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<td>(57) Abstract</td>
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Hitherto unknown compounds of formula (II) wherein Rⁱ is hydrogen or methyl; R² is hydrogen, methyl, CH₂OH, CH₂OR³, CHO, CH=CH₂, COOH or COOR⁴; R³ is alkyl, aralkyl, aryl, alkanesulfonyl, arenesulfonyl; alkanoyl or aroyl, optionally substituted; R⁴ is alkyl, alkenyl, alkynyl, aralkyl, aryl, alkanoyloxyalkyl or dialkylaminoethyld; Q¹ and Q² are hydrogen, hydroxy or OR³ or, together, oxygen; or Q¹ (Q²) and R¹ (R²), together, constitute a double bond; or Q² and R², with carbon atoms 3 and 4, form an oxetane ring. X is hydrogen; or X and Q¹ (Q²), taken together, form a double bond, C₂4,25-bond is a double or a single bond; one or more of the double bonds optionally being epoxidized or hydrated; and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof. The present compounds are cholesterol-lowering and anti-atherosclerosis agents.
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TETRACYCLIC TRITERPENES AS CHOLESTEROL-LOWERING AND ANTI-ATHEROSCLEROSIS AGENTS

This invention is directed to a class of tetracyclic triterpenes, in particular protostane and fusidane (29-des-methylprotostane) derivatives, that are useful as cholesterol-lowering and anti-atherosclerosis agents.

More particularly, the invention relates to a hitherto unknown metabolite of the fungus Fusidium coccineum represented by the formula (I)

![Chemical structure](image)

and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof, and to derivatives of said compound having the general formula (II)
wherein

R\(^1\) stands for hydrogen or methyl;

R\(^2\) is hydrogen, methyl, CH\(_2\)OH, CH\(_2\)OR\(^3\), CHO, CH=CH\(_2\),

COOH or COOR\(^4\);

R\(^3\) stands for straight or branched (C\(_1\)-C\(_6\)) alkyl,
aralkyl or aryl, optionally substituted with halogen,

hydroxy or carboxy; alkanesulfonyl or arenesulfonyl;

(C\(_1\)-C\(_4\)) alkanoyl or aroyl, optionally substituted with halogen,

hydroxy or carboxy;

R\(^4\) stands for straight or branched (C\(_1\)-C\(_6\)) alkyl,

(C\(_2\)-C\(_6\)) alkenyl, (C\(_2\)-C\(_6\)) alkynyl, aralkyl, aryl, alkanoyloxy-
alcohol or dialkylaminoethyl;

Q\(^1\) and Q\(^2\) are each independently hydrogen, hydroxy or

a group OR\(^3\); or, taken together, Q\(^1\) and Q\(^2\) stand for oxygen;

or Q\(^1\) (Q\(^2\)) and R\(^1\) (R\(^2\)), when taken together,

contribute a double bond that connects carbon atoms 3 and

4; or Q\(^2\) and R\(^2\), when taken together with carbon atoms 3

and 4, may form an oxetane ring.

X is hydrogen; or X and Q\(^1\) (Q\(^2\)), when taken together,

form a double bond connecting carbon atoms 2 and 3;

the C24,25-bond is a double bond or a single bond;
and, additionally, one or more of the double bonds connecting carbon atoms 2 and 3, 3 and 4, 17 and 20, and/or 24 and 25 may optionally be epoxidized with formation of an oxirane ring or hydrated to give a carbon-carbon single bond where one of the carbon atoms is substituted with hydroxy;

with the proviso that when, at the same time, the C24, 25-bond is a double bond, Q^1 is hydrogen, Q^2 is hydroxy, and R^1 is methyl, then R^2 cannot be methyl or hydroxymethyl;

and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof.

The compounds of the invention derived from the compounds of formula (II) by epoxidation or hydration can comprise several diastereomeric forms (e.g. R and S configuration at the carbon atoms which are part of the oxirane ring or at the carbon atom bearing the hydroxy group). The invention covers all these diastereoisomers in pure form as well as mixtures thereof.

Atherosclerosis, a chronic disease related to the vascular system, is one of the most common causes of death in the Western world, and a high cholesterol level in the blood is a key risk factor in its development.

The inhibition of the biosynthesis of cholesterol constitutes an important approach to lowering serum cholesterol, and several therapeutic agents based on this principle are already available.

These agents (e.g. lovastatin, simvastatin, pravastatin and fluvastatin) interfere with an early step in the cholesterol biosynthesis - namely the conversion of hydroxymethylglutaryl-CoA (HMG-CoA) to mevalonate (cf. Scheme A).
Scheme A is a schematic presentation of multivalent feedback regulation of HMG-CoA-reductase. The dashed lines indicate probable nonsterol regulators and the dotted lines indicate regulation by cholesterol which is derived from LDL uptake. This cholesterol suppresses HMG-CoA reductase and to a limited extent squalene synthetase (Brown & Goldstein, 1980, J. Lipid Research 21, 505-517).
However, mevalonate is also the obligate precursor of a number of non-steroidal isoprenoids such as dolichol, ubiquinone and isopentenyl t-RNA and the formation of these essential compounds will therefore also be inhibited by inhibitors of HMG-CoA reductase. This is an undesired effect and efforts have therefore been concentrated on the finding of cholesterol lowering compounds that interfere with a later step in the biosynthesis of cholesterol.

