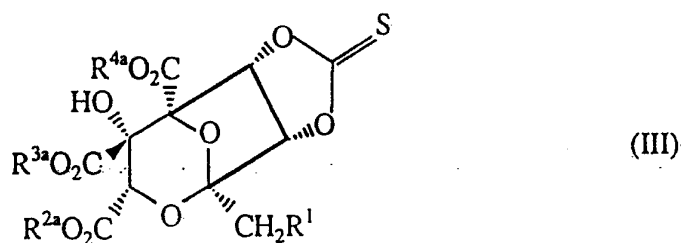
The compounds of the invention may be prepared by the processes described below.

Thus, a general process (A) for the preparation of compounds of formula (I) comprises hydrogenation of a compound of formula (II)



(wherein R^1 is as defined previously and R^{2a} , R^{3a} and R^{4a} are protecting groups) in the presence of an appropriate metal catalyst such as a suitable palladium catalyst [e.g. palladium-on-carbon in a solvent such as an alcohol (e.g. ethanol) or an ester (e.g. ethyl acetate)], followed by removal of the protecting groups present.

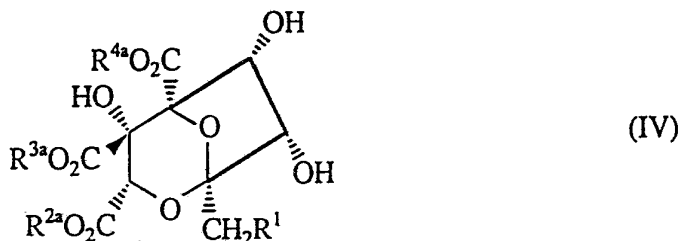
Compounds of formula (II) may be prepared by treating a compound of formula (III)



- 12 -

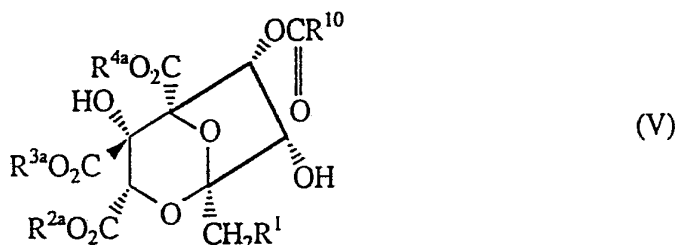
(wherein R^1 and R^{2a} - R^{4a} are as defined previously) with a phosphorus reagent such as a suitable 1,3,2-diazaphospholine (e.g. 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholine) at an elevated temperature (e.g. 30^0 - 70^0 C).

Compounds of formula (III) may be prepared by reacting a compound of formula (IV)



(wherein R^1 and R^{2a} - R^{4a} are as defined previously) with a suitable thiocarbonylating agent, such as thiocarbonyldiimidazole in a solvent such as an ether (e.g. tetrahydrofuran) at an elevated temperature (e.g. 30^0 - 70^0 C).

Compounds of formula (IV) may be prepared from compounds of formula (V)

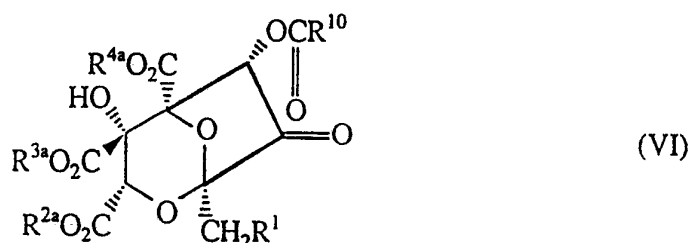


(wherein R^1 and R^{2a} - R^{4a} are as defined previously and R^{10} represents a group selected from -CH $\equiv$ CHCH(CH₃)(CH₂)₃CH₃, -CH $\equiv$ CHC(CH₃) $\equiv$ CHCH(CH₃)CH₂CH₃, -CH $\equiv$ CHCH(CH₃)CH₂CH(CH₃)CH₂CH₃, -CH₂CH(OH)CH(CH₃)CH₂CH(CH₃)CH₂CH₃, -CH $\equiv$ CHC(OH)(CH₃)CH₂CH(CH₃)CH₂CH₃, -CH₂CH(OH)CH₂CH₂CH(CH₃)CH₂CH₃ and -CH₂CH₂CH(CH₃)CH₂CH(CH₃)CH₂CH₃) by suitable deacylating means.

- 13 -

Deacylation to provide a compound of formula (IV) may conveniently be carried out by base-catalysed hydrolysis of a compound of formula (V) using a base such as aqueous sodium hydroxide in a solvent such as an alcohol (e.g. methanol). Alternatively, deacylation of α , β -unsaturated esters may be carried out by treating a compound of formula (V) with a hydroxylamine (e.g. N-methylhydroxylamine hydrochloride) optionally in the presence of a suitable base (e.g. a trialkylamine such as triethylamine) in a solvent such as dimethylformamide.

Compounds of formula (V) may be prepared from compounds of formula (VI)

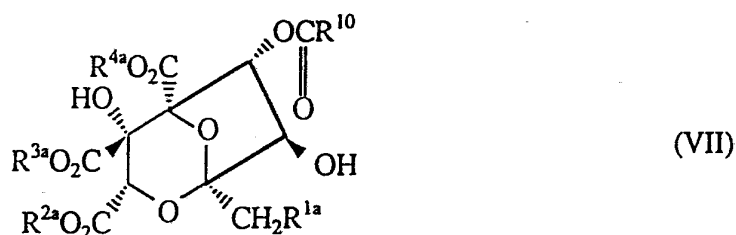


(wherein R^1 , R^{2a} - R^{4a} and R^{10} are as defined previously) by reduction with a suitable reducing agent.

Thus, for example, the reduction may conveniently be carried out using a borohydride such as lithium triethylborohydride in a solvent such as an ether (e.g. tetrahydrofuran) or sodium borohydride, optionally in the presence of a suitable metal halide (e.g. cerium trichloride), in a solvent such as an alcohol (e.g. methanol) or a mixture of an alcohol with another solvent such as an ether (e.g. tetrahydrofuran) at a temperature below 20°C (e.g. -70° to 10°C). Alternatively, the reduction may be effected using an aluminium hydride reducing agent such as diisobutylaluminium hydride in a solvent such as toluene at a low temperature (e.g. -70° to 0°C) or lithium tris[(3-ethyl-3-pentyl)oxy]aluminumhydride in a solvent such as an ether (e.g. tetrahydrofuran) at a low temperature (e.g. -70° to 0°C).

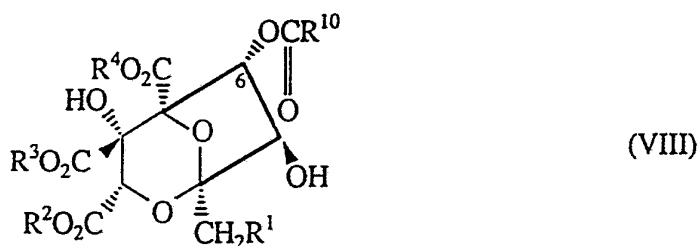
Compounds of formula (VI) may conveniently be prepared from compounds of formula (VII)

- 14 -



(wherein R^{2a} - R^{4a} and R^{10} are as defined previously and R^{1a} is as defined for R^1 above or is a protected derivative thereof) by treating (VII) with a suitable oxidising agent such as a chlorochromate (e.g. pyridinium chlorochromate) in the presence of powdered molecular sieves in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane), conveniently at room temperature.

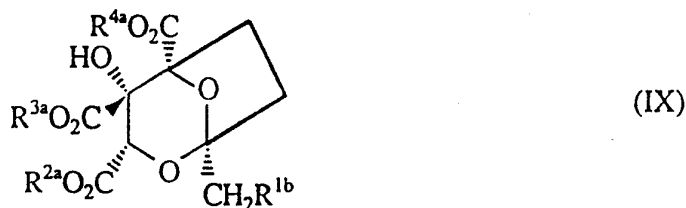
Compounds of formula (VII) may conveniently be prepared from compounds of formula (VIII)



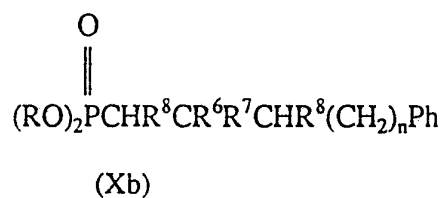
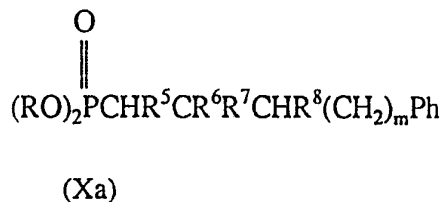
(wherein R^1 - R^4 are as defined in formula (I) above and R^{10} is as defined previously) by standard carboxylic acid protection methods with hydroxyl group protection within R^1 if necessary.

Another process (B) for the preparation of compounds of formula (I) in which R^1 represents a group $-\text{CH}=\text{CR}^5\text{CR}^6\text{R}^7\text{CHR}^8(\text{CH}_2)_m\text{Ph}$ or a group $-\text{CH}_2\text{CR}^9=\text{CR}^8\text{CR}^6\text{R}^7\text{CHR}^8(\text{CH}_2)_n\text{Ph}$ where R^5 is hydrogen or C_{1-4} alkyl comprises reacting a compound of formula (IX)

- 15 -



(wherein R^{1b} represents CHO or CH_2COR^9 as appropriate and R^{2a} , R^{3a} and R^{4a} are as defined for R^2 , R^3 and R^4 above or are protecting groups) with a compound of formula (Xa) or (Xb)



as appropriate (wherein R^8 is as defined previously, R^5 is hydrogen or C_{1-4} alkyl, CR^6R^7 forms a group $C=O$ and R represents a C_{1-6} alkyl group, e.g. methyl or an aryl group, e.g. phenyl) under Wadsworth-Emmons conditions, followed, where appropriate, by converting CR^6R^7 as a group $C=O$ to a group CHR^7 where R^7 is hydroxy, C_{1-6} alkoxy or acyloxy according to the general procedures described hereinafter, and thereafter removing any protecting groups present.

The reaction between compounds (IX) and (Xa) or (Xb) may conveniently be carried out in an ether solvent (e.g. tetrahydrofuran) in the presence of a strong base such as an alkali metal hydride (e.g. sodium hydride) at a temperature in the range of 0^0 to 20^0C .

Another general process (C) comprises converting a compound of formula (I) or a protected derivative thereof to a different compound of formula (I) or a protected derivative thereof, followed, if necessary, by the removal of any protecting groups present. Specific examples of interconversion reactions are described hereinafter.

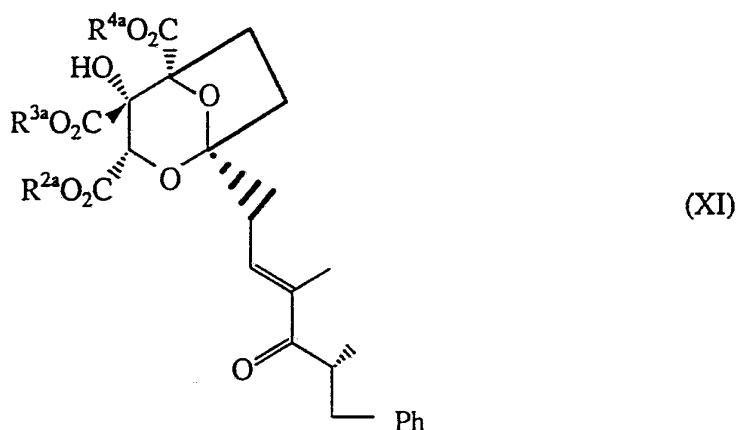
Compounds of formula (I) in which R^1 represents a group $-CH_2CHR^5CR^6R^7CHR^8(\text{CH}_2)_m\text{Ph}$ or $CH_2CHR^9CHR^8CR^6R^7CHR^8(\text{CH}_2)_n\text{Ph}$ may be prepared from the corresponding compounds of formula (I) in which R^1

- 16 -

represents a group $-\text{CH}=\text{CR}^5\text{CR}^6\text{R}^7\text{CHR}^8(\text{CH}_2)_m\text{Ph}$ or $\text{CH}_2\text{CR}^9=\text{CR}^8\text{CR}^6\text{R}^7\text{CHR}^8(\text{CH}_2)_n\text{Ph}$ or protected derivatives thereof under appropriate reducing conditions, for example by catalytic hydrogenation [e.g. hydrogenation in the presence of palladium-on-carbon in a suitable solvent such as an ester (e.g. ethyl acetate) or an alcohol (e.g. ethanol)], followed, where appropriate, by removing any protecting groups present.

