



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07D 493/08		A1	(11) International Publication Number: WO 92/12158 (43) International Publication Date: 23 July 1992 (23.07.92)
(21) International Application Number: PCT/EP92/00016 (22) International Filing Date: 5 January 1992 (05.01.92) (30) Priority data: 9100424.2 9 January 1991 (09.01.91) GB 9117151.2 7 August 1991 (07.08.91) 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]; Glaxo Group Research Limited, Berkeley Avenue, Greenford, Middlesex UB6 0HE (GB). BIONDI, Stefano [IT/IT]; Glaxo SpA, Via Alessandro Fleming, 2, I-37100 Verona (IT). RAMSAY, Michael, Vincent, John [GB/GB]; LIVERMORE, David, George, Hubert [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 With international search report.
(54) Title: CYCLIC KETAL DERIVATIVES (Ia) (Ib) (a) (b)			
(57) Abstract Compounds are described of formula (Ia), (Ib), wherein R¹ represents a hydrogen atom, a hydroxyl group or a group selected from -OCOCH=CHCH(CH₃)(CH₂)₃CH₃, -OCOCH=CHC(CH₃)=CHCH(CH₃)CH₂CH₃ or -OCO-X-CH₂CH(CH₃)CH₂CH₃ [where X is -CH=CHCH(CH₃)-, -CH₂CH(OH)CH(CH₃)-, -CH=CHC(OH)(CH₃)-, -CH₂CH(OH)CH₂- or -CH₂CH₂CH(CH₃)-]; R² represents a hydroxyl group, a group -OCOR⁷ or a group -OCO₂R⁷ (where R⁷ is a group selected from C₁₋₈alkyl, aryl, arylC₁₋₄alkyl and C₃₋₈cycloalkyl); R³ represents a group selected from (a) (where R⁸ is a hydrogen atom or an acetyl group), -C(CH₃)=CHCH(CH₂R⁹)CH₂Ph (where R⁹ is a hydrogen or a hydroxyl group), -C(CH₂OH)=CHCH(CH₃)CH₂Ph, -C(=CH₂)CH(OH)CH(CH₂OH)CH₂Ph, -C(=CH₂)CH(NHCOCH₃)CH(CH₃)CH₂Ph, -C(CH₂NHCOCH₃)=CHCH(CH₃)CH₂Ph and (b); R⁴, R⁵ and R⁶ 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.			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
DE	Germany	MC	Monaco	TG	Togo
DK	Denmark			US	United States of America

- 1 -

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

SUBSTITUTE SHEET

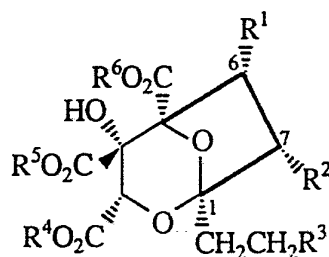
- 2 -

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.

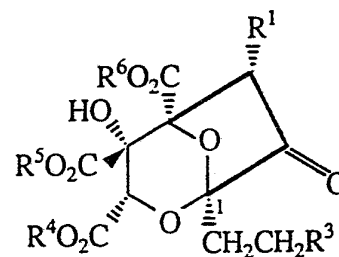
The biosynthesis of ergosterol, the major sterol component of fungal cell membranes, is analogous to that of cholesterol in mammals, including humans, and is thus mediated by the enzyme squalene synthase. Agents which act to inhibit the enzyme squalene synthase in fungal cells are therefore potential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



(Ia)



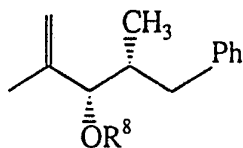
(Ib)

wherein R^1 represents a hydrogen atom, a hydroxyl group or a group selected from $-\text{OCOCH}=\text{CHCH}(\text{CH}_3)(\text{CH}_2)_3\text{CH}_3$, $-\text{OCOCH}=\text{CHC}(\text{CH}_3)=\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ or $-\text{OCO}-\text{X}-\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ [where X is $-\text{CH}=\text{CHCH}(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{OH})\text{CH}(\text{CH}_3)-$, $-\text{CH}=\text{CHC}(\text{OH})(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2-$ or $-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-$];

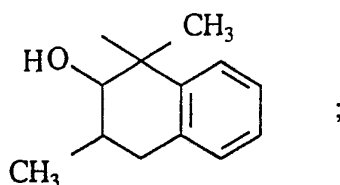
R^2 represents a hydroxyl group, a group $-\text{OCOR}^7$ or a group $-\text{OCO}_2\text{R}^7$ (where R^7 is a group selected from C_{1-8} alkyl, aryl, aryl C_{1-4} alkyl and C_{3-8} cycloalkyl);

R^3 represents a group selected from

- 3 -



(where R^8 is a hydrogen atom or an acetyl group), $-C(CH_3)=CHCH(CH_2R^9)CH_2Ph$
 (where R^9 is a hydrogen or a hydroxyl group), $-C(CH_2OH)=CHCH(CH_3)CH_2Ph$,
 $-C(=CH_2)CH(OH)CH(CH_2OH)CH_2Ph$,
 $-C(=CH_2)CH(NHCOCH_3)CH(CH_3)CH_2Ph$,
 $-C(CH_2NHCOCH_3)=CHCH(CH_3)CH_2Ph$ and



R^4 , R^5 and R^6 may each independently represent a hydrogen atom or a methyl group; and salts thereof;

The term aryl as a group or part of a group means phenyl or phenyl substituted by one or more moieties including for example halogen atoms, hydroxyl, C_{1-3} alkyl and C_{1-3} alkoxy groups.

The term alkyl within R^2 means a straight or branched alkyl chain. Examples of suitable alkyl groups include methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, n-hexyl and n-heptyl.

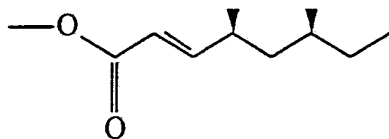
Examples of R^2 as C_{3-8} cycloalkyl include cyclopentyl and cyclohexyl.

It will be appreciated that R^2 in formula (I) may contain one or more chiral centres. It is to be understood that the present invention is intended to cover all optical isomers, including diastereoisomers, of such compounds of formula (I).