Recently, the isolation and characterisation of two new families of compounds, called squalstatins and zaragozic acids, respectively, have been reported. These compounds are potent inhibitors of the enzyme squalene synthetase (cf. Scheme A) and therefore lower the formation of the cholesterol-precursor squalene without interfering with the production of non-steroidal isoprenoids.

The conversion of 2,3-oxidosqualene into lanosterol - another intermediate in the biosynthesis of cholesterol - is another target for inhibition. This conversion, which is catalyzed by the enzyme oxidosqualene cyclase, is believed to take place as outlined in Scheme B.
Scheme B

2,3-Oxidosqualene → Protosterol Cation → Lanosterol
The 2,3-oxidosqualene, formed by enzymatic epoxidation of squalene, is folded in a pre-chair-boat-chair-boat conformation and the proton initiated cyclization proceeds through a series of rigidly-held carbocationic intermediates. The intermediate C-20 protosterol cation then undergoes backbone rearrangement to yield lanosterol.

Because of the similarity between the conformation of the protosterol cation and that of a protosterol (e.g. compound 6, Scheme 1) we hypothesized that certain compounds containing the protostane ring system might act as inhibitors of oxidosqualene cyclase and thereby inhibit the formation of cholesterol in a very specific way.

The effect of the compounds of the invention on cholesterol synthesis (\(^{14}\text{C}\)-acetate incorporation into cholesterol, separated by TLC) in human Hep G2 cells can be tested in vitro according to the method described by A. Boogards et al. (Biochem. J., 1987, 241, 345-351).

The effect of the compounds of the invention on cholesterol biosynthesis from \(^{14}\text{C}\)acetate or \(^{3}\text{H}\)mevalonate by isolated rat hepatocytes and by rat or mouse liver in vivo can be tested according to the method described by Y. Tsujita et al. (Biochem. Biophys. Acta, 1986, 877, 50-60).

Two of the compounds represented by the general formula (II), i.e. those in which the C24,25-bond is a double bond, Q is hydrogen, Q is hydroxy, R is methyl and R stands for either methyl or hydroxymethyl have been described previously. (S. Okuda et al., Tetrahedron Letters 1968, 4769-4772; T. Hattori et al., Tetrahedron Letters 1969, 1023-1026; G. Visconti, Ph.D. Thesis No. 4156, ETH Zurich, 1968). Both compounds have been isolated in small amounts from the mycelium of the helvolic acid-producing fungus Cephalosporium caerulens and, independently, from the mycelium of Fusidium cocccineum, the fungus known to produce fusidic acid, but an investigation of their biological activities has never been reported.

However, the discovery and recent isolation in substantial amounts of the compound of formula (I) offered the
possibility to prepare larger amounts of said two compounds of formula (II) by chemical means and to study their biological activities.

It has now been found that said two compounds of formula (II) and other compounds of the present invention show activity as inhibitors of hepatic cholesterol synthesis in vitro and in vivo.

The invention also relates to methods of preparing the compounds of the formulae (I) and (II) as defined above.

The compound of formula (I) is a hitherto unknown metabolite of the fungus Fusidium coccineum, formed during the fermentation process in addition to fusidic acid, and can be isolated in substantial amounts by fractionation of mother liquors from which fusidic acid has been recovered.

It is noteworthy in this context that the production of fusidic acid by fermentation of Fusidium coccineum has been described in detail (see Biotechnology of Industrial Antibiotics, E.J. Vandamme, ed.; Marcel Dekker, Inc., New York, 1984, 427-449, and references cited therein).

The new compound is a tetracyclic triterpenoid acid C_{30}H_{48}O_3, containing a secondary hydroxyl group and two isolated double bonds, one trisubstituted, the other tetrasubstituted. Chemical and spectral data obtained for this compound were in agreement with the structure shown in formula (I). The compound can be used as such or in the form of salts or in vivo hydrolysable esters.

The compounds of formula (II) may conveniently be prepared from the compound of formula (I) by the routes outlined in Schemes 1 to 5. The conversion of a C24,25 double bond into a C24,25 single bond is readily performed by hydrogenolysis in the presence of a palladium catalyst. The compounds of formula (II) wherein R^2 is COOH can be used as such or in the form of salts or in vivo hydrolysable esters.

The salts of the compounds are especially the pharmaceutically acceptable, non-toxic salts, such as alkali
metal salts and alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, as well as salts with ammonia or suitable non-toxic amines, such as lower alkyl amines, for example triethylamine, hydroxy-

lower alkylamines, for example 2-hydroxyethylamine, bis-(2-

hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine, cyclo-

alkylamines, for example dicyclohexylamine, or benzyl-

amines, for example N,N'-dibenzylethylenediamine, and di-

benzylamine.