Compounds of formulae (Xa) and (Xb) are either known compounds or may be prepared by methods analogous to those used to prepare the known compounds of formulae (Xa) and (Xb).

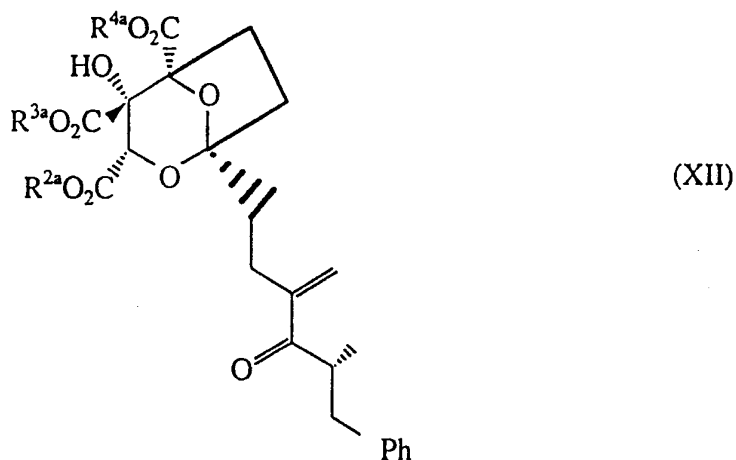
Compounds of formula (IX) in which R^{1b} represents CHO may be prepared by ozonolysis of a compound of formula (XI)



(wherein R^{2a} , R^{3a} and R^{4a} are as defined previously). Thus, for example, a compound of formula (XI) may conveniently be treated with ozone in a halogenated hydrocarbon solvent (e.g. dichloromethane) at a low temperature (e.g. -70°C to 0°C), followed by treatment with either a triarylphosphine such as triphenylphosphine or a dialkyl sulphide such as dimethyl sulphide to provide the desired compound of formula (IX).

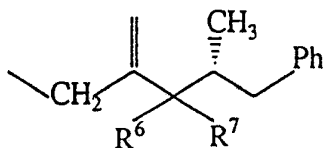
Compounds of formula (XI) may be prepared by isomerization of a compound of formula (XII)

- 17 -



(wherein R^{2a} , R^{3a} and R^{4a} are as previously defined). The isomerization may conveniently be effected by heating a compound of formula (XII) with a suitable transition metal catalyst such as rhodium trichloride in an aqueous alcoholic solvent (e.g. aqueous methanol).

Compounds of formula (I) in which R^1 represents a group

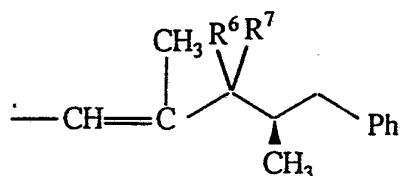


(wherein CR^6R^7 forms a group $C=O$) are either compounds of formula (XII) or may be prepared from compounds of formula (XII) by removing any protecting groups present. Compounds of formula (I) in which R^6 is hydrogen and R^7 is a hydroxyl group may be prepared by reducing the keto group in formula (XII) using, for example, sodium borohydride in an alcoholic solvent (e.g. methanol) at a temperature in the range 0^0 to 20^0C or using zinc dust in an aqueous ether (e.g. aqueous tetrahydrofuran), followed where necessary by removing any protecting groups present. Compounds of formula (I) in which R^6 is hydrogen and R^7 is an acyloxy group may be prepared from the corresponding compounds in which R^6 is hydrogen and R^7 is a hydroxyl group or protected derivatives thereof by conventional acylation means described hereinafter, followed where necessary by removal of any protecting groups present. Compounds of formula (I) in which R^6 is

- 18 -

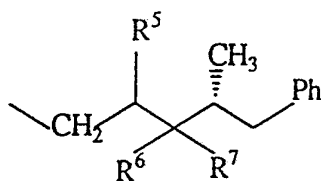
hydrogen and R^7 is a C_{1-6} alkoxy group may be prepared from the corresponding compounds in which R^6 is hydrogen and R^7 is a hydroxyl group or protected derivatives thereof by conventional etherification means described hereinafter, followed where necessary by removal of any protecting groups present.

Compounds of formula (I) in which R^1 represents a group



(wherein CR^6R^7 forms a group $C=O$) are either compounds of formula (XI) or may be prepared from compounds of formula (XI) by removing any protecting groups present. Compounds of formula (I) in which R^6 is hydrogen and R^7 is a hydroxyl group may be prepared by reducing the keto group in formula (XI) using, for example, sodium borohydride in an alcoholic solvent (e.g. methanol) at a temperature in the range 0^0 to 20^0C or using zinc dust in an aqueous ether (e.g. aqueous tetrahydrofuran), followed where necessary by removing any protecting groups present. Compounds of formula (I) in which R^6 is hydrogen and R^7 is an acyloxy group may be prepared from the corresponding compounds in which R^6 is hydrogen and R^7 is a hydroxyl group or a protected derivative thereof by conventional acylation means as described hereinafter, followed where necessary by removal of any protecting groups present. Compounds of formula (I) in which R^6 is hydrogen and R^7 is a C_{1-6} alkoxy group may be prepared from the corresponding compounds in which R^6 is hydrogen and R^7 is a hydroxyl group or protected derivatives thereof by conventional etherification means described hereinafter, followed where necessary by removal of any protecting groups present.

Compounds of formula (I) in which R^1 represents a group (wherein R^5 is

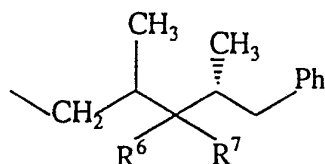


C_{2-4} alkyl, R^6 is a hydrogen atom and R^7 is a hydroxyl or acyloxy group or CR^6R^7 forms a group $C=O$) may be prepared from compounds of formula (XII). Thus,

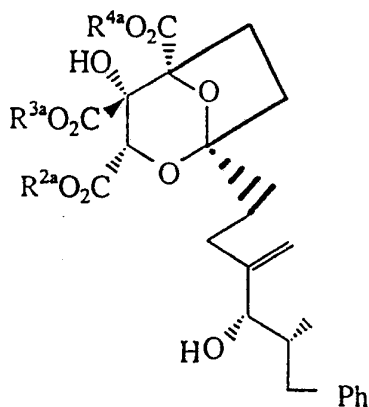
- 19 -

compounds in which CR^6R^7 represents $\text{C}=\text{O}$ may be prepared by treating the compound of formula (XII) with a suitable lithium cuprate $\text{LiCu}(\text{R}^{11})_2$ (where R^{11} is a C_{1-3} alkyl group, e.g. methyl) at reduced temperature (e.g. -20° to $+10^\circ\text{C}$) in an ether solvent (e.g. tetrahydrofuran), followed where necessary by removing any protecting groups present. The resulting keto compounds of formula (I) or protected derivatives thereof may then be converted to the corresponding compounds of formula (I) in which R^5 is C_{2-4} alkyl, R^6 is hydrogen and R^7 is a hydroxyl, C_{1-6} alkoxy or acyloxy group by reduction as described hereinabove, followed where appropriate by etherification or acylation as described hereinafter, followed, where necessary, by removal of any protecting groups present.

Compounds of formula (I) in which R^1 represents a group



(wherein R^6 represents a hydrogen atom and R^7 represents a hydroxyl, C_{1-6} alkoxy or acyloxy group or CR^6R^7 forms a group $\text{C}=\text{O}$) may be prepared from compounds of formula (XIII)



(XIII)

(wherein R^{2a} , R^{3a} and R^{4a} are as previously defined). Thus, compounds in which CR^6R^7 represents $\text{C}=\text{O}$ may be prepared by treating a compound of formula (XIII) with a suitable transition metal reagent such as a rhodium complex, e.g.

- 20 -

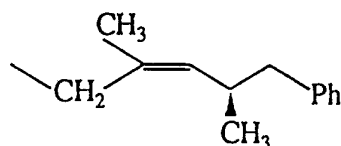
$\text{RhCl}(\text{PPh}_3)_3$ at an elevated temperature (e.g. at reflux), in a suitable solvent such as an aqueous alcohol (e.g. aqueous methanol), or using a suitable metal catalyst such as palladium-on-carbon in an organic solvent, for example an ester or an alcohol at ambient temperature, followed where necessary by removing any protecting groups present. The resulting keto compounds of formula (I) or-protected derivatives thereof may then be converted to the corresponding compounds of formula (I) in which R^6 is hydrogen and R^7 is a hydroxyl, C_{1-6} alkoxy or acyloxy group by reduction as described hereinabove, followed where appropriate by etherification or acylation as described hereinafter, followed by removal of any protecting groups present.

The aforementioned conversion of CR^6R^7 as CHOH to CHR^7 where R^7 is a C_{1-6} alkoxy group may be effected by reaction under conventional conditions for ether formation. Thus, for example, the conversion may conveniently be effected by reaction with a halide R^{12}Hal (where Hal is a halogen atom such as bromine or iodine and R^{12} is a C_{1-6} alkyl group) preferably in the presence of a suitable base such as an alkali metal hydroxide (e.g. potassium hydroxide), an alkali metal hydride (e.g. sodium hydride) or an alkali metal alkoxide (e.g. potassium tert-butoxide) in a solvent such as an ether (e.g. tetrahydrofuran) or a dialkylamide (e.g. dimethylformamide).

The aforementioned conversion of CR^6R^7 as CHOH to CHR^7 where R^7 is an acyloxy group may conveniently be effected by reaction with a suitable acylating agent under conventional conditions. Thus, for example the conversion may conveniently be effected by reaction with an acyl halide, for example an acyl chloride, in the presence of 4-dimethylaminopyridine with or without a suitable base such as a tertiary amine (e.g. triethylamine) or using an alkali metal carbonate or alkaline earth metal carbonate (e.g. calcium carbonate) in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane).

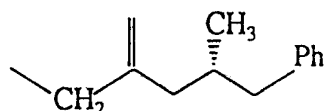
Compounds of formula (I) in which R^1 represents a group

- 21 -

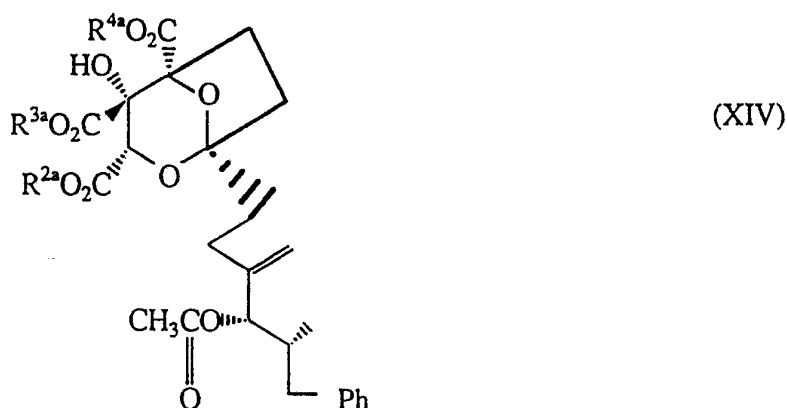


may be prepared from compounds of formula (XIII) by catalytic hydrogenolysis using a suitable palladium catalyst such as palladium-on-barium sulphate in a suitable solvent such as an alcohol (e.g. ethanol), followed where necessary by removing any protecting groups present.