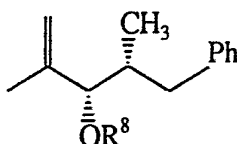
Compounds of formulae (Ia) and (Ib) in which R^4 , R^5 and R^6 represents hydrogen atoms and physiologically acceptable cations are generally preferred.

R^1 preferably represents a group

- 4 -



R^3 preferably represents a group



(where R^8 is a hydrogen atom or an acetyl group).

Compounds of formula (Ia) represent a preferred group of compounds of the present invention.

It is to be understood that this invention covers any combination of the abovementioned preferences.

Compounds of formulae (Ia) and (Ib) in which R^1 represents a hydroxyl group are particularly useful as intermediates for the preparation of related structures having squalene synthase inhibitory activity.

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

- 5 -

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 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.

SUBSTITUTE SHEET

- 6 -

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 the invention to inhibit cholesterol biosynthesis may be demonstrated by measuring inhibition from [¹⁴C]-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

SUBSTITUTE SHEET

- 7 -

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, 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

SUBSTITUTE SHEET

- 8 -

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.

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

SUBSTITUTE SHEET

- 9 -

(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 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

- 10 -

components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

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 (Ia) or (Ib) or a physiologically acceptable salt thereof or a pharmaceutical composition comprising a compound of formula (Ia) or (Ib) 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 (Ia) or (Ib) or a physiologically acceptable salt thereof or a pharmaceutical composition comprising a compound of formula (Ia) or (Ib) 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 (Ia) or (Ib) 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 (Ia) or (Ib) 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.

SUBSTITUTE SHEET

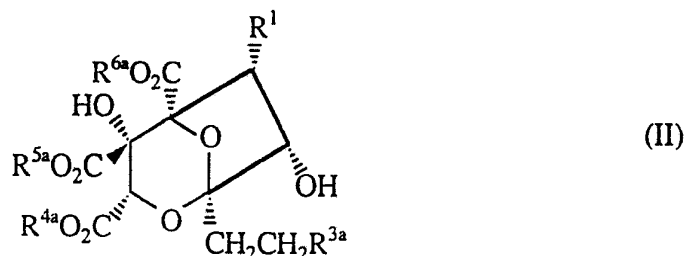
- 11 -

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 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 (Ia) or (Ib) 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 (Ia) in which R^2 represents a group $-\text{OCOR}^7$ or $-\text{OCO}_2\text{R}^7$ comprises reacting a compound of formula (II)



(wherein R^1 is as defined previously, R^{3a} is as defined for R^3 above or is a protected derivative thereof and R^{4a} , R^{5a} and R^{6a} are protecting groups) under conventional conditions for ester or carbonate formation followed by removal of the protecting groups present. Thus, for example, the reaction may conveniently be effected by treating a compound of formula (II) with a compound $R^7\text{COHal}$ or $R^7\text{OCOHal}$ as appropriate, wherein Hal represents a halogen atom such as chlorine or bromine.

The reaction with a compound $R^7\text{COHal}$ or $R^7\text{OCOHal}$ may conveniently be effected in the presence of 4-dimethylaminopyridine with or without a suitable base

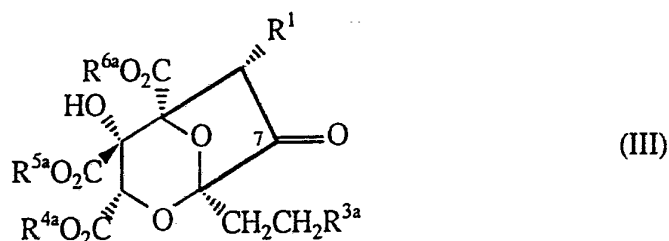
- 12 -

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).

It will be appreciated that when R^1 in the compounds of formula (II) represents a hydroxyl group this hydroxyl group will also be susceptible to ester and carbonate formation. Thus, in the preparation of compounds of formula (Ia) in which R^1 is a hydroxyl group it may be appropriate to have protected the R^1 hydroxyl group in compounds of formula (II) or utilise a compound of formula (II) in which R^1 is an acyloxy group as defined in formula (Ia) above, and following the reaction to form the 7-position ester or carbonate remove the protecting group or deacylate as appropriate to provide the desired compound of formula (Ia) in which R^1 is hydroxyl.

Compounds of formula (Ia) in which R^2 represents an acetoxy group may conveniently be prepared from compounds of formula (II) by reaction with acetic anhydride followed by removal of the protecting groups present. The acetylation reaction may conveniently be effected in the presence of a suitable base such as a tertiary amine (e.g. triethylamine) in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane).

Another process (B) for the preparation of compounds of formula (Ia) in which R^2 represents a hydroxyl group comprises reducing compounds of formula (III)



(wherein R^1 and R^{3a} - R^{6a} are as defined previously) with a suitable reducing agent, followed by removal of the protecting groups present. Thus, for example, the reduction may conveniently be carried out using a borohydride such as lithium

- 13 -

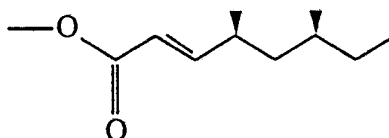
triethylborohydride in a solvent such as an ether (e.g. tetrahydrofuran) or sodium borohydride, optionally in the presence of a suitable metal halide (e.g. cerium trichloride), in a solvent such as an alcohol (e.g. methanol) or a mixture of an alcohol with another solvent such as an ether (e.g. tetrahydrofuran) at a temperature below 20°C (e.g. -70°C 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°C 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°C to 0°C).

If it is desired to prepare compounds of formula (Ia) in which R¹ represents a hydroxyl group from compounds of formula (III) then R¹ in formula (III) conveniently represents an acyloxy group as defined in formula (Ia) above which is converted to a hydroxyl group (under deacylation condition described hereinafter) following the reduction reaction and prior to or subsequent to the removal of the protecting groups.

Compounds of formula (Ib) may conveniently be prepared from compounds of formula (III) by removal of the protecting groups present.

Another 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.