The in vivo hydrolysable esters can e.g. be alkanoyl-

oxyalkyl, aralkanoyloxyalkyl, aroyloxyalkyl esters, such as actoxymethyl, pivaloyloxyethyl, benzoyloxyethyl esters, and the corresponding 1'-oxyethyl derivatives, or alkoxy-
carbonyloxyalkyl esters, such as methoxycarbonyloxyethyl, ethoxycarbonyloxyalkyl esters, and the corresponding 1'-

-oxyethyl derivatives, or lactonyl esters, such as phthal-
idyl esters, or dialkylaminoalkyl esters, such as diethyl-
aminoethyl esters.
Scheme 1  Synthesis of compounds of formula (II) where $R^1$ is methyl and $R^2$ is methyl or hydroxymethyl

5 Notes to Scheme 1
a) Esterification with methyl iodide in the presence of base (e.g. potassium carbonate).
b) Reduction (e.g. with lithium aluminium hydride).
c) Tosylation with p-toluenesulfonyl chloride in the presence of base (e.g. pyridine).
d) Reduction (e.g. with lithium aluminium hydride).
e) Oxydation (e.g. with Jones reagent).
f) Reduction (e.g. with potassium selectride).
g) Alkylation, acylation etc.
Scheme 2  Synthesis of compounds of formula (II) where R¹ is methyl and R² is hydrogen or R¹ is hydrogen and R² is methyl

(l) \[\begin{array}{c}
11 \xrightarrow{a} 12 + 13 \\
\xrightarrow{cord} 14 \text{ 3β-OH} \\
\xrightarrow{e} 15 \text{ 3α-OH} \\
\xrightarrow{f} 19 + 20 \\
\end{array}\]
Notes to Scheme 2

a) Oxidation (e.g. with Jones reagent).
b) Decarboxylation of β-keto acid (e.g. by thermolysis in 95% ethanol).

c) Reduction (e.g. with sodium borohydride).
d) Reduction (e.g. with potassium selectride)
e) Elimination (e.g. via tosylate in the presence of base).
f) Epoxidation (e.g. with m-chloroperbenzoic acid).
Synthesis of ring A modified compounds of formula (II) where R₁ is methyl by reduction/elimination of sulfonates.
Notes to Scheme 3

a) Tosylation with p-toluenesulfonyl chloride in the presence of base (e.g. pyridine).

b) Elimination of p-toluenesulfonic acid (e.g. with lithium aluminium hydride).

c) Sulfonation/elimination (e.g. with triflic anhydride in pyridine).

d) Reduction (e.g. with lithium aluminium hydride).

e) Mesylation with methanesulfonyl chloride in the presence of base (e.g. pyridine).

f) Treatment with lithium triethylhydroborate ("Superhydride").
Scheme 4

Synthesis of compounds of the invention by epoxidation of compounds of formula II having C17(20) and C24,25 double bonds followed by reduction or rearrangement.

\[ \text{R} = \text{CH}_3, \text{CH}_2\text{OH}, \text{COOH}, \text{COOCH}_3, \text{H} \]

\( \text{Ra} = \text{H}, \text{Rb} = \text{OH} \) or \( \text{Ra} = \text{OH}, \text{Rb} = \text{H} \)
Notes to Scheme 4

a) Epoxidation (e.g. with \textit{m}-chloroperbenzoic acid).
b) Rearrangement (e.g. with aluminium chloride as catalyst).

c) Reduction (e.g. with lithium aluminium hydride).
Synthesis of compounds of the invention by hydroboration/oxidation or oxymercuration of C17(20) and C24,25 double bonds.

Scheme 5

R = CH₃, OH, COOH, COOCH₃, H
Notes to Scheme 5

Hydroboration (e.g. with borane, monoalkylboranes, dialkylboranes or catecholborane) followed by oxidation (e.g. with 30% hydrogen peroxide/sodium hydroxide).

Oxymercuration (e.g. with mercury(II)acetate or trifluoroacetate) followed by demercuration (e.g. reduction with sodium borohydride or sodium trimethoxyborohydride).

It is a further object of the present invention to provide pharmaceutical compositions of (I) and (II) which are useful in the treatment of the above mentioned diseases.

The amount required of a compound of formula (I) or (II) (hereinafter referred to as the active ingredient) for therapeutic effect will, of course, vary both with the particular compound, the route of administration and the mammal under treatment. A suitable daily dose of a compound of formula (I) for systemic treatment is 0.05 to 20 mg per kilogram mammal bodyweight, a more preferred daily dosage being 0.1 to 7 mg per kg of mammal bodyweight administered in one or more doses.

By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active material as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.

The formulations for human medical use of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredient(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.
The formulations include those in a form suitable for enteral, parenteral (including subcutaneous, intramuscular, intravenous and intraperitoneal) administration.

The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient into association with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active ingredient into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion. The active ingredient may also be administered in the form of a bolus.

A tablet may be made by compressing or moulding the active ingredient optionally with one or more accessory ingredient. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form, such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Moulded tablets may be made by moulding, in a suitable machine, a mixture of the powdered active ingredient and a suitable carrier moistened with an inert liquid diluent.

Formulations suitable for rectal administration may be in the form of suppositories.

Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation
of the active ingredient which is preferably isotonic with the blood of the recipient.

In addition to the aforementioned ingredients, the formulations of this invention may include one or more additional ingredients, such as diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives, e.g. methylhydroxybenzoate (including anti-oxidants), emulsifying agents and the like.

The compositions may further contain other therapeutically active compounds usually applied in the treatment of the above-mentioned pathological conditions.