Compounds of formula (I) in which R^1 represents

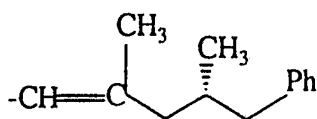


may be prepared from compounds of formula (XIV)



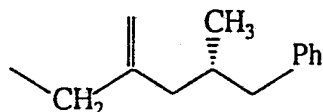
(wherein R^{2a} , R^{3a} and R^{4a} are as defined previously) by heating the compound of formula (VII) with ammonium formate in the presence of a suitable palladium catalyst such as $(Ph_3P)_2PdCl_2$ and in a suitable solvent such as an ether (e.g. dioxan), followed where necessary by removing any protecting groups present.

Compounds of formula (I) in which R^1 represents



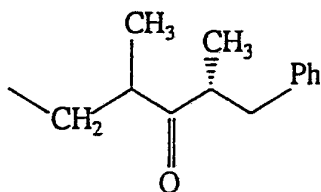
may be prepared from a compound of formula (I) in which R^1 represents

- 22 -

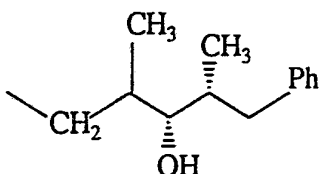


by isomerization using, for example, a hydrogen halide (e.g. hydrogen chloride) in a solvent such as an ether (e.g. dioxan).

Compounds of formula (I) in which R^1 represents

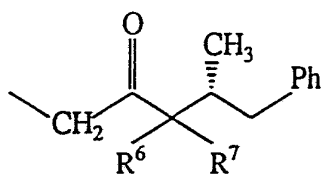


may also be prepared from compounds of formula (I) in which R^1 represents

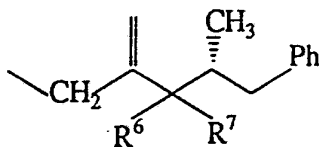


or protected derivatives thereof using a suitable oxidising agent such as pyridinium chlorochromate in a halogenated hydrocarbon solvent (e.g. dichloromethane), followed where necessary by removal of any protecting groups present.

Compounds of formula (I) in which R^1 represents



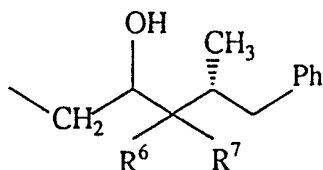
may be prepared from corresponding compounds in which R^1 represents



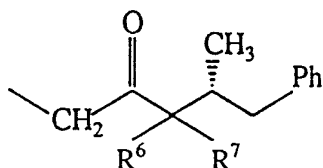
by ozonolysis using for example ozone in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane) at a low temperature (e.g. -70°C to 0°C), followed by treatment with either a triarylphosphine such as triphenylphosphine or a dialkyl sulphide such as dimethyl sulphide, and thereafter where necessary followed by removing any protecting groups present.

- 23 -

Compounds of formula (I) in which R^1 represents

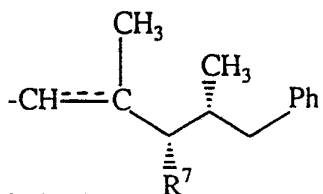


(where R^6 and R^7 are as defined previously except that CR^6R^7 may not represent $C=O$) may be prepared from compounds of formula (I) in which R^1 represents



or protected derivatives thereof by reduction, for example using a suitable reducing agent such as sodium borohydride in an appropriate solvent (e.g. an alcohol such as methanol) at reduced temperature (e.g. -10^0C to $+10^0C$), followed where necessary by removal of any protecting groups present.

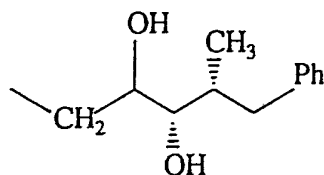
Compounds of formula (I) in which R^1 represents a group



(in which R^7 is hydrogen, hydroxy or acetoxy) may also be prepared from compounds of formulae (XIII) and (XIV) as appropriate by catalytic hydrogenation using hydrogen in the presence of a suitable palladium catalyst (e.g. palladium-on-carbon) in a solvent such as ethyl acetate, or a mixture of an alcohol (e.g. ethanol) and a halogenated hydrocarbon (e.g. dichloromethane) in the presence of triethylamine, or by isomeriation using a suitable palladium catalyst as defined above under the conditions described just above, optionally in the presence of hydrogen, followed where appropriate by removing any protecting groups present.

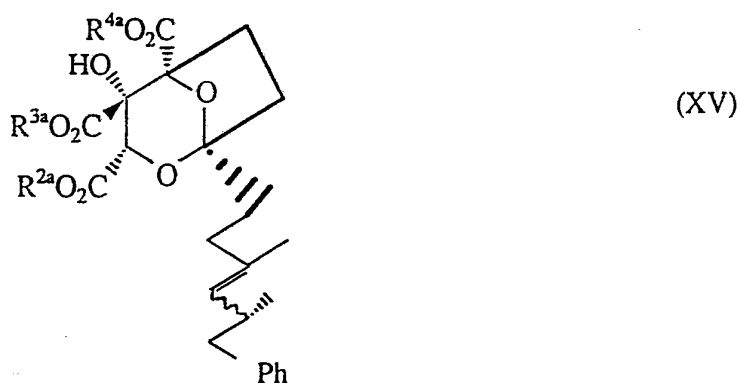
Compounds of formula (IX) in which R^{1b} represents CH_2CHO may conveniently be prepared from the aforementioned compounds of formula (I) in which R^1 represents

- 24 -



by oxidation using a suitable oxidising agent such as a periodate (e.g. sodium periodate) in an aqueous ether (e.g. aqueous tetrahydrofuran) conveniently at a temperature in the range 0° - 20° C.

Compounds of formula (IX) in which R^{1b} represents CH_2COCH_3 may conveniently be prepared from compounds of formula (XV)



(wherein R^{2a} , R^{3a} and R^{4a} are as defined previously) by ozonolysis using for example ozone in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane) at a low temperature (e.g. -70° C to 0° C), followed by treatment with either a triarylphosphine such as triphenylphosphine or a dialkyl sulphide such as dimethyl sulphide.

Compounds of formula (XV) in which R^{2a} , R^{3a} and R^{4a} represent protecting groups may be prepared from the corresponding compounds of formula (XV) in which R^{2a} , R^{3a} and R^{4a} are hydrogen atoms using standard methods of carboxylic acid group protection. Such compounds of formula (XV) in which R^{2a} , R^{3a} and R^{4a} represent hydrogen atoms may conveniently be prepared from compounds of formula (XIII) in which R^{2a} , R^{3a} and R^{4a} are hydrogen atoms by catalytic hydrogenolysis using palladium-on-barium sulphate in a suitable solvent such as an alcohol (e.g. ethanol).

- 25 -

Compounds of formula (XII) may conveniently be prepared from compounds of formula (XIII) by oxidation, using for example a suitable oxidising agent such as pyridinium chlorochromate in a halogenated hydrocarbon solvent (e.g. dichloromethane).

Compounds of formulae (XIII) and (XIV) in which one or more of R^{2a} , R^{3a} and R^{4a} represents a protecting group may be prepared from the corresponding carboxylic acid of formula (XIII) or (XIV) using standard protection methods.

Compounds of formulae (XIII) and (XIV) may conveniently be prepared from compounds of formula (VIII) by appropriate manipulation of the 6 and 7 position groupings using the procedures described hereinabove.

It will be appreciated that the hydrogenation reaction to modify the R^1 grouping may also be effected on a compound of formula (II), thereby reducing the ring double bond and modifying the R^1 grouping in a one-pot reaction, to provide an appropriate compound of formula (I) or a protected derivative thereof.

Suitable carboxylic acid protecting groups for R^{2a} , R^{3a} and R^{4a} and hydroxyl protecting groups for R^{1a} include any conventional protecting group, for example as described in 'Protective Groups in Organic Chemistry', Ed. J. F. W. McOmie (Plenum Press, 1973) or 'Protective Groups in Organic Synthesis' by Theodora W. Greene (John Wiley and Sons, 1981). Examples of suitable carboxylic acid protecting groups include alkyl groups such as t-butyl, 2-methoxyethoxymethyl or aralkyl groups such as benzyl, diphenylmethyl or p-nitrobenzyl. Examples of suitable hydroxyl protecting groups include groups such as 2-methoxyethoxymethyl.

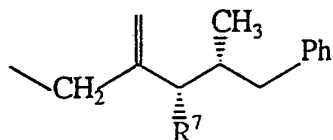
The protecting groups may be removed using conventional techniques. Thus, an alkyl group such as t-butyl may, for example, be removed under anhydrous acid conditions (for example using hydrogen chloride in a solvent such as an ether, e.g. dioxan). An aralkyl group may conveniently be removed by catalytic hydrogenation using for example a suitable metal catalyst such as palladium-on-carbon. Alternatively, a p-nitrobenzyl group may conveniently be removed using zinc metal and hydrochloric acid in a solvent such as an ether (e.g. tetrahydrofuran or aqueous tetrahydrofuran). A diphenylmethyl group or a 2-methoxyethoxymethyl group may conveniently be removed using aqueous formic acid or trifluoroacetic acid.

- 26 -

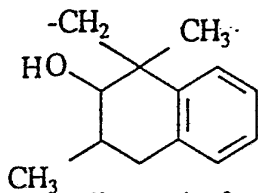
Esterification of carboxylic acids of formula (VIII) to the corresponding methyl esters may conveniently be effected by treatment with a methylating agent such as a methyl halide (e.g. methyl iodide) or dimethyl sulphate in a suitable organic solvent such as an amide (e.g. dimethylacetamide or preferably dimethylformamide) in the presence of a base such as a bicarbonate (e.g. sodium bicarbonate). The reaction may conveniently be carried out at a temperature ranging from 0⁰ to 100⁰C, preferably 20⁰ to 30⁰C. Alternatively, the esterification may be effected by treatment with an ethereal solution of diazomethane in a suitable solvent such as methanol. The esterification may also be effected by treatment with methanol in the presence of a suitable acid such as a mineral acid (e.g. hydrochloric acid) at about room temperature.

Conversion of one methyl ester of formula (VIII) to a different methyl ester may be carried out by appropriate esterification/deesterification steps. The deesterification may be effected under standard conditions, for example by base hydrolysis.

Compounds of formula (VIII) in which R¹ represents (where R⁷ is a hydroxyl



or acetoxy group), $-\text{CH}_2\text{C}(\text{CH}_3)=\text{CHCH}(\text{CH}_2\text{OH})\text{CH}_2\text{Ph}$,
 $-\text{CH}_2\text{C}(\text{CH}_2\text{OH})=\text{CHCH}(\text{CH}_3)\text{CH}_2\text{Ph}$,
 $-\text{CH}_2\text{C}(=\text{CH}_2)\text{CH}(\text{OH})\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{Ph}$,
 $-\text{CH}_2\text{C}(=\text{CH}_2)\text{CH}(\text{NHCOCH}_3)\text{CH}(\text{CH}_3)\text{CH}_2\text{Ph}$,
 $-\text{CH}_2\text{C}(\text{CH}_2\text{NHCOCH}_3)=\text{CHCH}(\text{CH}_3)\text{CH}_2\text{Ph}$ or



may be prepared according to the fermentation process described hereinafter or may be prepared from products of the fermentation process by acylation or deacylation at the 6-position according to suitable acylation methods described hereinafter and

- 27 -

deacylation methods described hereinabove. Other compounds of formula (VIII) may be prepared from the fermented compounds of formula (VIII) or acylated or deacylated derivatives thereof by modification of the R¹ grouping according to the general procedures described hereinabove. The fermentation process comprises cultivating a microorganism capable of producing one or more of the compounds of formula (VIII). Thereafter the desired compound from the culture may be isolated and, if desired, acylated and/or esterified to the corresponding methyl ester.