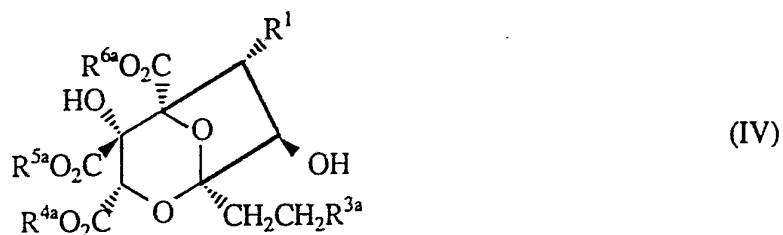
Compounds of formula (III) in which R¹ represents a hydrogen atom may be prepared from corresponding compounds of formula (III) in which R¹ represents



by reaction with aqueous chromous chloride in a suitable solvent such as a ketone (e.g. acetone), conveniently at about room temperature.

Compounds of formula (III) may conveniently be prepared from compounds of formula (IV)

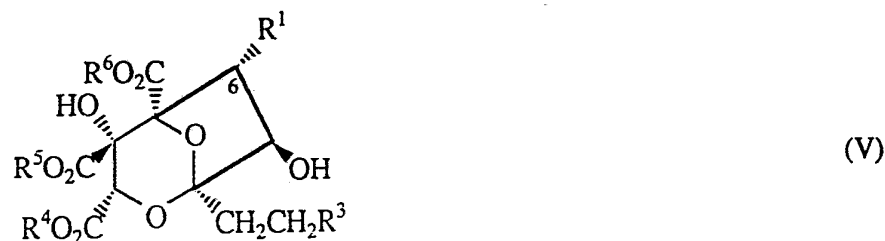
- 14 -



(wherein R^1 and R^{3a} - R^{6a} are as defined previously) by treating (IV) 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 (II) in which R^1 represents a hydroxyl group may also be prepared from compounds of formula (III) in which R^1 represents a hydroxyl group according to the reduction procedure described above.

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



(wherein R^1 and R^3 - R^6 are as defined in formula (I) above) by standard carboxylic acid protection methods with hydroxyl group protection within R^3 where appropriate.

Suitable carboxylic acid protecting groups for R^{4a} , R^{5a} and R^{6a} and hydroxyl protecting groups for R^{3a} 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

- 15 -

aralkyl groups such as 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). 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.

Esterification of carboxylic acids of formula (V) 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 (V) 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 (V) 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 as appropriate according to suitable acylation and deacylation methods. Suitable acylation methods are described hereinafter. Deacylation may conveniently be effected by base-catalysed hydrolysis 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

SUBSTITUTE SHEET

- 16 -

be carried out using 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. The fermentation process comprises cultivating a microorganism capable of producing one or more of the compounds of formula (V). Thereafter the desired compound from the culture may be isolated and, if desired, acylated or deacylating 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 9 hereinafter.

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

- 17 -

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

- 18 -

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

- 19 -

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 macroreticular 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 (V) 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.

SUBSTITUTE SHEET

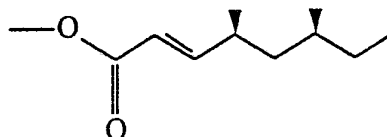
- 20 -

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.

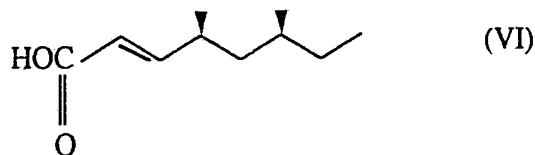
Where a product of the fermentation process is obtained in the form solution in an organic solvent, for example after purification by chromatography, the solvent may be removed by conventional procedures, e.g. by evaporation, to yield the required compound. If desired, the compound may be further purified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

Acylation to provide a compound of formula (V) in which R^1 represents an acyloxy group as defined in formulae (Ia) and (Ib) above may be effected by treating a corresponding compound of formula (V) in which R^1 is a hydroxyl group or a protected derivative thereof with a suitable acylating agent such as a carboxylic acid or an activated derivative thereof such as an acyl halide, e.g. an acyl chloride, under conventional esterification conditions followed by removal of any protecting groups present.

Thus, for example a compound of formula (V) in which R^1 represents



may be prepared by treating a compound of formula (V) in which R^1 is a hydroxyl group or a protected derivative thereof with an acid of formula (VI)



or an activated derivative thereof such as the corresponding acid chloride.

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

- 21 -

(e.g. calcium carbonate) in a solvent such as a halogenated hydrocarbon (e.g. dichloromethane).

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

Salts of compounds of formulae (Ia) and (Ib) may be conveniently formed by treating a compound of formula (Ia) or (Ib) with an appropriate salt or base. Thus, for example, salts may conveniently be prepared by treating a compound of formula (Ia) or (Ib) 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) and (III) are novel intermediates and form a further aspect of the present invention.

Isomeric 7- α -(4,6-dimethyl-2-octenoate) compounds of Examples 3, 4 and 5 hereinafter are also novel compounds which act to inhibit the enzyme squalene synthase in mammalian and/or fungal cells. These compounds and salts thereof 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)

- 22 -

(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
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 :

- 23 -

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

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.

- 24 -

(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 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.

- 25 -

(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 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 -20⁰C. 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 25⁰C 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.

SUBSTITUTE SHEET

- 26 -

(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 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

- 27 -

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.

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 deuterio-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 deuterio-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*),7B]] 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-dimethyl-ethyl) ester

- 28 -

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 (7.21ml) 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 (200ml) and washed with brine (3x100ml). The organic phase was dried (MgSO₄) 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; $\nu_{\max}$ (CHBr₃) ca 3400-3600 (OH), 1755 (ester C=O), 1730 (ester C=O) and 1250cm⁻¹ (ester C=O); δ (CDCl₃) includes 1.43(s, Me₃C-), 1.48(s, Me₃C-), 1.60(s, Me₃C-), 2.08(s, CH₃CO₂-), 2.93(d, J=3Hz, 7-OH), 4.00(broad s, 7-H), 4.08(s, 4-OH), 4.95(bs, C=CH₂), 5.05(s, 3-H), 5.11(d, J=5Hz, CH₃CO₂CH), 5.77(d, J=15Hz, CH=CH.CHMe), 6.01(d, J=2Hz, 6-H), 6.91(dd, J=15Hz, 7Hz, CH=CH.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 20⁰C 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 (CDCl₃) includes 0.80(m, 9H, CH₃), 1.43(s, 18H, C(CH₃)₃), 1.65(s, 9H, C(CH₃)₃), 2.10(s, OCOCH₃), 4.22(s, OH), 4.69(s, 3H), 4.99, 5.01(2s, =CH₂), 5.11(d, 1H, CHOCOCH₃),

- 29 -

5.76(d,1H,J=15Hz,CH=CHCO), 6.42(s,1H,6H), 6.95(dd,1H,J=15 and 8Hz,CH=CHCO), 7.1-7.3(m,5H,aromatic protons).