According to the invention, the present compounds are administered to a patient suffering from one of the above mentioned pathological conditions in a daily dose (for adults) from 3.5 mg to 1400 mg, preferably from 10 - 500 mg.

The invention will now be further described in the following Examples:

20 **General**

For nuclear magnetic resonance spectra (300 Mhz) chemical shift values (δ) are quoted for deuteriochloroform solutions relative to internal tetramethylsilane (δ = 0) or chloroform (δ = 7.25). The value for a multiplet, either defined (doublet (d), triplet (t), quartet (q)) or not (m) at the approximate mid point is given unless a range is quoted (s = singlet, b = broad). Coupling constants (J) are given in Hertz, and are sometimes approximated to the nearest unit.

Electron ionization mass spectrometry (EIMS) was used to determine molecular weights, M⁺ corresponding to the molecular ion.

Ether is diethyl ether, and was dried over sodium. THF was dried over sodium/benzophenone. Petroleum ether refers to the pentane fraction. Reactions were run at room temperature unless otherwise noted. The work-up procedure referred to involves dilution with the specified solvent.
(otherwise the organic reaction solvent), extraction with water and then brine, drying over anhydrous MgSO₄, and concentration in vacuo to give a residue.

Example 1

3β-Hydroxyprotosta-17(20)Z,24-dien-29-oic Acid (I)

Crude fusidic acid methanol solvate (200 g) containing 12.6% of the title compound, as determined by HPLC analysis, was dissolved in ethyl acetate (2.5 liter) at 40°C.

Addition of dicyclohexylamine (100 ml) to the stirred solution caused precipitation of a white crystalline product. After stirring for 30 minutes at room temperature, the crystals were filtered off, washed with ethyl acetate, followed by petroleum ether, and dried to afford 207.5 g of dicyclohexylammonium fusidate, C₃₀H₄₈O₆, C₁₂H₂₃N.

The mother liquor was concentrated to about 500 ml, water (250 ml) was added, and the apparent pH of the mixture was adjusted to 3 with concentrated sulfuric acid. The organic phase was separated, washed with water (2 x 100 ml), dried (MgSO₄) and evaporated. The residual oil (54.2 g) was crystallized from methanol to give 26.0 g of the title compound which according to TLC contained traces of fusidic acid. Recrystallization from acetone afforded 21.2 g of the pure compound, mp 179-180°C, [α]D²⁰ +38.2° (c1, CHCl₃).

Anal. Calculated for C₃₀H₄₈O₃:  C 78.90, H 10.59

Found: C 79.01, H 10.64

¹H NMR δ 0.77 (s,3H), 0.90 (s,3H), 1.12 (s,3H), 1.45 (s,3H), 1.10-1.65 (m,12H), 1.58 (s,3H), 1.60 (s,3H), 1.68 (s,3H), 1.75-2.20 (m,12H), 2.25 (dd,1H), 3.17 (dd,1H), 5.10 (bt,1H)

Example 2

Methyl 3β-hydroxyprotosta-17(20)Z,24-dien-29-oate

To a stirred solution of 3β-hydroxyprotosta-17(20)Z,24-dien-29-oic acid (45.7 g, 100 mmol) in dimethylformamide (250 ml) was added potassium carbonate (20.7 g,
150 mmol) and methyl iodide (10 ml, 150 mmol). The reaction mixture was stirred at room temperature overnight, insoluble material was removed by filtration, and the filtrate was transferred to a separating funnel with ethyl acetate (500 ml), washed with water (2 x 250 ml, 2 x 125 ml), dried (MgSO₄) and evaporated. The residual oil was crystallized from ether-methanol to give 43.4 g (92.2%) of the title compound, mp 99-100°C.

Anal. Calculated for C₃₁H₅₀O₃ :  C 79.10, H 10.71
10 Found: C 79.19, H 10.77

¹H NMR δ 0.77 (s,3H), 0.79 (s, 3H), 1.12 (s,3H), 1.39 (s,3H),1.10-1.60 (m,12H), 1.58 (bs,3H), 1.60 (s,3H), 1.68 (s,3H),1.75-2.35 (m,12H), 3.10 (m,1H), 3.65 (s,3H), 5.10 (bt, 1H)

Example 3

3β,29-Dihydroxyprostasta-17(20)Z,24-diene

In a 3-necked round-bottom 250ml-flask, equipped with a reflux condenser, a dropping funnel and a thermometer, lithium aluminium hydride (1.52 g, 40 mmol) was dissolved in dry ether (40 ml), and a solution of methyl 3β-hydroxyprostasta-17(20)Z,24-dien-29-oate (9.80 g, 20 mmol) in dry ether (40 ml) was added dropwise with stirring over 15 minutes. After stirring for a further 15 minutes, excess lithium aluminium hydride was removed by dropwise addition of ethyl acetate (40 ml) followed by 2N sulfuric acid (40 ml). The mixture was filtered through a celite pad, washed with ethyl acetate (2 x 10 ml), and the filtrate was transferred to a separating funnel. The aqueous layer was extracted with ethyl acetate (20 ml), and the combined organic phases were washed with water (2 x 10 ml) and brine (10 ml), dried (MgSO₄) and evaporated. The residue thus obtained was crystallized from ether to afford 8.15 g (92.1%) of the title compound, mp 139-140°C.