Suitable microorganisms may readily be identified by using a small scale test and analysing a test sample obtained from fermentation of the microorganism using standard methodology.

In particular the microorganism to be conventionally used is a strain of microorganism deposited on 31st May 1989 in the culture collection of Glaxo Group Research Limited, Microbiology Division, Greenford Road, Greenford, Middlesex, England, UB6 0HE (collection number 202 in the World Directory of Collections of Cultures of Microorganisms, 1982; curator : Miss A M Harris) under accession no. C2932 or a mutant thereof. It is to be understood that the above mentioned culture collection centre has given its unreserved and irrevocable consent to the microorganism deposited being made available to any person making a valid request therefor to the culture collection in accordance with Rule 17 of the UK Patents Rules 1982.

The strain deposited at Greenford under accession no. C2932 has also been deposited in the permanent culture collection of the CAB International Mycological Institute, Ferry Road, Kew, Surrey, England. The strain was received by the Institute on 25th May 1989 and was subsequently given the accession no. IMI 332962 and a deposit date of 27th June 1989 (date of confirmation of viability). The deposited strain is identified herein by reference to the Institute accession no. IMI 332962. The characteristics thus far identified for IMI 332962 are given in Example 4 hereinafter.

It will be appreciated that the desired intermediates may also be prepared from a mutant of IMI 332962.

- 28 -

Mutants of the IMI 332962 may arise spontaneously or may be produced by a variety of methods including those outlined in Techniques for the Development of Micro-organisms by H. I. Adler in 'Radiation and Radioisotopes for Industrial Microorganisms', Proceedings of the Symposium, Vienna 1973, p241, International Atomic Energy Authority. Such methods include ionising radiation, chemical methods e.g. treatment with N-methyl-N'-nitro-N-nitrosoguanidine (NTG), heat, genetic techniques, such as recombination and transformation, and selective techniques for spontaneous mutants.

The fermentation may be effected by conventional means i.e. by culturing the organism in the presence of assimilable sources of carbon, nitrogen and mineral salts.

Assimilable sources of carbon, nitrogen and minerals may be provided by either simple or complex nutrients. Sources of carbon will generally include glucose, maltose, starch, glycerol, molasses, dextrin, lactose, sucrose, fructose, galactose, myo-inositol, D-mannitol, soya bean oil, carboxylic acids, amino acids, glycerides, alcohols, alkanes and vegetable oils. Sources of carbon will generally comprise from 0.5 to 10% by weight of the fermentation medium. Fructose, glucose and sucrose represent preferred sources of carbon.

Sources of nitrogen will generally include soya bean meal, corn steep liquors, distillers solubles, yeast extracts, cottonseed meal, peptones, ground nut meal, malt extract, molasses, casein, amino acid mixtures, ammonia (gas or solution), ammonium salts or nitrates. Urea and other amides may also be used. Sources of nitrogen will generally comprise from 0.1 to 10% by weight of the fermentation medium.

Nutrient mineral salts which may be incorporated into the culture medium include the generally used salts capable of yielding sodium potassium, ammonium, iron, magnesium, zinc, nickel, cobalt, manganese, vanadium, chromium, calcium, copper, molybdenum, boron, phosphate, sulphate, chloride and carbonate ions.

Cultivation of the organism will generally be effected at a temperature of from 20 to 40°C preferably from 20 to 35°C, especially around 25 to 28°C, and will desirably take place with aeration and agitation e.g. by shaking or stirring. The

- 29 -

medium may initially be inoculated with a small quantity of mycelium and/or spores. The vegetative inoculum obtained may be transferred to the fermentation medium, or to one or more seed stages where further growth takes place before transfer to the principal fermentation medium. The fermentation will generally be carried out in the pH range 3.5 to 9.5, preferably 4.5 to 7.5. It may be necessary to add a base or an acid to the fermentation medium to keep the pH within the desired range. Suitable bases which may be added include alkali metal hydroxides such as aqueous sodium hydroxide or potassium hydroxide. Suitable acids include mineral acids such as hydrochloric, sulphuric or phosphoric acid.

The fermentation may be carried out for a period of 4-30 days, preferably about 7-18 days. An antifoam may be present to control excessive foaming and added at intervals as required. Carbon and/or nitrogen sources may also be fed into the fermentation medium as required.

The products of the fermentation process may be present in both the fermentation liquor and the mycelial fraction, which may conveniently be separated by filtration or centrifugation. The liquor may be optionally thereafter treated with an acid such as sulphuric acid in the presence of an organic solvent until the pH is below pH 6 (e.g. about pH 3).

The products of the fermentation process may be separated from the fermentation broth by conventional isolation and separation techniques. It will be appreciated that the choice of isolation techniques may be varied widely.

The products of the fermentation process may be isolated and purified by a variety of fractionation techniques, for example adsorption-elution, precipitation, fractional crystallisation, solvent extraction and liquid-liquid partition which may be combined in various ways.

Adsorption onto a solid support followed by elution has been found to be suitable for isolating and purifying compounds of the invention.

The products of the fermentation process may be extracted from the cells and the aqueous phase with an appropriate organic solvent such as a ketone (e.g. acetone, methyl ethyl ketone or methyl isobutyl ketone), a halogenated hydrocarbon, an alcohol, a diol (e.g. propane-1,2-diol or butane-1,3-diol) or an ester (e.g. methyl

- 30 -

acetate or ethyl acetate). Generally, more than one extraction may be desirable to achieve optimum recovery. The water-immiscible solvent extracts may themselves be extracted with basic aqueous solutions, and after acidification of these basic solutions the desired compounds may be reextracted into water-immiscible organic phase. Removal of the solvent from the organic extracts (e.g. by evaporation) yields a material containing the desired compounds.

Chromatography (including high performance liquid chromatography) may be effected on a suitable support such as silica; a non-functional macroporous adsorption resin for example cross-linked styrene divinyl benzene polymer resins such as Amberlite XAD-2, XAD-4, XAD-16 or XAD-1180 resins (Rohm & Haas Ltd) or Kastell S112 (Montedison); a substituted styrene-divinyl benzene polymer, for example a halogenated (e.g. brominated) styrene-divinyl benzene polymer such as Diaion SP207 (Mitsubishi); an anion exchanger (e.g. IRA-35 or IRA-68) an organic solvent-compatible cross-linked dextran such as Sephadex LH20 (Pharmacia UK Ltd), or on reverse phase supports such as hydrocarbon linked silica e.g. C₁₈-linked silica. An alternative chromatographic means for the purification/separation of the products of the fermentation process is countercurrent chromatography using a coil extractor such as a multi-layer coil extractor.

The products of the fermentation process may also be isolated and purified by the use of a liquid anion exchanger such as LA 2.

When IRA-68 or, particularly, IRA-35 is used as the solid adsorbant the cell extracts may be loaded directly without removal of solvent. The extract may either be loaded directly at about pH3 or at about pH8 following filtration of solid impurities.

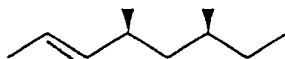
Suitable solvents/eluates for the chromatographic purification/ separation of compounds of formula (VIII) will, of course, depend on the nature of the column type and support. When using countercurrent chromatography we have found a solvent system comprising ethyl acetate, hexane, methanol and an aqueous acid (e.g. aqueous sulphuric acid) to be particularly suitable. When using an anion exchanger such as IRA-35 the resin may conveniently be washed with aqueous acetone followed by elution with sulphuric acid in aqueous acetone.

- 31 -

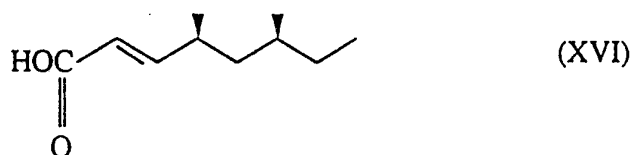
The presence of the products of the fermentation process during the extraction/isolation procedures may be monitored by conventional techniques such as h.p.l.c. or UV spectroscopy or by utilising the properties of the compounds.

Where a product of the fermentation process is obtained in the form solution in an organic solvent, for example after purification by chromatography, the solvent may be removed by conventional procedures, e.g. by evaporation, to yield the required compound. If desired, the compound may be further purified by the aforementioned chromatographic techniques.

Acylation to provide a compound of formula (VIII) in which R¹⁰ represents

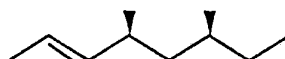


may be effected by treating a corresponding compound of the fermentation process in which the 6-positional substituent is a hydroxyl group or a protected derivative thereof with an acid of formula (XVI)



or an activated derivative thereof such as the corresponding acid chloride under conventional esterification conditions followed by removal of any protecting groups present. The acylation reaction may conveniently be carried out in the presence of 4-dimethylaminopyridine with or without a suitable base such as a tertiary amine (e.g. triethylamine) or using an alkali metal carbonate or alkaline earth metal carbonate (e.g. calcium carbonate) in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane).

The compound of formula (XVI) may conveniently be prepared by hydrolysis of a compound of formula (VIII) in which R¹⁰ represents



- 32 -

for example by base catalysed hydrolysis using a base such as aqueous sodium hydroxide in a solvent such as an alcohol (e.g. methanol).

It is to be understood that the acylation and esterification processes may be combined as sequential or simultaneous reaction steps as appropriate.

Salts of compounds of formula (I) may be conveniently formed by treating a compound of formula (I) with an appropriate salt or base. Thus, for example, salts may conveniently be prepared by treating a compound of formula (I) with a salt or a base selected from sodium or potassium hydroxide, hydrogen carbonate, carbonate or acetate (e.g. potassium hydroxide, potassium hydrogen carbonate, sodium hydrogen carbonate or potassium acetate), ammonium acetate, calcium acetate and L-lysine as appropriate. The salt may, for example, be prepared by adding the appropriate salt or base (if necessary as an aqueous solution) to a solution or suspension of the compound of formula (I) in a suitable solvent such as water and/or a cosolvent such as an alcohol (e.g. methanol), a nitrile (e.g. acetonitrile) or a ketone (e.g. acetone) at temperatures of for example 0°C to 80°C and conveniently at about room temperature.

Compounds of formulae (II), (III), (IX), (XI), (XII), (XIII), (XIV) and (XV) are novel intermediates and form a further aspect of the present invention.

The following examples are provided by way of illustrating the invention and are not intended to limit the invention in any way.

Intermediate 1

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α ,6 α (2E,4R*,6R*),7 β]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid, 6-(4,6-dimethyl-2-octenoate)

(a) IMI 332962 was grown on agar plates of the following composition:

Malt extract (Oxoid L39)	30g
Mycological peptone (Oxoid L40)	5g
Yeast extract (Oxoid L21)	0.5g

- 33 -

Agar (Oxoid No 3)	20g
Distilled water to 1 litre	

The pH of the medium before autoclaving was in the range of 5.3-5.5. The inoculated plates were incubated at 28⁰C for 14 days. Several 6mm diameter plugs of agar covered with fungal mycelium were cut from the growing edge of the culture and two plugs were transferred into each of several cryotubes containing 1.6ml of sterile distilled water. The tubes were capped and stored at room temperature until required.

Two agar plugs were used to inoculate each of eight 50ml aliquots of seed medium (A) contained in 250ml Erlenmeyer flasks :

Seed medium (A) : Peptone (Oxoid L34)	10g
Malt extract (Oxoid L39)	21g
Glycerol	40g
Junlon 110 (Honeywill & Stein Ltd., Wallington, Surrey)	1g
Distilled water to 1 litre	

The pH of the medium was adjusted to 6.3-6.5 with aqueous sodium hydroxide before autoclaving

The flasks of inoculated seed medium were incubated at 25⁰C on a shaker platform, which rotated at 250rpm with a 50mm diameter orbital motion, for 5 days.