Intermediate 4

[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

- 30 -

to give the title compound and the corresponding 7 β -hydroxy isomer in the approximate ratio of 8:1 (400mg); δ (CDCl_3) includes 0.8-0.9 (m, 9H, CH_3), 1.45 (s, 18H, $\text{C}(\text{CH}_3)_3$), 1.63 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.10 (s, OCOCH_3), 4.95 (m, 2H, $=\text{CH}_2$), 5.81 (d, 1H, $J=15\text{Hz}$, $\text{CH}=\text{CHCO}$), 6.52 (d, 1H, $J=6.3\text{Hz}$, 6H), 6.85-7.02 (m, $\text{CH}=\text{CHO}$), 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 (CDCl_3) includes 5.16 (obscured d, $J=6\text{Hz}$, CHOAc) and 6.84-6.89 (m, $\text{CH}=\text{CHCO}$).

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-

- 31 -

dimethyl-2-octenoate) and the 7 β -hydroxy isomer in the approximate ratio of 9:1:10 (460mg).

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 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) and the 7 β -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 7 β -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

- 32 -

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 0.85(d,3H,J=7.3Hz, CH_3), 1.45(s,9H, $\text{C}(\text{CH}_3)_3$), 1.5(s,9H, $\text{C}(\text{CH}_3)_3$), 1.60(s,9H, $\text{C}(\text{CH}_3)_3$), 2.16(s,3H, OCOCH_3), 3.90(s, OH), 4.22(m,1H,7H), 4.47(s,1H,3H), 4.91 and 5.05(2s,2H, $=\text{CH}_2$), 5.11(m,1H,6H), 5.18(d,1H,J=4.8Hz, CHOCOCH_3), 7.1-7.3(m,5H,aromatic protons).

Intermediate 6

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

A solution of Intermediate 3 (2.09g) in deoxygenated acetone (100ml) was treated under nitrogen with an aqueous solution of chromous chloride (0.85M; 80ml) over 2h. The mixture was diluted with acetone (40ml) and further treated with chromous chloride solution (0.85M; 40ml). After 1h the mixture was diluted with ethyl acetate (500ml). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (200ml). The combined organic solutions were washed with brine (4x100ml), dried and chromatographed on silica gel (Merck 7734; 100g) eluting with ethyl acetate : cyclohexane (1:9 increasing to 1:4) to give the title compound (863mg); δ (CDCl_3) includes 0.80 (d,J=7Hz, CH_3), 1.44, 1.51, 1.58 each (s, (CH_3)₃C), 2.10 (s,AcO), 2.68 and 3.45 each (d,J=19Hz, CH_2CO), 4.13 (s,4-OH), 4.67 (s,3-H), 4.99 (brs,C= CH_2), 5.10 (d,J=5Hz, CHOAc), 7.10-7.30 (m,C₆H₅).

Example 1

[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

Intermediate 5 (110mg) was treated at 20°C with a solution of anhydrous hydrogen chloride in dioxane (6N; 0.33ml). The solution was kept for 18h in the absence of moisture then diluted with ether (20ml) and evaporated to dryness in

- 33 -

vacuo at 40°C. Ether dilution and evaporation was repeated twice to yield a foam. Purification was carried out by preparative HPLC on a 1" spherisorb ODS-2 column eluting with 40% acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to give the title compound (55mg); δ (CD₃OD) includes 0.85(d,3H,J=7.3Hz,CH₃), 2.10(s,3H,OCOCH₃), 4.37(d,1H,J=7.5Hz,7H), 4.98 and 5.04(2s,2H,=CH₂), 5.11(d,1H,J=6.8Hz,CHOCOCH₃), 5.35(d,1H,J=7.5Hz,6H), 7.1-7.3(m,5H,aromatic protons); analytical high performance liquid chromatography (spherisorb ODS-2 column) eluting with 35% acetonitrile in water containing 0.15ml/L conc. H₂SO₄ gave a retention time of 3.78min.

Example 2

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

Intermediate 6 (125mg) was dissolved in anhydrous hydrogen chloride solution in dioxan (6M; 2.5ml) and the mixture was stood at 20°C for 7.5h. The solvent was then evaporated under reduced pressure and the residue was purified by reverse-phase HPLC (1 inch Spherisorb ODS-2, flow rate 15ml/min eluting with 40% increasing to 95% acetonitrile - water containing 0.15ml concentrated sulphuric acid/L). Appropriate fractions were combined, the acetonitrile was removed by evaporation under reduced pressure and the aqueous solution was extracted with ethyl acetate (x3), washed with brine (x2), dried and evaporated to dryness to give the title compound (47mg); δ (CD₃OD) includes 0.84 (d,J=7Hz,CH₃), 2.10 (s,AcO), 2.85 and 3.66 each (d,J=19Hz,CH₂CO), 4.9 and 5.00 each (s,C=CH₂), 5.05 (d,J=5Hz,CHOAc), 7.1-7.3 (m,C₆H₅); approximate molecular weight 520.5; -FAB mass spectrometry [M-H]⁻519.