Anal. Calculated for C₃₀H₅₀O₂ :  C 81.39, H 11.38

Found: C 81.52, H 11.47
\[
^{1}H \text{ NMR} \delta 0.74 \text{ (s, 3H), 0.89 (s, 3H), 1.12 (s, 3H), 1.22 (s, 3H), 1.57 (bs, 3H), 1.60 (s, 3H), 1.68 (s, 3H), 1.10-2.35 (m, 23H), 2.68 (d, 1H), 2.73 (d, 1H), 3.29 (dd, 1H), 3.34 (m, 1H), 4.22 (d, 1H), 5.10 (bt, 1H).}
\]

5

Example 4

29-Mono- and 3β-29-Ditosylate of 3β,29-Dihydroxyprotosta-17(20)Z,24-diene

To a stirred solution of 3β,29-dihydroxyprotosta-17(20)Z,24-diene (8.86 g, 20 mmol) in pyridine (50 ml) was added at 0°C p-toluenesulfonyl chloride (7.62 g, 40 mmol) in one portion. The reaction mixture was stirred at 0 - 5°C for 2 hours and then kept in a refrigerator overnight. The yellowish mixture was poured onto ice-water (200 ml) and extracted with ethyl acetate (2 x 100 ml). The combined organic phases were washed with 4N hydrochloric acid (200 ml), water (2 x 25 ml) and brine (20 ml), dried (MgSO₄) and evaporated to yield 12.6 g of an approximate 3:1 mixture of the mono and the ditosylate, respectively, as a foam.

20

A. 3β,29-Dihydroxyprotosta-17(20)Z,24-diene 3β,29-Ditosylate

The above mixture was separated by column chromatography on silicagel. Elution with 25% ethyl acetate in petroleum ether followed by evaporation and crystallization (ether) gave 2.86 g (19.0%) of the 3β,29-ditosylate, mp 135-136°C.

Anal. Calculated for C₄₄H₆₂O₆S₂: C 70.36, H 8.32, S 8.54
Found: C 70.40, H 8.35, S 8.59

\[
^{1}H \text{ NMR} \delta 0.73 \text{ (s, 3H), 0.83 (s, 3H), 0.88 (s, 3H), 1.08 (s, 3H), 1.57 (s, 3H), 1.59 (s, 3H), 1.68 (s, 3H), 1.10-2.30 (m, 23H), 2.45 (s, 3H), 2.46 (s, 3H), 3.88 (d, 1H), 4.21 (d, 1H), 4.27 (m, 1H), 5.09 (m, 1H), 7.33 (m, 4H), 7.74 (m, 4H)
\]
B. 3β,29-Dihydroxyprotosta-17(20)Z,24-diene 29-Mono-tosylate

Subsequent elution of the column with 50% ethyl acetate in petroleum ether afforded, after evaporation and crystallization (ether), 7.60 g (63.6%) of the 29-mono-tosylate, mp 147-148°C.

Anal. Calculated for C_{37}H_{56}O_{4}S: C 74.45, H 9.46, S 5.37

Found: C 74.44, H 9.43, S 5.34

$^{1}H$ NMR δ 0.74 (s,3H), 0.87 (s,3H), 1.08 (s,3H), 1.09 (s,3H), 1.58 (s,3H), 1.60 (s,3H), 1.68 (s,3H), 1.05-1.75 (m,15H), 1.85-2.35 (m,9H), 2.44 (s,3H), 3.32 (m,1H), 4.15 (ABq,2H), 5.10 (m,1H), 7.34 (d,2H), 7.78 (d,2H)

Example 5

3β-Hydroxyprotosta-17(20)Z,24-diene

In a 3-necked round-bottom 250 ml-flask, equipped with a reflux condenser, a dropping funnel and a thermometer, lithium aluminium hydride (0.95 g, 25 mmol) was dissolved in dry ether (75 ml), and a solution of 3,29-dihydroxyprotosta-17(20)Z,24-diene 29-tosylate (2.98 g, 5 mmol) in dry tetrahydrofuran (25 ml) was added dropwise with stirring. After the addition was finished (15 minutes), the mixture was stirred at room temperature for a further 30 minutes and then refluxed for one hour. Excess reagent was removed by dropwise addition of ethyl acetate (40 ml) followed by 2N sulfuric acid (40 ml). After filtration through a celite pad, the filtrate was transferred to a separating funnel. The aqueous layer was extracted with ethyl acetate, and the combined organic phases were washed with water (3 x 10 ml) and brine (10 ml), dried (MgSO$_4$) and evaporated. The resulting gum was crystallized from ether-hexane to give 2.06 g (96.6%) of the title compound, mp 97-98°C.