The contents of the flasks were pooled and homogenised. The homogenised seed culture was used at 3% (v/v) to inoculate 120, 50ml aliquots of fermentation medium (B) in 250ml Erlenmeyer flasks :

Fermentation medium (B) : Glycerol	50g
Soyabean oil	30g
Cottonseed flour (Sigma)	10g
Distilled water to 1 litre	

- 34 -

The pH of the medium before autoclaving was in the range 6.1-6.3. The flasks were incubated as above with shaking for 8 days.

The fermentation broth (approximately 6L) from flasks incubated for 8 days was filtered to remove the mycelium and the filtrate adjusted to pH 2.8 with sulphuric acid (20% v/v) and extracted with 3 x 2 volumes of ethyl acetate. The ethyl acetate extracts were bulked and back extracted with 2 x 400ml of aqueous sodium hydrogen carbonate solution (1% w/v). The aqueous back extracts were bulked, adjusted to pH 2.8 as above and re-extracted into 2 x 800ml of ethyl acetate. These extracts were combined and evaporated to dryness to yield a brown oil. This oil was further processed by countercurrent chromatography using an Ito Multi-layer Coil Extractor (P. C. Inc., Potomac, Maryland, USA). The coil used was the standard preparative coil consisting of approximately 70 metres of 2.6mm internal diameter PTFE tubing giving a total volume of about 380ml. The solvent system used was a mixture of ethyl acetate, hexane, methanol and N/100 sulphuric acid (6:5:5:6 by volume). The lower phase was kept stationary. The coil was filled with the lower phase using a Gilson Model 303 pump and a Model 804C Manometric Module (Gilson, Villiers Le Bel, France). The oil (497mg in 4ml of the upper phase +4ml of the lower phase) was then injected at the "tail" end of the column. The centrifuge was then operated at 800 rev./min. and the mobile (upper) phase pumped at 4ml/min. from the "tail" end of the column. 20ml fractions were collected and monitored by measuring inhibition of squalene synthase.

Consecutive fractions showing activity against squalene synthase were bulked. The earlier fractions were evaporated to dryness to yield the title compound (90mg) as a pale yellow oil.

(b) The mycelium separated from 6L broth, from flasks incubated for 8 days according to the procedure in part (a) above, was extracted with methanol (2 x 3L) and filtered. The filtrate was concentrated by evaporation to ca. 500ml, adjusted to pH 3.0 with formic acid and extracted with 3 x 500ml of ethyl acetate. The ethyl acetate extracts were bulked and back extracted with 2 x 200ml of sodium hydrogen

- 35 -

carbonate solution (1% w/v). The aqueous back extracts were bulked, adjusted to pH 3.0 and re-extracted into 2 x 500ml of ethyl acetate. All the organic fractions were combined and reduced to dryness using a rotary evaporator to yield a brown oil. The oil (578mg) was further processed by high performance liquid chromatography (HPLC) using a Gilson autopreparative system composed of 3 Gilson solvent delivery pumps (model 303), an 811 Dynamic mixer and an 802C manometric module. The chromatography was carried out on a Dynamax Microsorb C18 (5 μ m) semi-preparative column (250 x 10mm). The mobile phase was a gradient composed of acetonitrile and 0.1% v/v formic acid to pH 3.15 with ammonium acetate (1:3 $\rightarrow$ 4:1 $\rightarrow$ 1:3) pumped at 2.8-5.6ml/min with a run time of 65 minutes. This method was repeated 16 times. 13 x 4.95 minute fractions were collected and monitored by measuring inhibition of squalene synthase. Fraction number 5 from each run was bulked, acidified to pH 3.0 with formic acid and extracted with 2 x 100ml ethyl acetate. The organic phase was removed and evaporated to dryness to yield the title compound (172mg) as a pale yellow oil.

(c) (i) Eight 0.5ml aliquots from a 5 day old fermentation carried out as in part (a) above were used to inoculate eight 50ml aliquots of seed medium (A) contained in 250ml Erlenmeyer flasks. The flasks were incubated at 25⁰C on a shaker platform, which rotated at 250rpm with a 50mm diameter orbital motion, for 4 days. The contents of the flasks were pooled and homogenised.

The homogenised seed culture was used at 3% (v/v) to inoculate 120, 50ml aliquots of fermentation medium (B) in 250ml Erlenmeyer flasks. The flasks were incubated with shaking as above for 10 days.

(c) (ii) Homogenised seed culture prepared as in part (c)(i) above were used at 3% (v/v) to inoculate two fermentation vessels, each of 5 litres capacity, containing 3 litres of fermentation medium (B). The inoculated medium was maintained at 25⁰C and agitated with two six bladed turbine impellers (70mm diameter) rotating at 500 rpm. The culture was aerated by sparging with sterile air at 3 Lpm. Provision was made for control of excessive foaming of the culture by the

- 36 -

addition of silicone antifoam (Dow Corning 1520). The contents of the two culture vessels were combined after 11 days growth and further processed by countercurrent chromatography according to the procedure in part (a) above to give the title compound (137mg); 500MHz proton nmr in deuterio-methanol includes signals at about δ 0.84-0.90 (m,9H), 1.03 (d,7,3H), 1.09-1.19 (m,2H), 2.10 (s,3H), 2.24 (m,1H), 2.34 (m,1H), 2.68 (dd,13,6,1H), 4.04 (d,2,1H), 4.97 (s,1H), 5.02 (s,1H), 5.08 (d, 5,1H), 5.27 (s,1H), 5.80 (d,16,1H), 6.31 (d,2,1H), 6.85 (dd,16,8,1H), 7.14 (t,7,1H), 7.19 (d,7,2H), 7.26 (t,7,2H); composite pulse decoupled 125.75 MHz carbon-13 nmr in deuterio-methanol includes peaks at about δ 172.5 (0), 172.1(0), 170.1(0), 168.5(0), 166.5 (0), 157.6 (1), 147.7 (0), 141.6 (0), 130.2 (1), 129.3 (1), 126.9 (1), 119.8 (1), 111.5 (2), 106.8 (0), 91.1 (0), 82.5 (1), 81.0 (1), 80.1 (1), 76.6 (1), 75.6 (0), 44.4 (2), 40.9 (2), 37.7 (1), 35.6 (1), 34.9 (2), 33.1 (1), 30.8 (2), 26.5 (2), 20.9 (3), 20.5 (3), 19.2 (3), 14.1 (3), 11.4 (3).

(d) (i) Frozen stocks of inoculum were prepared from a 5 day old fermentation carried out as in part (a) above. Samples of culture were centrifuged for 10 min and the mycelium resuspended to the original volume in 15% glycerol and 0.01% Tween 80. The mycelium was spun down and resuspended again before being distributed in 1.8ml amounts in plastic tubes and stored at -20°C . Eight 0.5ml aliquots of frozen inoculum were used to inoculate eight 50ml aliquots of seed medium (A) contained in 250ml Erlenmeyer flasks. The flasks were incubated at 25°C on a shaker platform, which rotated at 250rpm with a 50mm diameter orbital motion, for 4 days. The contents of the seed flasks were pooled and used at 3% (v/v) to inoculate 120 50ml aliquots of fermentation medium (B) in 250 ml Erlenmeyer flasks. The flasks were incubated with shaking as above for 9 days.

(d) (ii) The contents of 4 final stage flasks grown as in part (d)(i) above were pooled after 7 days incubation and homogenised to provide the seed for 120 50ml aliquots of fermentation medium (B) which were incubated for 8 days as in parts (c)(i) and (d)(i) above. The fermentation broth (approximately 6L) from flasks incubated for 8 days was filtered to remove the mycelium. The filtrate was adjusted

- 37 -

to pH 2.8 with sulphuric acid (20% v/v) and extracted into ethyl acetate, back extracted into sodium hydrogen carbonate and re-extracted into ethyl acetate at pH 2.8 as described in part (a) above. The ethyl acetate extract was concentrated under reduced pressure to a yellow oil which was dissolved in methanol (10ml). This solution was evaporated to 3ml and applied to a column (32 x 2.5cm) of ODS-3 (Whatman Partisil Bioprep 40, 75 Angstrom, slurry packed in acetonitrile-water, 20:80). The column was eluted with a stepwise gradient of a mixture of acetonitrile and water, increasing the proportion of acetonitrile as follows : 1:4, 3:7, 2:3, 1:1, 3:2. Fractions were monitored by HPLC and those containing the title compound were evaporated to remove acetonitrile. The resulting aqueous suspensions were pooled and freeze dried overnight to yield the title compound (59mg) as an off-white solid.

(e) The procedure in part (d)(i) was followed except that the pooled seed flasks were used at 3% (v/v) to inoculate 4 litres of seed medium (A) in a 7L fermenter. The culture was incubated with agitation as above at 500rpm for 2 days with the culture aerated at 4L/min. 1.2L of the culture was removed and used to inoculate a 70L fermenter filled with 40L seed medium (A). The culture was incubated as above at 500rpm for 2 days with the culture aerated at 40L/min. 15L of the culture was removed and added to a 780L fermenter filled with 500L fermentation medium (C).

Fermentation medium (C) :	Fructose	50g
	Soyabean oil	30g
	Cottonseed flour (sigma)	20g

Natural pH

The culture was incubated with shaking as above at 200rpm for 450h with the culture aerated at 500L/min and fed at 120h with a 50% (w/v) solution of fructose at 5g/L/day increasing to 7.5g/L/day at 162h. Analysis of the broth at 450h indicated a yield of the title compound of 1056 mg/L.

- 38 -

The above procedure was repeated on a reduced scale but replacing fructose with other sources of carbon selected from glucose, galactose, sucrose, maltose, lactose, myo-inositol, D-mannitol and soyabean oil. Analysis of the broth from each experiment at 450h indicated a substantial titre of the title compound.

The title compound prepared according to the above procedures was consistent with a product having the following characterising features :

Approximate molecular weight 690; -FAB mass spectrometry [M-H]- 689.2789; +FAB mass spectrometry [M+Na]+ 713.2753; Molecular formula $C_{35}H_{46}O_{14}$.

500 MHz proton nmr spectrum in deutero-chloroform [δ values with multiplicities, coupling constants (H_z) and integration values in parenthesis] : 0.79 to 0.85 (m,9H), 1.00 (d,7,3H), 1.04 to 1.15 (m,2H), 2.09 (s,3H), 2.40 (m,1H), 2.69 (dd,13,5,1H), 4.05 (s,1H), 4.94 (s,1H), 4.96 (s,1H), 5.06 (d,4,1H), 5.30 (s,1H), 5.78 (d,16,1H), 5.92 (s,1H), 6.88 (dd,16,8,1H), 7.11 (d,7,2H), 7.14 (t,7,1H), 7.24 (t,7,2H).

Composite pulse decoupled 125.75MHz carbon-13 nmr spectrum in deutero-chloroform [δ values with the number of attached protons in parenthesis] : 171.5 (0), 171.0 (0), 169.1 (0), 167.0 (0), 166.7 (0), 157.9 (1), 145.4 (0), 140.1 (0), 128.9 (1), 128.1 (1), 125.8 (1), 117.8 (1), 111.4 (2), 105.8 (0), 88.5 (0), 81.6 (1), 80.7 (1), 79.3 (1), 75.1 (1), 74.2 (0), 42.9 (2), 39.7 (2), 36.7 (1), 34.2 (1), 33.6 (2), 31.6 (1), 29.4 (2), 25.4 (2), 20.9 (3), 19.8 (3), 18.8 (3), 13.5 (3), 10.9 (3).