Example 3

[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), 7-methylcarbonate

- 34 -

To a solution of Intermediate 4 as an 8:1 mixture with the corresponding 7 β -hydroxy isomer (200mg) in dry dichloromethane (20ml) under nitrogen was added methyl chloroformate (29mg) and 4-N,N-dimethylaminopyridine (37mg). After stirring the mixture at 20 °C for 22h further quantities of methyl chloroformate (7mg) and 4-N,N-dimethylaminopyridine (8.6mg) were added and the reaction allowed to proceed for 3h. The mixture was poured into saturated aqueous ammonium chloride (30ml) and the organic material extracted with ether (3 x 50ml) and the ethereal solution then washed sequentially with water (30ml), 5% aqueous sodium bicarbonate solution (30ml) and brine (30ml). The dried organic phase was evaporated to an oil which was purified by chromatography on silica gel (Merck silica gel 60, 230-400 mesh, 10g). The column was eluted with a mixture (4:1) of light petroleum and ethyl acetate and the resulting material was dissolved in a solution of anhydrous hydrogen chloride in dioxane (6.9M, 2.5ml) and then set aside under a nitrogen atmosphere for 32h at 20 °C. Removal of solvents gave a yellow wax which was separated by preparative HPLC on a Spherisorb ODS-2 column eluting with 67% acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to provide the title compound (50mg); retention time 6.02 min (Spherisorb ODS-2 analytical column, 65% acetonitrile in water containing 0.15ml of conc. H₂SO₄); δ (CD₃OD) includes 2.10 (s, 3H, OCOCH₃), 2.36 (dd, 1H, J=15 and 10Hz, HCHPh), 2.71 (dd, 1H, J=15 and 5Hz, HCHPh), 3.64 (s, 3H, OCO₂CH₃), 4.98, 5.00 and 5.02 (3s, each 1H, =CH₂ and CHCO₂H), 5.07 (d, 1H, J=5Hz, CH=CHCO), 6.69 (d, 1H, J=6Hz, CHOCO.CH=CH), and 6.82 (dd, 1H, J=15 and 8Hz, CH=CHO). The less mobile component (16mg) was [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, 6-methylcarbonate, 7-(4,6-dimethyl-2-octenoate); retention time 7.32min (Spherisorb ODS-2 analytical column, 65% acetonitrile in water containing 0.15ml of conc. H₂SO₄); δ (CD₃OD) includes 2.08 (s, 3H, OCOCH₃), 2.36 (dd, 1H, J=15 and 10Hz, HCHPh), 2.69 (dd, 1H, J=15 and 5Hz; HCHPh), 3.68 (s, 3H, OCO₂CH₃), 4.95, 4.97 and 4.99 (three singlets, each 1H, =CH₂ and CHCO₂H), 5.06 (d, 1H, J=5Hz, CHOCOCH₃), 5.76 (d, 1H, J=15Hz, CH=CHCO), 5.82 (d, 1H,

- 35 -

J=6Hz, $\underline{\text{CH}}\text{OCOCH}=\text{CH}$), 6.51 (d, 1H, J=6Hz, $\underline{\text{CH}}\text{OCO}_2\text{CH}_3$), and 6.87 (dd, 1H, J=15 and 8Hz, $\underline{\text{CH}}=\text{CHCO}$).

Example 4

[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, 6-phenoxyacetate, 7-(4,6-dimethyl-2-octenoate)

To a solution of Intermediate 4 as a 9:1:10 mixture with the corresponding 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) and 7 β -hydroxy isomers (380mg) in dichloromethane (25ml) under a nitrogen atmosphere was added phenoxyacetyl chloride (123mg) and 4-N,N-dimethylaminopyridine (88mg). After 32h at 20°C the mixture was poured into aqueous ammonium chloride solution and was then extracted with dichloromethane. The organic phase was washed successively with 1M hydrochloric acid (50ml) and a saturated aqueous solution of sodium bicarbonate (50ml) and was then dried and evaporated to an oil which was purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 40g). Elution of the column with a mixture (4:1) of petroleum ether and ethyl acetate gave a gum (370mg). A solution of this material (360mg) in anhydrous hydrogen chloride/dioxane (6.9M, 4ml) was set aside at 20°C under a nitrogen atmosphere for 40h. The solvents were evaporated and the residue purified by preparative HPLC on a Spherisorb ODS-2 column eluting with 65% acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to give the title compound (30mg); retention time 14.1 min (Spherisorb ODS-2 analytical column, 75% acetonitrile in water with 0.15ml/L of conc. H_2SO_4); δ (CD_3OD) includes 2.08 (s, 3H, $\text{OCO}\underline{\text{CH}}_3$), 2.36 (dd, 1H, J=14 and 9Hz, $\underline{\text{HCHPh}}$), 2.70 (dd, 1H, J=14 and 6Hz, $\text{HCH}\underline{\text{Ph}}$), 4.50 (d, 1H, J=16Hz, $\underline{\text{HCHOPh}}$), 4.59 (d, 1H, J=16Hz, $\text{HCH}\underline{\text{OPh}}$), 4.96, 4.98 and 5.06 (three singlets, each 1H, $=\text{CH}_2$ and $\underline{\text{CHCO}}_2\text{H}$), 5.07 (d, 1H, J=6Hz, $\underline{\text{CH}}\text{OCOCH}_3$), 5.75 (d, 1H, J=6Hz, $\underline{\text{CH}}\text{OCOCH}=\text{CH}$), 5.81 (dd, 1H, J=15 and 1Hz, $\text{CH}=\underline{\text{CHCO}}$), 6.84 (partially obscured d, 1H, J=6Hz, $\underline{\text{CH}}\text{OCOCH}_2\text{OPh}$) and 6.91 (dd, 1H, J=15 and 8Hz, $\underline{\text{CH}}=\text{CHCO}$).

- 36 -

Example 5

[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), 7-acetate

To a solution of Intermediate 4 as a 9:1:1 mixture with the corresponding 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) and 7 β -hydroxy isomers (170mg) in dry dichloromethane (6ml) under nitrogen was added acetyl chloride (24mg) and 4-N,N-dimethylaminopyridine (40mg). The mixture was stirred at 20°C for 16h and was then diluted with ethyl acetate (30ml) and the organic phase washed sequentially with 1M hydrochloric acid (5ml) and 5% aqueous sodium bicarbonate (2 x 5ml). Evaporation of the dried organic layer gave an oil which was purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 10g) eluting with a mixture (4:1) of light petroleum and ethyl acetate. A solution of this material (110mg) in anhydrous hydrogen chloride/dioxane (6.8M, 2ml) was set aside at 20°C under a nitrogen atmosphere for 40h. The mixture was evaporated and the residue separated by preparative HPLC on a Spherisorb ODS-2 column eluting with 65% acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to give the title compound (28mg); retention time 14.7 min (Spherisorb ODS-2 analytical column, 65% acetonitrile in water containing 0.15ml/L of conc. H₂SO₄); δ (CD₃OD) includes 1.92 (s, 3H, OCOCH₃), 2.10 (s, 3H, OCOCH₃), 2.40 (dd, 1H, J=15 and 9Hz, HCHPh), 2.70 (dd, 1H, J=15 and 5Hz, HCHPh), 4.98, 4.99 and 5.01 (three singlets, each 1H, =CH₂ and and CHCO₂H), 5.08 (d, 1H, J=5Hz, CHOCOCH₃), 5.72 (d, 1H, J=6Hz, 7 α -CHOAc), 5.79 (dd, 1H, (dd, 1H, J=15 and 8Hz; CH=CHCO).