Anal. Calculated for C$_{30}$H$_{50}$O: C 84.44, H 11.81

Found: C 83.97, H 11.98

$^{1}H$ NMR δ 0.75 (s,3H), 0.79 (s,3H), 0.85 (m,1H), 0.93 (s,3H), 0.98 (s,3H), 1.13 (s,3H), 1.10-1.75 (m,14H), 1.58
(s, 3H), 1.61 (s, 3H), 1.68 (s, 3H), 1.90-2.35 (m, 9H), 3.24 (dd, 1H), 5.11 (m, 1H)

Example 6

5 3-Oxoprotostat-17(20)Z,24-dien-29-oic Acid

To a stirred solution of 3β-hydroxyprotosta-17(20)Z,24-dien-29-oic acid (13.70 g, 30 mmol) in acetone (420 ml) was added dropwise at 0-5°C Jones reagent (13.5 ml). After the addition was finished (about 20 minutes), the cooling bath was removed, and the reaction mixture was transferred to a separating funnel. Water (600 ml) was added, and the mixture was extracted with ether (1 x 500 ml, 1 x 250 ml). The combined organic phases were washed with water (3 x 100 ml), dried (MgSO₄), and evaporated to give 12.76 g (93.5%) of the title compound as a foam which was used in the next step without further purification (see Example 7).

¹H NMR δ 0.79 (s, 3H), 1.08 (s, 3H), 1.09 (s, 3H), 1.43 (s, 3H), 1.58 (s, 3H), 1.60 (s, 3H), 1.68 (s, 3H), 1.00-2.38 (m, 21H), 2.52 (m, 1H), 2.80 (m, 1H), 5.11 (m, 1H)

Example 7

3-Oxofusida-17(20)Z,24-diene and 4-epi-3-oxofusida-17(20)Z,24-diene

A solution of crude 3-oxoprotostat-17(20)Z,24-dien-29-oic acid (4.32 g, 9.5 mmol) in 95% ethanol (100 ml) was refluxed for 2 hours. After cooling to room temperature, the reaction mixture was evaporated to give 3.88 g of a gum which consisted of two compounds, as revealed by TLC. These could be separated by column chromatography on silica gel eluting with 5% and 10% ether in petroleum ether.

A. 3-Oxofusida-17(20)Z,24-diene

Elution of the less polar minor compound followed, by evaporation and crystallization from ether-methanol, gave pure 3-oxofusida-17(20)Z,24-diene, mp 91-92°C, [α]D²⁰ +56.6° (c0.5, CHCl₃).
Anal. Calculated for C_{29}H_{46}O:  C 84.81, H 11.29  
Found:  C 84.67, H 11.27  

$^1$H NMR δ 0.78 (s,3H), 1.01 (s,3H), 1.02 (d,3H), 1.10 (s,3H), 1.59 (s,3H), 1.61 (s,3H), 1.69 (s,3H), 0.95-1.78 (m,12H), 1.85 (m,1H), 1.90-2.36 (m,9H), 2.43 (m,2H), 5.11 (m,1H)

B. 4-Epi-3-oxofusida-17(20)Z,24-diene

Evaporation of the eluate containing the more polar major compound, followed by crystallization of the resulting gum from ether-methanol, afforded pure 4-epi-3-oxofusida-17(20)Z,24-diene, mp 67-68°C, [α]_{D}^{20} +93.0° (c0.5, CHCl₃)

Anal. Calculated for C_{29}H_{46}O:  C 84.81, H 11.29  
Found:  C 84.25, H 11.24  

$^1$H NMR δ 0.79 (s,3H), 0.81 (s,3H), 1.10 (d,3H), 1.17 (s,3H), 1.59 (bs,3H), 1.60 (s,3H), 1.68 (s,3H), 1.00-1.75 (m,10H), 1.90-2.67 (m,14H), 5.11 (m,1H)

Example 8

3-Oxofusida-17(20)Z,24-diene

To a solution of 4-epi-3-oxofusida-17(20)Z,24-diene (2.67 g, 6.5 mmol) in tetrahydrofuran (35 ml) was added 1N methanolic potassium hydroxide (13 ml), and the mixture was kept at room temperature overnight. The yellowish solution was transferred to a separating funnel with ethyl acetate (70 ml) and repeatedly washed with water (4 x 35 ml) followed by brine (2 x 15 ml). The organic phase was dried (Na₂SO₄) and evaporated to leave 2.64 g of an oil. The residue was purified by column chromatography on silicagel (4% ether in petroleum ether as eluant) to give 2.04 g (76.4%) of the pure title compound, mp 90-91°C, identical in every respect with the compound prepared in Example 7A.
Example 9

3β-Hydroxyfusida-17(20)Z,24-diene

To a stirred solution of 3-oxofusida-17(20)Z,24-diene (2.88 g, 7.0 mmol) in tetrahydrofuran (50 ml) was added solid sodium borohydride (0.32 g, 8.4 mmol) and, dropwise over 10 minutes, methanol (25 ml). After stirring for a further 15 minutes, the mixture was transferred to a separating funnel with ethyl acetate (150 ml), washed with water (4 x 50 ml), followed by brine (20 ml), dried (Na₂SO₄) and evaporated. The residue was purified by flash chromatography on silicagel (20% ether in petroleum ether) to give after evaporation and crystallization from ether-methanol, 1.44 g (49.8%) of the title compound, mp 123-124°C, \([\alpha]_D^{20} +22.1^\circ\) (c0.5, CHCl₃).