Intermediate 2

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α ,6 α (2E,4R*,6R*),7 β]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid, 6-(4,6-dimethyl-2-octenoate), 3,4,5-tris(1,1-dimethylethyl) ester

A freeze-dried product of Intermediate 1 (7.5g) in dry dichloromethane (32ml) was heated at reflux, under nitrogen and treated dropwise over 20 min with N,N-dimethylformamide di-tert.butyl acetal (31.3ml). The mixture was heated under reflux for 1h when a further addition of N,N-dimethylformamide di-tert.butyl acetal (7.2ml) was made over 3 min. The mixture was heated under reflux for a further 4h and was then allowed to cool to room temperature, diluted with diethyl ether

- 39 -

(200ml) and washed with brine (3x100ml). The organic phase was dried (MgSO_4) and evaporated to give a red-brown foam (10.75g). This was subjected to flash chromatography on silica gel (Merck 9385, 1100ml) eluting with ethyl acetate:cyclohexane (1:6). Fractions which contained the major component were combined and evaporated to give the title compound (6.55g) as a cream-yellow foam; ν_{max} (CHBr_3) ca 3400-3600 (OH), 1755 (ester C=O), 1730 (ester C=O) and 1250cm^{-1} (ester C=O); δ (CDCl_3) includes 1.43(s, Me_3C -), 1.48(s, Me_3C -), 1.60(s, Me_3C -), 2.08(s, CH_3CO_2 -), 2.93(d, $J=3\text{Hz}$, 7-OH), 4.00(broad s, 7-H), 4.08(s, 4-OH), 4.95(bs, $\text{C}=\text{CH}_2$), 5.05(s, 3-H), 5.11(d, $J=5\text{Hz}$, $\text{CH}_3\text{CO}_2\text{CH}$), 5.77(d, $J=15\text{Hz}$, $\text{CH}=\text{CH}\cdot\text{CHMe}$), 6.01(d, $J=2\text{Hz}$, 6-H), 6.91(dd, $J=15\text{Hz}, 7\text{Hz}$, $\text{CH}=\text{CH}\cdot\text{CHMe}$) and 7.10-7.30(m, aromatic protons).

Intermediate 3

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α ,6 α (2E,4R*,6R*)]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4,6-dihydroxy-7-oxo-2,8-dioxabicyclo[3.2.1] octane-3,4,5-tricarboxylic acid, 6-(4,6-dimethyl-2-octenoate), 3,4,5-tris(1,1-dimethylethyl) ester

To a solution of Intermediate 2 (6.9g) in dichloromethane (69ml) was added powdered 3A molecular sieves (6.9g) and pyridinium chlorochromate (6.9g). The mixture was stirred at 20°C for 14h then diluted with a 3:1 mixture of light petroleum and ethyl acetate (2 litres) and filtered through Kieselguhr. The resultant clear orange solution was washed with water (3x500ml), dried and evaporated to give the title compound, (6g); δ (CDCl_3) includes 0.80(m, 9H, CH_3), 1.43(s, 18H, $\text{C}(\text{CH}_3)_3$), 1.65(s, 9H, $\text{C}(\text{CH}_3)_3$), 2.10(s, OCOCH_3), 4.22(s, OH), 4.69(s, 3H), 4.99, 5.01(2s, $=\text{CH}_2$), 5.11(d, 1H, CHOCOCH_3), 5.76(d, 1H, $J=15\text{Hz}$, $\text{CH}=\text{CHCO}$), 6.42(s, 1H, 6H), 6.95(dd, 1H, $J=15$ and 8Hz , $\text{CH}=\text{CHCO}$), 7.1-7.3(m, 5H, aromatic protons).

Intermediate 4

- 40 -

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α ,6 α (2E,4R*,6R*),7 α]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4,6,7-trihydroxy-2,8-dioxabicyclo [3.2.1]octane-3,4,5-tricarboxylic acid, 6-(4,6-dimethyl-2-octenoate), 3,4,5-tris(1,1-dimethylethyl) ester

(a) To a solution of Intermediate 3 (6g) in methanol (50ml) cooled in ice was added sodium borohydride (265mg) portionwise keeping the temperature below 20°C. After 5 minutes further sodium borohydride (70mg) was added. The reaction mixture was kept for 1 minute before quenching by addition of excess aqueous citric acid and extraction with ether (2x200ml). The extracts were washed with brine, dried and evaporated to give a product which was purified by chromatography on silica gel (Merck silica gel 60, 230-400 mesh; 300g) eluting with a 3:1 mixture of light petroleum and ethyl acetate to give the title compound and the corresponding 7 β -hydroxy isomer in the approximate ratio of 3:1 (3g); δ (CDCl₃) includes 0.8-0.9(m,9H,CH₃), 1.45(s,18H,C(CH₃)₃), 1.63(s,9H,C(CH₃)₃), 2.10(s,OCOCH₃), 4.95(m,2H,=CH₂), 5.81(d,1H,J=15Hz,CH=CHCO), 6.52(d,1H,J=6.3Hz,6H), 6.85-7.02(m,CH=CHCO), 7.1-7.3(m,5H,aromatic protons).

(b) To a solution of Intermediate 3 (1g) in tetrahydrofuran (80ml) and methanol (20ml) cooled in ice under a nitrogen atmosphere was added cerium chloride heptahydrate (560mg). After 20 minutes the mixture was then cooled to -70°C and treated portionwise with sodium borohydride (370mg), keeping the temperature below -60°C. After 20 minutes, further sodium borohydride (100mg) was added. The reaction mixture was kept for 10 minutes before quenching by addition of excess saturated aqueous ammonium chloride and extraction with ethyl acetate (2 x 100ml). The extracts were washed sequentially with 0.2M hydrochloric acid, saturated sodium bicarbonate and brine and were then dried and evaporated to give a product which was purified by chromatography on silica gel (Merck silica gel 60, 230-400 mesh; 100g) eluting with a 4:1 mixture of light petroleum and ethyl acetate to give the title compound and the corresponding 7 β -hydroxy isomer in the approximate ratio of 8:1 (400mg); δ (CDCl₃) includes 0.8-0.9 (m,9H,CH₃), 1.45 (s,18H,C(CH₃)₃), 1.63 (s,9H,C(CH₃)₃), 2.10 (s,OCOCH₃), 4.95 (m,2H,=CH₂),

- 41 -

5.81 (d, 1H, J=15Hz, CH=CHCO), 6.52 (d, 1H, J=6.3Hz, 6H), 6.85-7.02 (m, CH=CHO), 7.1-7.3 (m, 5H, aromatic protons).

c) To a solution of Intermediate 3 (3g) in tetrahydrofuran (100ml) cooled to -65 °C under a nitrogen atmosphere was added a tetrahydrofuran solution (0.5M, 7ml) of lithium tris [(3-ethyl-3-pentyloxy)]aluminumhydride. After 3h at -65 °C, the temperature was allowed to rise to 0 °C and after a further 5 min the reaction mixture was cooled to -60 °C, quenched with 1M hydrochloric acid (100ml) and then extracted with ethyl acetate (3 x 100ml). The organic phase was washed with brine (100ml), then dried and evaporated to an oil which was introduced on to a silica gel column (Merck silica gel 60, 230-400 mesh; 400g). Gradient elution with a mixture of light petroleum and ethyl acetate (from 4:1 to 7:3) gave the title compound and the isomeric [1S-[1 α (4R*,5S*),3 α ,4 β ,5 α ,6 α ,7 α (2E,4R*,6R*)]] 1-(4-acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid, 7-(4,6-dimethyl-2-octenoate), 3,4,5-tris(1,1-dimethylethyl) ester in the approximate ratio of 9:1 (2.8g), δ (CDCl₃) includes 5.16 (obscured d, J=6Hz, CH₃COAc) and 6.84-6.89 (m, CH=CHCO).

d) To a solution of diisobutylaluminium hydride (0.5M) in toluene (21ml) cooled to 0 °C under a nitrogen atmosphere was added a toluene solution (30ml) of 2,6-di-*t*-butyl-4-methylphenol (2.2g). When gas evolution ceased, the solution was cooled to -70 °C and Intermediate 3 (860mg) in toluene (5ml) was added dropwise. After 20 min at -70 °C, the reaction mixture was quenched with 1M hydrochloric acid and was then extracted with ether (3 x 100ml). The organic phase was washed with 5% aqueous sodium bicarbonate solution (100ml) and brine (100ml) and was evaporated to an oil which was purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 120g). Gradient elution with a mixture of light petroleum and ether (from 9:1 to 2:1) provided the title compound, the isomeric 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) and the 7 β -hydroxy isomer in the approximate ratio of 9:1:10 (460mg).

- 42 -

e) To a solution of Intermediate 3 (900mg) in dry tetrahydrofuran (50ml) cooled to -70°C under an atmosphere of nitrogen was added a 1M solution of lithium triethylborohydride in the same solvent (2.2ml). The reaction mixture was stirred for 2h and was then quenched with dilute acetic acid and extracted with ethyl acetate (3 x 150ml). The organic phase was washed well with 5% aqueous sodium bicarbonate solution and was then dried and stripped of solvent to afford an oil which was purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 120g). Elution of the column with light petroleum/ethyl acetate (3:1) gave a mixture of the title compound, the isomeric 6α -hydroxy- 7α -(4,6-dimethyl-2-octenoate) and the 7β -hydroxy isomer in the approximate ratio of 9:1:1 (520mg).

(f) To a solution of Intermediate 3 (449mg) in dry tetrahydrofuran (20ml) at -20°C under nitrogen was added a 0.5M solution of lithium tris[(3-ethyl-3-pentyl)oxy]aluminumhydride in tetrahydrofuran (10.49ml) and the resultant left to stir at -20°C for 15min. The mixture was quenched with water (2ml) and allowed to warm up to room temperature with stirring over 3/4h. Magnesium sulphate was added and the suspension filtered in vacuo. The filtrate was evaporated to give exclusively the title compound (450mg) as a yellow oil.

Intermediate 5

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α ,6 α ,7 α]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4,6,7-trihydroxy-2,8-dioxabicyclo[3.2.1] octane-3,4,5-tricarboxylic acid, 3,4,5-tris(1,1-dimethylethyl) ester

To a solution of Intermediate 4 as a 3:1 mixture with the corresponding 7β -hydroxy isomer (3g) in dry dimethylformamide (28ml) was added N-methylhydroxylamine hydrochloride (720mg) followed by triethylamine (1.9ml). The mixture was stirred at 20°C for 18h in the absence of moisture and then diluted with ether (250ml), washed with 2N-hydrochloric acid (10ml) and brine, dried and evaporated. The product was purified by chromatography on silica gel (Merck silica gel 60, 230-400 mesh; 160g) eluting with a 3:2 mixture of light petroleum and ethyl acetate to give the title compound (1.6g); δ (CDCl_3) includes

- 43 -

0.85(d,3H,J=7.3Hz,CH₃), 1.45(s,9H,C(CH₃)₃), 1.5(s,9H,C(CH₃)₃), 1.60(s,9H,C(CH₃)₃), 2.16(s,3H,OCOCH₃), 3.90(s,OH), 4.22(m,1H,7H), 4.47(s,1H,3H), 4.91 and 5.05(2s,2H,=CH₂), 5.11(m,1H,6H), 5.18(d,1H,J=4.8Hz,CHOCOCH₃), 7.1-7.3(m,5H,aromatic protons).