The less mobile component (15 mg) was [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, 6-acetate, 7-(4,6-dimethyl-2-octenoate) retention time 16.6 min (Spherisorb ODS-2 analytical column, 65% acetonitrile in water containing 0.15ml/L of conc. H₂SO₄); δ (CD₃OD) includes 1.95 (s, 3H, OCOCH₃), 2.10 (s, 3H, OCOCH₃), 2.40 (dd, 1H, J=15 and 9Hz; HCHPh), 2.70 (dd, 1H, J=15 and 5Hz, HCHPh), 4.95, 4.96

- 37 -

and 5.03 (three singlets, each 1H, =CH₂ and CHCO₂H), 5.07 (d, 1H, J=15Hz, CHOCOCH₃), 5.74 (d, 1H, J=6Hz, CHOCOCH=CH), 5.82 (d, 1H, J=15Hz, CH=CHCO), 6.64 (d, 1H, J=6Hz, 6 α .CHOCOCH₃), 6.90 (dd, 1H, J=15 and 8Hz, CH=CHCO).

Example 6

[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), 7-phenylcarbonate

To a solution of Intermediate 4 as a 9:1 mixture with the corresponding 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) isomer (200mg) in dry dichloromethane (15ml) under a nitrogen atmosphere was added phenyl chloroformate (47mg) and 4-N,N-dimethylaminopyridine (40mg). After 16h at 20° C further quantities of phenyl chloroformate (24mg) and 4-N,N-dimethylaminopyridine (20mg) were added and the reaction then allowed to proceed for 60h. The mixture was worked up for neutral material which was purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 20g). The column was eluted with a 7:3 mixture of petroleum ether and ethyl acetate and the resulting material (120mg) was dissolved in a solution of anhydrous hydrogen chloride in dioxane (6.8M, 4ml) and the mixture set aside under a nitrogen atmosphere at 20° for 36h. After concentrating the mixture, it was then extracted with ethyl acetate and the organic phase then washed with brine. Evaporation of the dried ethyl acetate solution gave a residue which was purified by preparative HPLC on a Spherisorb ODS-2 column eluting with an 80% solution of acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to give the title compound which was isolated as a freeze dried solid (60mg); retention time 6.45 min (Spherisorb ODS-2 analytical column, 90% acetonitrile in water containing 0.15ml/L of conc. H₂SO₄); δ (CD₃OD) includes 2.10 (s, 3H, OCOCH₃), 2.36 (dd, 1H, J=15 and 9Hz, HCHPh), 2.72 (dd, 1H, J=15 and 5Hz; HCHPh), 5.04, 5.06 and 5.08 (three singlets, each 1H, =CH₂ and CHCO₂H), 5.12 (d, 1H, J=5Hz, CHOCOCH₃), 5.72 (d, 1H, J=6Hz, CHOCO₂Ph),

- 38 -

5.86 (dd, 1H, J=15 and 1Hz, CH=CHCO), 6.67 (d, 1H, J=6Hz, CHOCOCH=CH), 6.89 (dd, 1H, J=15 and 8Hz, CH=CHCO).

Example 7

[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), 7-hexanoate

To a solution of Intermediate 4 as a 9:1 mixture with the corresponding 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) isomer (200mg) in dry dichloromethane (10ml) under a nitrogen atmosphere was added hexanoyl chloride (47mg) and 4-N,N-dimethylaminopyridine (43mg). After 14h at 20°C further quantities of hexanoyl chloride (14mg) and 4-N,N-dimethylaminopyridine (13mg) were added and the reaction allowed to proceed for 2h. The mixture was then poured into 2M hydrochloric acid/dichloromethane and the organic phase washed with saturated aqueous bicarbonate solution, dried and evaporated. The residue was purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 10g) eluting with a 3:1 mixture of light petroleum and ethyl acetate and this material (170mg) was dissolved in a solution of anhydrous hydrogen chloride in dioxane (6.8M, 2.5ml) and the solution set aside under a nitrogen atmosphere at 20°C for 40h. The mixture was then concentrated and the concentrate purified by preparative HPLC on a Spherisorb ODS-2 column eluting with a 90% solution of acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to give the title compound which was isolated as a freeze-dried solid (50mg); retention time 8.46 min (Spherisorb ODS-2 analytical column, 85% acetonitrile in water containing 0.15ml/L conc. (H₂SO₄); δ (CD₃OD) includes 2.10 (s, 3H, OCOCH₃), 2.39 (dd, 1H, J=15 and 9Hz, HCHPh), 2.71 (dd, 1H, J=15 and 5Hz, HCHPh), 4.98, 5.00 and 5.05 (three singlets, each 1H, =CH₂ and CHCO₂H), 5.07 (d, 1H, J=5Hz, CHOCOCH₃), 5.74 (d, 1H, J=6Hz, CHOCOC₅H₁₁), 5.77 (dd, 1H, J=15 and 1Hz, CH=CHCO), 6.57 (d, 1H, J=6Hz, CHOCOCH=CH) and 6.85 (dd, 1H, J=15 and 8Hz, CH=CHCO).