Anal. Calculated for C₂₉H₄₈O:  C 84.40, H 11.72

Found:  C 84.39, H 11.76

\(^1H\) NMR \(\delta\) 0.76 (s,3H), 0.90 (s,3H), 0.95 (d,3H), 1.09 (s,3H), 1.58 (m,3H), 1.60 (s,3H), 1.69 (s,3H), 1.00-1.75 (m,15H), 1.75-2.38 (m,10H), 3.09 (m,1H), 5.11 (m,1H)

Example 10

4-Epi-3β-hydroxyfusida-17(20)Z,24-diene

By following the procedure described in Example 9 and substituting 4-epi-3-oxofusida-17(20)Z,24-diene for the 3-oxofusida-17(20)Z,24-diene, 4-epi-3β-hydroxyfusida-17(20)Z,24-diene, mp 105-106°C, \([\alpha]_D^{20} +9.0^\circ\) (c0.5, CHCl₃), was prepared.

\(^1H\) NMR \(\delta\) 0.76 (s,3H), 0.89 (d,3H), 0.92 (s,3H), 1.12 (s,3H), 1.58 (m,3H), 1.60 (s,3H), 1.68 (s,3H), 1.02-2.35 (m,25H), 3.79 (m,1H), 5.11 (m,1H)

Examples 11-16

Protost-17(20)Z-ene Derivatives

24,25-Dihydro derivatives of the compounds described in Examples 1, 2, 3, 4A, 4B and 5 were obtained by the following procedure: To a solution of the corresponding protost-a-17(20)Z,24-diene (10 mmol) in ethanol (50 ml) was added
10% palladium on calcium carbonate catalyst (500 mg), and the mixture was shaken in a hydrogen atmosphere until the consumption of hydrogen ceased (about 30 minutes). The catalyst was filtered off, washed with ethanol, and the combined filtrate and washings were evaporated to dryness. The residue was purified by crystallization or chromatography, and the pure compound thus obtained was characterized.

The following compounds were prepared by the above procedure.

<table>
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<tr>
<th>Example</th>
<th>Name</th>
<th>mp (°C)</th>
<th>Formula</th>
<th>Elemental analysis</th>
<th>¹H NMR data (δ values)</th>
</tr>
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<tr>
<td>11</td>
<td>3β-Hydroxyprost-17(20)Z-en-29-oic Acid</td>
<td>163-166</td>
<td>C₃₀H₅₀O</td>
<td>Calcd.: C 78.55, H 10.99</td>
<td>Found: C 78.39, H 10.97</td>
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<td>0.77 (s,3H), 0.87 (d,6H), 0.91 (s,3H), 1.12</td>
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<td>(s,3H), 1.45 (s,3H), 1.58 (s,3H), 1.05-2.38</td>
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<td>(m,27H), 3.18 (dd,1H)</td>
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<td>12</td>
<td>Methyl 3β-hydroxyprost-17(20)Z-en-29-oate</td>
<td>83-84</td>
<td>C₃₁H₅₂O₃</td>
<td>Calcd.: C 78.76, H 11.09</td>
<td>Found: C 78.78, H 11.14</td>
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<td>0.76 (s,3H), 0.79 (s,3H), 0.87 (d,6H), 1.12</td>
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<td>(s,3H), 1.39 (s,3H), 1.56 (bs,3H), 1.05-2.35</td>
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<td>(m,26H), 3.12 (m,1H), 3.65 (s,3H), 3.72 (d,1H)</td>
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<td>13</td>
<td>3β,29-Dihydroxyprost-17(20)Z-ene</td>
<td>119-120</td>
<td>C₃₀H₅₂O₂·0.5H₂O</td>
<td>Calcd.: C 79.41, H 11.77</td>
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<td>Found: C 79.38, C 11.63</td>
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<td>0.74 (s,3H), 0.87 (d,6H), 0.90 (s,3H), 1.12</td>
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<td>(s,3H), 1.22 (s,3H), 1.56 (m,3H), 1.05-2.37</td>
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<td>Example</td>
<td>Name</td>
<td>mp (°C)</td>
<td>Formula</td>
<td>Elemental analysis</td>
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<td>1H NMR data (δ values)</td>
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<tr>
<td>5</td>
<td>3β,29-Dihydroxyprotost-17(20)Z-ene, 3β,29-Ditosylate</td>
<td>103-106</td>
<td>C₄₄H₆₄O₂S₂</td>
<td>Calcd.: C 70.17, H 8.57, S 8.51</td>
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<td>Found: C 70.17, H 8.67, S 8.50</td>
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<td>15</td>
<td>3β,29-Dihydroxyprotost-17(20)Z-ene, 29-Monotosylate</td>
<td>129-132</td>
<td>C₃₇H₅₈O₄S</td>
<td>Calcd.: C 74.20, H 9.76, S 5.35</td>
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<td></td>
<td>Found: C 74.16, H 9.80, S 5.39</td>
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<td>16</td>
<td>3β-Hydroxyprotost-17(20)Z-ene</td>
<td>Cryst.</td>
<td>C₃₀H₅₂O</td>
<td>Calcd.: C 84.04, H 12.22</td>
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<td>Found: C 83.72, H 12.30</td>
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<td>0.99 (s, 3H), 1.13 (s, 3H), 1.57 (m, 3H), 1.05-1.80 (m, 20H), 1.87-2.38 (m, 7H), 3.25 (dd, 1H)</td>
<td></td>
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<tr>
<td>30</td>
<td>Example 17</td>
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<tr>
<td></td>
<td>Methyl 3β-Tosyloxyprotosta-17(20)Z,24-dien-21-oate</td>
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<tr>
<td></td>
<td>To an icecold solution of methyl 3β-hydroxyprotosta-</td>
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<tr>
<td></td>
<td>17(20)Z,24-dien-21-oate (4.71 g, 10 mmol) in pyridine (25</td>
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<td></td>
<td>ml) was added 4-dimethylaminopyridine (DMAP; 0.24 g, 2</td>
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<tr>
<td></td>
<td>mmol) and p-toluenesulfonyl chloride (3.81 g, 20 mmol), and</td>
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<td></td>
<td>the mixture was stirred at 0 - 5°C for another hour before</td>
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<td></td>
<td>kept at room temperature overnight. After addition of meth-</td>
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<td>anol (6 ml), the mixture was stirred for 30 minutes,</td>
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<td>diluted with ethyl acetate (250 ml), washed with 2 N hydro-</td>
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<td></td>
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</table>
chloric acid (160 ml), water (2 x 50 ml) and brine (20 ml),
dried (MgSO₄) and evaporated. The residual solid (6.16 g)
was crystallized from dichloromethane - ethyl acetate to
give 5.62 g (89.9%) of the title compound, mp 162-164°C.