Intermediate 6

[3aS-[4α(4R*,5S*),6α,7β,8α,8aβ]] 4-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-3a,4,6,7,8,8a-hexahydro-7-hydroxy-2-thioxo-4,8-epoxy-1,3-dioxolo[4,5-c]oxepin-6,7,8-tricarboxylic acid, 6,7,8-tris(1,1-dimethylethyl) ester

To a solution of Intermediate 5 (1.6g) in dry tetrahydrofuran (38ml) was added thiocarbonyldiimidazole (512mg) and the mixture was warmed at 55⁰C for 18h. Removal of the solvent in vacuo followed by purification of the residue by chromatography on silica gel (Merck silica gel 60, 230-400 mesh; 150g) eluting with a 5:1 mixture of light petroleum and ethyl acetate afforded the title compound (1.4g); δ (CDCl₃) includes 0.82(d,3H,J=7.5Hz,CH₃), 1.43(s,9H,C(CH₃)₃), 1.51(s,9H,C(CH₃)₃), 1.63(s,9H,C(CH₃)₃), 2.13(s,3H,OCOCH₃), 4.05(s,1H,OH), 4.39(s,1H,3H), 5.05(broad s,2H,=CH₂), 5.15(d,1H,J=6.3Hz,CHOCOCH₃), 5.19(d,1H,J=6.5Hz,7H), 6.21(d,1H,J=6.5Hz,6H), 7.1-7.3(m,5H,aromatic protons).

Intermediate 7

[1S-[1α(4R*,5S*),3α,4β,5α]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]oct-6-ene-3,4,5-tricarboxylic acid, 3,4,5-tris(1,1-dimethylethyl) ester

Intermediate 6 (1.4g) was treated at 55⁰C for 18h with 1,3-dimethyl-2-phenyl-1,3,2-diaza-phospholine (1.4ml). Purification by chromatography on silica gel (Merck silica gel 60, 230-400 mesh; 150g) eluting with a 5:1 mixture of light petroleum and ethyl acetate afforded the title compound (1.0g); δ (CDCl₃) includes 0.85(s,3H,CH₃), 1.40(s,9H,C(CH₃)₃), 1.46(s,9H,C(CH₃)₃), 1.58(s,9H,C(CH₃)₃), 2.10(s,3H,OCOCH₃), 3.91(s,1H,OH), 4.85(s,1H,3H), 4.98(s,2H,=CH₂), 5.09(d,1H,J=6.5Hz,CHOCOCH₃), 5.99(d,1H,J=6.5Hz,7 olefinic proton), 6.60(d,1H,J=6.5Hz,6 olefinic proton), 7.1-7.3(m,5H,aromatic protons).

- 44 -

Intermediate 8

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid, 3,4,5-tris(1,1-dimethylethyl) ester

A solution of Intermediate 7 (1g) in ethanol (100ml) was hydrogenated at 20⁰C and atmospheric pressure over 10% palladium on carbon (200mg). When saturation of the 6,7-double bond was complete (after about 0.5h; monitored by sampling at intervals for nmr spectra) hydrogenation was discontinued. Removal of the catalyst by filtration through Kieselguhr and evaporation of the filtrate afforded the title compound (1g); δ (CDCl₃) includes 0.81(d,3H,J=7.5Hz,CH₃), 1.49(s,9H,C(CH₃)₃), 1.50(s,9H,C(CH₃)₃), 1.59(s,9H,C(CH₃)₃), 2.10(s,3H,OCOCH₃), 3.9(s,1H,OH), 4.69(s,1H,3H), 4.99(s,2H,=CH₂), 5.13(d,1H,J=7.3Hz,CHOCOCH₃), 7.1-7.3(m,5H, aromatic protons).

Intermediate 9

[1S-[1 α (3R*S*,4S*,5S*),3 α ,4 β ,5 α]] 1-(4-Acetyloxy-3,5-dimethyl-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid, 3,4,5-tris(1,1-dimethylethyl) ester [Compound 1] and [1S-[1 α (3R*S*,5R*),3 α ,4 β ,5 α]] 1-(3,5-Dimethyl-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid, 3,4,5-tris(1,1-dimethylethyl) ester [Compound 2]

A solution of Intermediate 8 (200mg) in ethanol (40ml) containing glacial acetic acid (0.1ml) was hydrogenated at 20⁰C and atmospheric pressure over 10% palladium on carbon (40mg) for 3 days. The catalyst was removed by filtration and the filtrate was evaporated. The residue was chromatographed on silica gel (Merck silica gel 60, 230-400 mesh; 20g) eluting with a 5:1 mixture of light petroleum and ethyl acetate. From early fractions was eluted the title compound 2 (42mg); δ (CDCl₃) 0.80-0.95(m,6H,CH₃), 1.42,1.45(2s,18H,C(CH₃)₃), 1.51(s,9H,C(CH₃)₃), 3.86(2s,1H,OH), 4.69(s,1H,3H), 7.1-7.3(m,5H,aromatic protons). From later fractions was eluted title compound 1 (45mg); δ (CDCl₃) includes 0.8-0.95(m,6H,CH₃), 1.45,1.49(2s,18H,C(CH₃)₃), 1.60(s,9H,C(CH₃)₃),

- 45 -

2.10(2s,3H,OCOCH₃), 3.85(2s,1H,OH), 4.66(s,1H,3H), 4.81(m,1H,CHOCOCH₃), 7.1-7.3(m,5H,aromatic protons).

Example 1

[1S-[1 α (4R*,5S*),3 α ,4 β ,5 α]] 1-(4-Acetyloxy-5-methyl-3-methylene-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid

Intermediate 8 (200mg) was treated at 20⁰C with a solution of anhydrous hydrogen chloride in dioxane (6N; 0.61ml) for 18h then diluted with ether (20ml) and evaporated to dryness in vacuo at 40⁰C. Dilution with ether and evaporation was repeated twice to give a foam. Purification of the foam by preparative HPLC on a 1" spherisorb ODS-2 column eluting with 50% acetonitrile/water containing 0.15ml/L of concentrated sulphuric acid afforded the title compound (76mg); δ (CD₃OD) includes 0.86(d,3H,J=7.5Hz,CH₃), 2.10(s,3H,OCOCH₃), 4.95(s,1H,3H), 4.98 and 5.01(2s,2H,=CH₂), 5.09(d,1H,J=5.0Hz,CHOCOCH₃), 7.1-7.3(m,5H,aromatic protons); mass spectrum (thermospray negative) gave 505(M-H)⁻, 447 (M-AcO)⁻.

Example 2

[1S-[1 α (3R*S*,4S*,5S*),3 α ,4 β ,5 α]] 1-(4-Acetyloxy-3,5-dimethyl-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid

Title Compound 1 of Intermediate 9 (45mg) was treated at 20⁰C with a solution of anhydrous hydrogen chloride in dioxane (6N; 0.14ml) for 18h then diluted with ether (15ml) and evaporated to dryness in vacuo at 40⁰C. Dilution with ether and evaporation was repeated twice to afford a product which was purified by preparative HPLC on a 1" spherisorb ODS-2 column eluting with 50% acetonitrile/water containing 0.15ml/L conc. sulphuric acid to give the title compound (18mg); δ (CD₃OD) includes 0.8-0.95(m,6H,CH₃), 2.10,2.11(2s,3H,OCOCH₃), 4.93(s,1H,3H), 7.1-7.3(m,5H,aromatic protons); mass spectrum (thermospray negative) gave 507(M-H)⁻.

Example 3

- 46 -

[1S-[1 α (3R*S*,5R*),3 α ,4 β ,5 α]] 1-(3,5-Dimethyl-6-phenylhexyl)-4-hydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid

Title Compound 2 of Intermediate 9 (42mg) was treated at 20⁰C with a solution of anhydrous hydrogen chloride in dioxane (6N; 0.14ml) for 18h then diluted with ether (15ml) and evaporated to dryness in vacuo at 40⁰C. Dilution with ether and evaporation was repeated twice to afford a product which was purified by preparative HPLC on a 1" sphersorb ODS-2 column eluting with 50% acetonitrile/water containing 0.15ml/L conc. sulphuric acid to give the title compound (22mg); δ (CD₃OD) includes 0.8-0.95(m,6H,CH₃), 4.91(s,1H,3H), 7.1-7.3(m,5H,aromatic protons); mass spectrum, (thermospray negative) gave 449(M-H)⁻.

Example 4

Characteristics of IMI 332962

After 2-3 weeks growth at 25⁰C on oatmeal agar the colonies were smoke grey to mouse grey in colour (Rayner's Mycological Colour Chart, 1970; published by the Commonwealth Agricultural Bureaux) on both the surface and reverse of the colony.

Morphological observations of the strain grown at 25⁰C on oatmeal agar were made under an optical microscope. The fungus had no sexual reproductive stage but formed pycnidia, thereby placing it in the class Coelomycetes. The fungus produced rostrate pycnidia with loose hyphae and both aseptate and one-septate conidia. The conidia were approximately 5-9 x 1.5-3 μ M in dimensions (usually 7-9 x 1.502.5 μ M). Numerous multiseptate/multicellular, globose structures resembling chlamydospores or pycnidial initials were also observed. Distinct dictyochlamydospores were absent.

The isolate has been identified as a species of the genus Phoma, and the identity confirmed by the CAB International Mycological Institute.

Example 5

IN VITRO RESULTS

- 47 -

The ability of compounds of the invention to inhibit the enzyme squalene synthase was demonstrated using [2-¹⁴C] farnesylpyrophosphate as substrate under assay conditions similar to those described by S. A. Biller *et al* in J Medicinal Chemistry 31(10), 1869-1871 (1988). [¹⁴C] Squalene was separated from unreacted substrate on thin layer chromatography plates and determined by liquid scintillation counting. Inhibition of squalene synthase was quantified by incubating rat liver homogenate with various concentrations of the test compound in the presence of [2-¹⁴C] farnesylpyrophosphate. The concentration of compound giving 50% inhibition of [¹⁴C] squalene production in a 30 minute assay was taken as the IC₅₀ value.

In this test Example 1 had a mean (n=2) IC₅₀ value of 57nM.

Pharmaceutical Examples

In the following examples the term 'Active Ingredient' refers to a compound of the present invention, for example a compound of Examples 1 to 3 hereinabove.

Example 1 - Tablets

a) Active Ingredient	5.0mg
Lactose	95.0mg
Microcrystalline Cellulose	90.0mg
Cross-linked Polyvinylpyrrolidone	8.0mg
Magnesium Stearate	<u>2.0mg</u>
Compression Weight	200.0mg

The active ingredient, microcrystalline cellulose, lactose and cross-linked polyvinylpyrrolidone are sieved through a 500 micron sieve and blended in a suitable mixer. The magnesium stearate is sieved through a 250 micron sieve and blended with the active blend. The blend is compressed into tablets using suitable punches.

- 48 -

b)	Active Ingredient	5.0mg
	Lactose	165.0mg
	Pregelatinised Starch	20.0mg
	Cross-linked Polyvinylpyrrolidone	8.0mg
	Magnesium Stearate	<u>2.0mg</u>
	Compression weight	200.0mg

The active ingredient, lactose and pregelatinised starch are blended together and granulated with water. The wet mass is dried and milled. The magnesium stearate and cross-linked polyvinylpyrrolidone are screened through a 250 micron sieve and blended with the granule. The resultant blend is compressed using suitable tablet punches.

Example 2 - Capsules

a)	Active Ingredient	5.0mg
	Pregelatinised Starch	193.0mg
	Magnesium Stearate	<u>2.0mg</u>
	Fill weight	200.0mg

The active ingredient and pregelatinised starch are screened through a 500 micron mesh sieve, blended together and lubricated with magnesium stearate (meshed through a 250 micron sieve). The blend is filled into hard gelatin capsules of a suitable size.

b)	Active Ingredient	5.0mg
	Lactose	177.0mg
	Polyvinylpyrrolidone	8.0mg

- 49 -

Cross-linked Polyvinylpyrrolidone	8.0mg
Magnesium Stearate	<u>2.0mg</u>
Fill weight	200.0mg

The active ingredient and lactose are blended together and granulated with a solution of polyvinylpyrrolidone. The wet mass is dried and milled. The magnesium stearate and cross-linked polyvinylpyrrolidone are screened through a 250 micron sieve and blended with the granule. The resultant blend is filled into hard gelatin capsules of a suitable size.