Example 8

- 39 -

[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), 7-n-hexylcarbonate

To a solution of Intermediate 4 as a 9:1 mixture with the corresponding 6 α -hydroxy-7 α -(4,6-dimethyl-2-octenoate) isomer (200mg) in dichloromethane (10ml) under a nitrogen atmosphere was added n-hexylchloroformate (57.5mg) and 4-N,N-dimethylaminopyridine (43mg). After 14h at 20 °C further quantities of n-hexylchloroformate (19mg) and 4-N,N-dimethylaminopyridine (14mg) were added and the reaction allowed to proceed for 2h. The mixture was then quenched with 2M hydrochloric acid and was extracted with dichloromethane. The organic phase was washed with saturated aqueous sodium bicarbonate solution, dried and concentrated and the concentrate purified by chromatography over silica gel (Merck silica gel 60, 230-400 mesh, 10g) eluting with 3:1 mixture of petroleum spirit and ethyl acetate. This material (180mg) was dissolved in a solution of anhydrous hydrogen chloride in dioxane (6.8M, 3ml) and the reaction mixture set aside under a nitrogen atmosphere at 20 °C for 36h. The solution was then concentrated and the concentrate purified by preparative HPLC on a Spherisorb ODS-2 column eluting with 85% acetonitrile in water containing 0.15ml/L of concentrated sulphuric acid to give the title compound which was isolated as a freeze-dried solid (50mg); retention time 10.8 min (Spherisorb ODS-2 analytical column, 85% acetonitrile in water containing 0.15ml/L conc. (H₂SO₄); δ (CD₃OD) includes 2.09 (s, 3H, OCOCH₃), 2.36 (dd, 1H, J=15 and 9Hz, HCHPh), 2.73 (dd, 1H, J=15 and 5Hz, HCHPh), 3.96 (m, 1H, O.HCHC₅H₁₁), 4.03 (m, 1H, O.HCHC₅H₁₁), [5.00 (s, 2H) and 5.02 (s, 1H) =CH₂ and CHCO₂H], 5.09 (d, 1H, J=5Hz, CHOCOCH₃), 5.61 (d, 1H, J=6Hz, CHOCO₂C₆H₁₃), 5.75 (dd, 1H, J=15 and 1Hz, CH=CHCO), 6.65 (d, 1H, J=6Hz, CHOCOCH=CH) and 6.85 (dd, 1H, J=15 and 8Hz, CH=CHCO).

Example 9

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

- 40 -

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 1.5-3µM in dimensions (usually 7-9 x 1.502.5µ 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 10

IN VITRO RESULTS

(1) 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 the title compounds in Examples 1, 2, 4, 5 and 6 had IC₅₀ values of less than 100nM.

(2) The in vitro evaluation of the antifungal activity of compounds of the invention was performed by determining the minimum concentration (MIC) of the test compound at which growth of the particular microorganism in a suitable

- 41 -

medium failed to occur. In practice, a series of agar plates, each having the test compound incorporated at a particular concentration, was inoculated with a standard culture of a clinically relevant pathogen, for example Candida albicans, and each plate was then incubated at 37⁰C for 24 to 48 hours depending on the pathogen. The plates were then examined for the presence or absence of growth of the fungus and the appropriate MIC was noted.

In this test the title compounds in Examples 4, 6, 7 and 8 had MICs in the range of 2 to 16 µg/ml against a variety of clinically relevant pathogens.

Pharmaceutical Examples

In the following examples the term 'Active Ingredient' refers to a compound of the present invention, for example a compound described in Examples 1 to 8 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.

b)	Active Ingredient	5.0mg
----	-------------------	-------

- 42 -

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

- 43 -

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.

Example 4 - Intranasal Solution

- 44 -

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.

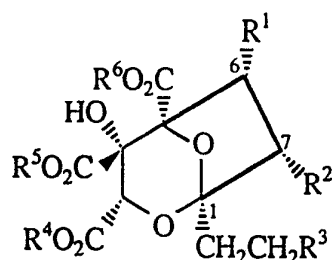
Alternatively, the solution may be provided as a sterile unit dose presentation such that the preservatives are omitted from the formulation.

b) Unpreserved sterile solution

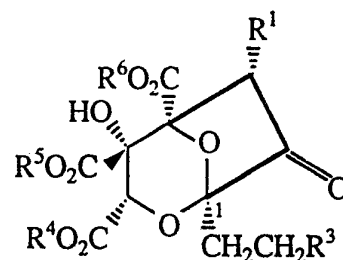
	<u>% w/w</u>
Active Ingredient	0.1
Dextrose (Anhydrous)	5.0
Suitable Buffers	qs
Purified Water	to 100

CLAIMS

1. Compounds having the formulae (Ia) and (Ib)



(Ia)

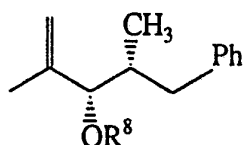


(Ib)

wherein R^1 represents a hydrogen atom, a hydroxyl group or a group selected from $-\text{OCOCH}^{\text{E}}\text{CHCH}(\text{CH}_3)(\text{CH}_2)_3\text{CH}_3$, $-\text{OCOCH}^{\text{E}}\text{CHC}(\text{CH}_3)^{\text{E}}\text{CHCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ or $-\text{OCO}-\text{X}-\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ [where X is $-\text{CH}^{\text{E}}\text{CHCH}(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{OH})\text{CH}(\text{CH}_3)-$, $-\text{CH}^{\text{E}}\text{CHC}(\text{OH})(\text{CH}_3)-$, $-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2-$ or $-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)-$];

R^2 represents a hydroxyl group, a group $-\text{OCOR}^7$ or a group $-\text{OCO}_2\text{R}^7$ (where R^7 is a group selected from C_{1-8} alkyl, aryl, aryl C_{1-4} alkyl and C_{3-8} cycloalkyl);

R^3 represents a group selected from



(where R^8 is a hydrogen atom or an acetyl group), $-\text{C}(\text{CH}_3)^{\text{E}}\text{CHCH}(\text{CH}_2\text{R}^9)\text{CH}_2\text{Ph}$

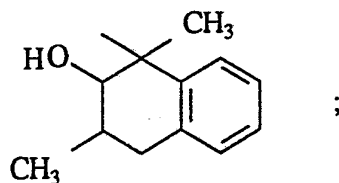
(where R^9 is a hydrogen or a hydroxyl group), $-\text{C}(\text{CH}_2\text{OH})^{\text{Z}}\text{CHCH}(\text{CH}_3)\text{CH}_2\text{Ph}$,

$-\text{C}(=\text{CH}_2)\text{CH}(\text{OH})\text{CH}(\text{CH}_2\text{OH})\text{CH}_2\text{Ph}$,

$-\text{C}(=\text{CH}_2)\text{CH}(\text{NHCOCH}_3)\text{CH}(\text{CH}_3)\text{CH}_2\text{Ph}$,

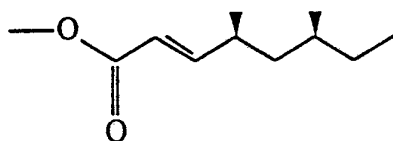
$-\text{C}(\text{CH}_2\text{NHCOCH}_3)^{\text{E}}\text{CHCH}(\text{CH}_3)\text{CH}_2\text{Ph}$ and

- 46 -

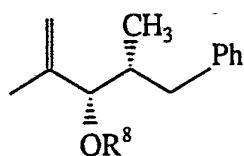


R^4 , R^5 and R^6 may each independently represent a hydrogen atom or a methyl group; and salts thereof.

2. Compounds having the formula (Ia) as defined in Claim 1.
3. Compounds according to Claim 1 or Claim 2 in which R^4 , R^5 and R^6 represent hydrogen atoms.
4. Compounds according to any preceding claim in which R^1 represents a group



5. Compounds according to any preceding claim in which R^3 represents the group



where R^8 represents a hydrogen atom or an acetyl group.

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.

- 47 -

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 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.

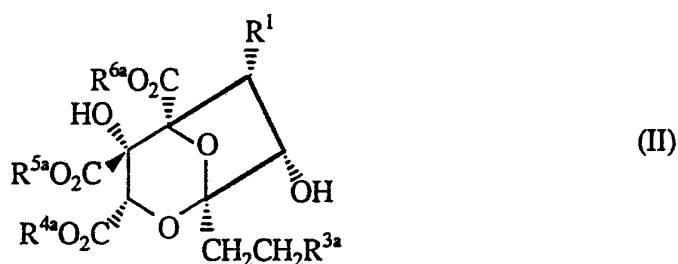
- 48 -

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.

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

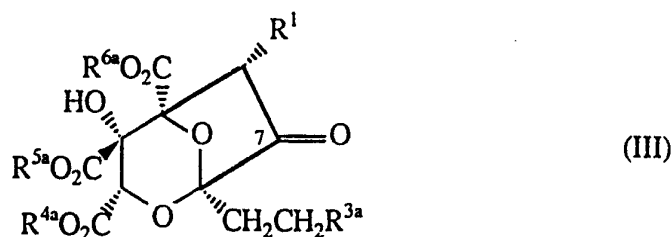
(A) (in the preparation of compounds of formula (Ia) in which R^2 represents a group $-\text{OCOR}^7$ or $-\text{OCO}_2\text{R}^7$) reacting a compound of formula (II)



(wherein R^1 is as defined in Claim 1, R^{3a} is as defined for R^3 in Claim 1 or is a protected derivative thereof and R^{4a} , R^{5a} and R^{6a} are protecting groups) under conditions for ester or carbonate formation, followed by removal of the protecting groups present;

(B) (in the preparation of compounds of formula (Ia) in which R^2 represents a hydroxyl group) reducing a compound of formula (III)

- 49 -



(wherein R^1 and R^{3a} - R^{6a} are as defined herein), and thereafter removing the 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) and (III).

19. [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, 6-methylcarbonate, 7-(4,6-dimethyl-2-octenoate);

[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, 6-phenoxyacetate, 7-(4,6-dimethyl-2-octenoate); and

[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, 6-acetate, 7-(4,6-dimethyl-2-octenoate); and salts thereof.

20. Compounds according to any of Claims 1 to 6 substantially as herein described.

21. Compositions according to any one of Claims 10 to 14 substantially as herein described.

ANNEX M3

International Application No: PCT/EP 92 100016

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page _____, line _____ of the description ¹	
A. IDENTIFICATION OF DEPOSIT ¹	
Further deposits are identified on an additional sheet ☐ ²	
Name of depositary institution ⁴	
CAB INTERNATIONAL MYCOLOGICAL INSTITUTE	
Address of depositary institution (including postal code and country) ⁴	
FERRY ROAD, KEW, SURREY, TW9 3AF, <u>ENGLAND</u>	
Date of deposit ⁴	Accession Number ⁴
25 MAY 1989 (25.05.89)	IMI 332962
B. ADDITIONAL INDICATIONS ³ (leave blank if not applicable). This information is continued on a separate attached sheet ☐	
IN RESPECT OF THOSE DESIGNATIONS IN WHICH A EUROPEAN PATENT IS SOUGHT, A SAMPLE OF THE DEPOSITED MICROORGANISM WILL BE MADE AVAILABLE UNTIL THE PUBLICATION OF THE MENTION OF THE GRANT OF THE EUROPEAN PATENT OR UNTIL THE DATE ON WHICH THE APPLICATION HAS BEEN REFUSED OR WITHDRAWN OR IS DEEMED TO BE WITHDRAWN, ONLY BY THE ISSUE OF SUCH A SAMPLE TO AN EXPERT NOMINATED BY THE PERSON REQUESTING THE SAMPLE (RULE 28(4)EPC). A SIMILAR REQUEST IS MADE IN RESPECT OF THE DESIGNATIONS DK, FI, NL, NO, SE AND GB.	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ⁵ (If the indications are not for all designated States)	
EP, DK, FI, NL, NO, SE AND GB.	
D. SEPARATE FURNISHING OF INDICATIONS ⁶ (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later ⁶ (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)	
(Authorized Officer)	
☐ The date of receipt (from the applicant) by the International Bureau ¹⁰	
was 14 FEBRUARY 1992 (14.02.92)	
(Authorized Officer) J. L. Buzon	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 92/00016

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: C 07 D 493/08

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System

Classification Symbols

IPC⁵ : C 07 D 493/00

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
------------------------	--	-------------------------------------

A	CHEMICAL ABSTRACTS, vol. 91, no. 11, issued 1979, September 2, (Columbus, Ohio, USA), KOELL, P. et al. "1,6-anhydro-3-deoxy-alpha- -D-xylo-hexofuranose" see page 803, column 2, the abstract-no. 91 866e Chem. Ber. 1979, 112(6), 2337-41. ----	1, 8, 10-17
---	--	------------------------

* Special categories of cited documents: ¹⁴

"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.

"Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

19 March 1992

Date of Mailing of this International Search Report

03.04.92

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

Nicole De Bie



FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS 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 9 because they relate to subject matter not required to be searched by this Authority, namely:
(Method for treatment of the human or animal body by surgery or therapy as well as diagnostic methods)
Rule 39.1 (iv) PCT
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 8.4(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.