Example 3 - Syrup

a)	Active Ingredient	5.0mg
	Hydroxypropyl Methylcellulose	45.0mg
	Propyl Hydroxybenzoate	1.5mg
	Butyl Hydroxybenzoate	0.75mg
	Saccharin Sodium	5.0mg
	Sorbitol Solution	1.0ml
	Suitable Buffers	qs
	Suitable Flavours	qs
	Purified Water	to 10.0ml

The hydroxypropyl methylcellulose is dispersed in a portion of hot purified water together with the hydroxybenzoates and the solution is allowed to cool to room temperature. The saccharin sodium, flavours and sorbitol solution are added to the bulk solution. The active ingredient is dissolved in a portion of the remaining water and added to the bulk solution. Suitable buffers may be added to control the pH in the region of maximum stability. The solution is made up to volume, filtered and filled into suitable containers.

- 50 -

Example 4 - Intranasal Solution

a) Preserved solution

	<u>% w/w</u>
Active Ingredient	0.1
Dextrose (Anhydrous)	5.0
Benzalkonium Chloride	0.02
Suitable buffers	qs
Purified Water	to 100

The active ingredient and dextrose are dissolved in a portion of the bulk solution. Suitable buffers may be added to control the pH in the region of maximum stability. The solution is made up to volume, filtered and filled into suitable containers.

Alternatively, the solution may be provided as a sterile unit dose presentation such that the preservatives are omitted from the formulation.

b) Unpreserved sterile solution

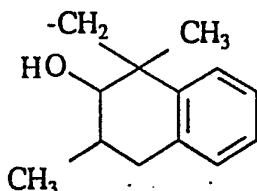
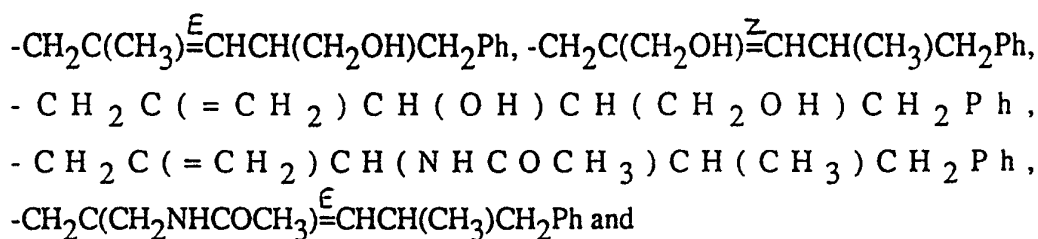
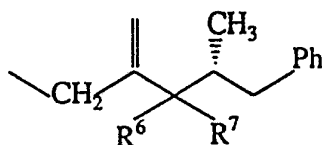
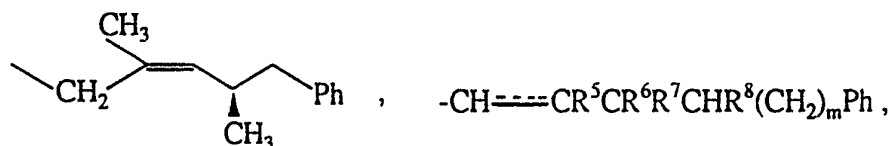
	<u>% w/w</u>
Active Ingredient	0.1
Dextrose (Anhydrous)	5.0
Suitable Buffers	qs
Purified Water	to 100

CLAIMS

1. Compounds having the formula (I)



wherein R^1 represents a group selected from



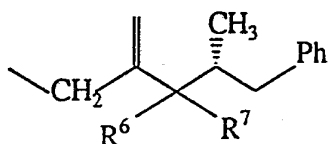
(where the dotted line represents the absence or presence of a single bond, R^5 represents a hydrogen atom or a hydroxyl, acyloxy, C_{1-6} alkoxy or C_{1-4} alkyl group, R^6 represents a hydrogen atom and R^7 represents a hydrogen atom or a hydroxyl, C_{1-6} alkoxy or acyloxy group or CR^6R^7 forms a group $\text{C}=\text{O}$, R^8 represents a hydrogen atom or a C_{1-4} alkyl group, R^9 represents a hydrogen atom or a methyl group, m represents 1 or 2 and n represents zero or 1);

- 52 -

R^2 , R^3 and R^4 may each independently represent a hydrogen atom or a methyl group; and salts thereof;

2. Compounds according to Claim 1 in which R^2 , R^3 and R^4 represent hydrogen atoms.

3. Compounds according to Claim 1 or Claim 2 in which R^1 represents $-\text{CH}-\text{CR}^5\text{CR}^6\text{R}^7\text{CHR}^8\text{CH}_2\text{Ph}$ or



4. Compounds according to any preceding claim in which within R^1 the group $-\text{CH}-\text{CR}^5-$ represents $-\text{CH}=\text{C}(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{OH})-$, $-\text{CH}_2\text{C}(=\text{O})-$, $-\text{CH}_2\text{CH}_2-$ or $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)-$.

5. Compounds according to any preceding claim in which within R^1 the group $-\text{CR}^6\text{R}^7-$ represents $-\text{CH}(\text{OH})-$, $-\text{C}(=\text{O})-$, $-\text{CH}_2-$ or $-\text{CH}(\text{OCOCH}_3)-$.

6. A compound according to any preceding claim for use in therapy.

7. A compound according to any preceding claim for use in the treatment of conditions where a lowering of the level of blood plasma cholesterol in animals, including humans, would be beneficial.

8. A compound according to any of Claims 1 to 6 for use in the treatment of fungal infections in a human or non-human animal patient.

9. A method of treatment of the human or non-human animal body to combat diseases associated with hypercholesterolemia and/or hyperlipoproteinemia or to

- 53 -

combat fungal diseases, which method comprises administering to said body an effective amount of a compound as claimed in any of Claims 1 to 6 which inhibits squalene synthase.

10. A pharmaceutical composition comprising a compound according to any of Claims 1 to 6 together with one or more carriers and/or excipients.

11. A pharmaceutical composition comprising an active amount of a compound as claimed in any of Claims 1 to 6 for use in the treatment of conditions where a lowering of the level of blood plasma cholesterol in animals, including humans, would be beneficial.

12. A pharmaceutical composition comprising an active amount of a compound as claimed in any of Claims 1 to 6 for use in the treatment of fungal infections in a human or non-human animal patient.

13. A pharmaceutical composition according to any one of Claims 10 to 12 in a form suitable for oral, buccal, topical, parenteral, implant, rectal, ophthalmic or genito-urinary administration or in a form suitable for administration by inhalation or insufflation.

14. A pharmaceutical composition according to any one of Claims 10 to 13 in unit dosage form.

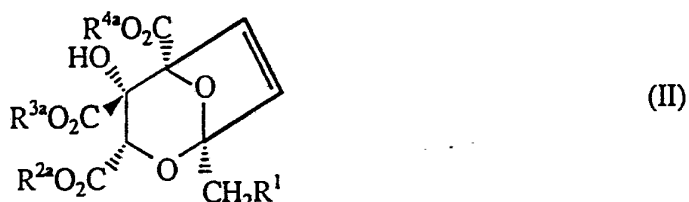
15. Use of a compound according to any of Claims 1 to 6 in the manufacture of a medicament for the treatment of hypercholesterolemia and/or hyperlipoproteinemia in a human or non-human animal patient.

16. Use of a compound according to any of Claims 1 to 6 in the manufacture of a medicament for the treatment of fungal infections in a human or non-human animal patient.

- 54 -

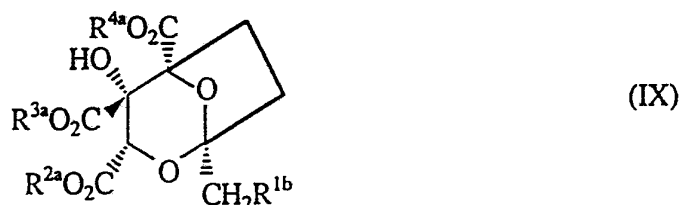
17. A process for the preparation of a compound as claimed in Claim 1 which comprises :

(A) hydrogenation of a compound of formula (II)

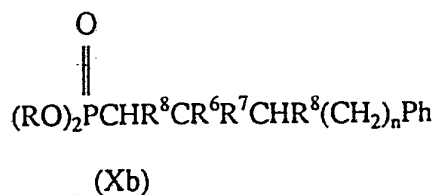
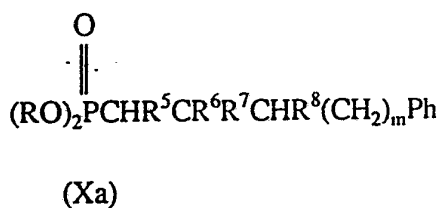


(wherein R¹ is as defined in Claim 1 and R^{2a}, R^{3a} and R^{4a} are protecting groups) in the presence of an appropriate metal catalyst, followed by removal of the protecting groups present;

(B) (in the preparation of compounds of formula (I) in which R¹ represents a group -CH=CR⁵CR⁶R⁷CHR⁸(CH₂)_mPh or a group -CH₂CR⁹=CR⁸CR⁶R⁷CHR⁸(CH₂)_nPh where R⁵ is hydrogen or C₁₋₄alkyl) reacting a compound of formula (IX)



(wherein R^{1b} represents CHO or CH₂COR⁹ as appropriate and R^{2a}, R^{3a} and R^{4a} are as defined for R², R³ and R⁴ or are protecting groups) with a compound of formula (Xa) or (Xb)



as appropriate (wherein R⁸ is as defined in Claim 1, R⁵ is hydrogen or C₁₋₄alkyl, CR⁶R⁷ forms a group C=O and R represents a C₁₋₆alkyl group, e.g. methyl or an

- 55 -

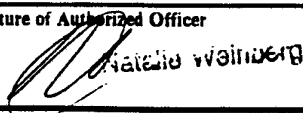
aryl group, e.g. phenyl) under Wadsworth-Emmons conditions, followed, where appropriate, by converting CR^6R^7 as a group $\text{C}=\text{O}$ to a group CHR^7 where R^7 is hydroxy, C_{1-6} alkoxy or acyloxy, and thereafter removing any protecting groups present; or

(C) converting a compound of formula (I) or a protected derivative thereof to a different compound of formula (I) or a protected derivative thereof, followed, if necessary, by the removal of any protecting groups present.

18. Compounds of formulae (II), (III), (IX), (XI), (XII), (XIII), (XIV) and (XV).
19. Compounds according to any of Claims 1 to 6 substantially as herein described.
20. Compositions according to any one of Claims 10 to 14 substantially as herein described.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/00015

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl.5	C 07 D 493/08	A 61 K 31/34
C 07 H 19/01	C 12 P 19/02	C 07 D 493/18
		C 12 P 17/18
		A 61 K 31/71 //
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl.5	C 07 D 493/00	C 12 P 17/00 C 07 H 19/00
	C 12 P 19/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	CH,A, 606071 (CIBA-GEIGY) 13 October 1978, see column 1 - column 2, line 23 ---	1,11,12
A	EP,A,0259087 (MERCK) 9 March 1988, see claims 1,9 ---	1,11,12
P,A	US,A,5026554 (K.F. BARTIZAI et al.) 25 June 1991, see column 1, line 50 - column 4, line 19 -----	1,11,12
¹⁰ Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
30-03-1992	06.05.92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	 Nathalie Waindberg	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers _____ because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 15 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claim numbers _____ because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers _____ because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims: _____
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: _____
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

EP 9200015

SA 54743

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 22/04/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CH-A- 606071	13-10-78	None	
EP-A- 0259087	09-03-88	US-A- 4837205	06-06-89
		CA-A- 1284497	28-05-91
		JP-A- 63068584	28-03-88
		US-A- 4943588	24-07-90
US-A- 5026554	25-06-91	EP-A- 0475706	18-03-92