Recrystallization from dichloromethane - ether afforded the
analytical sample, mp 164-165°C.

Anal. Calculated for C₃₈H₅₆O₅S:  C 73.03,  H 9.03,  S 5.13
    Found:  C 73.07,  H 9.00,  S 5.15
¹H NMR δ 0.74 (s,3H),  0.77 (s,3H),  1.11 (s,3H),  1.26
    (s,3H),  1.58 (s,3H),  1.59 (s,3H),  1.67 (s,3H),  2.44
    (s,3H),  1.10-2.45 (m,23H),  3.61 (s,3H),  4.29 (dd,1H),  5.09
    (m,1H),  7.32 (d,2H),  7.82 (d,2H)

Example 18

Methyl Protosta-2,17(20)Z,24-trien-29-oate

To a solution of methyl 3β-hydroxyprotosta-17(20)Z,-
-24-dien-21-oate (4.72 g, 10 mmol) in pyridine (50 ml) was
added at 0-5°C triflic anhydride (2.46 ml, 15 mmol), and
the mixture was stirred at the low temperature for 2 hours
before kept in the refrigerator overnight. After addition
of methanol (10 ml), the mixture was stirred for 15 min-
utes, diluted with ethyl acetate (250 ml), washed with 2 N
hydrochloric acid (320 ml), water (2x50 ml) and brine (20
ml), dried (MgSO₄) and evaporated. The residual oil was
purified by column chromatography on silica gel eluting
with 5% ether in petroleum ether to give 3.06 g (67.6%) of
the title compound, mp 107-108°C (from ether-methanol).

Anal. Calculated for C₃₁H₄₈O₂:  C 82.24,  H 10.69
    Found:  C 82.43,  H 10.75
¹H NMR δ 0.78 (s,6H),  1.09 (s,3H),  1.28 (s,3H),  1.58
    (bs,3H),  1.60 (s,3H),  1.68 (s,3H),  1.10-2.38 (m,21H),  3.62
    (s,3H),  5.11 (m,1H),  5.65 (m,2H)

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Example 19
29-Hydroxyprotosta-2,17(20)Z,24-triene

A. From Methyl 3β-Tosyloxyprotosta-17(20)Z,24-dien-21-oate
Lithium aluminium hydride (0.38 g, 10 mmol) was dissolved in ether (40 ml) in a two-necked 250 ml round-bottom flask equipped with a reflux condenser and a dropping funnel, and a solution of methyl 3β-tosyloxyprotosta-17(20)Z,24-dien-21-oate (3.0 g, 4.8 mmol) in tetrahydrofuran - ether 1:1 (40 ml) was added dropwise with stirring. After the addition was finished (15 minutes), the mixture was refluxed for 4 hours, and then cooled to room temperature. Ethyl acetate (15 ml) and 2 N sulfuric acid (15 ml) were added, and the mixture was filtered through a celite pad. The aqueous layer was separated, and the organic phase was washed with water (2x15 ml) and brine (10 ml), dried (Na₂SO₄) and evaporated. The residual oil was subjected to column chromatography on silica gel eluting with 10% ether in petroleum ether to give 1.16 g (57.0%) of the pure title compound, mp 95-97°C (from ether-methanol).

1H NMR δ 0.77 (s,3H), 0.94 (s,3H), 1.10 (s,3H), 1.12 (s,3H), 1.58 (bs,3H), 1.59 (s,3H), 1.69 (s,3H), 1.05-2.40 (m,22H), 3.52 (d,1H), 3.82 (d,1H), 5.11 (m,1H), 5.60 (m,2H)

B. From Methyl Protosta-2,17(20)Z,24-trien-29-oate
In a two-necked 250 ml round-bottom flask equipped with a reflux condenser and a dropping funnel, lithium aluminium hydride (0.28 g, 7.5 mmol) was dissolved in dry ether (30 ml), and a solution of methyl protosta-2,17(20)Z,24-trien-29-oate (1.36 g, 3 mmol) in dry ether (30 ml) was added dropwise with stirring. After the addition was finished, the mixture was stirred for a further 30 minutes and then refluxed for two hours. Excess reagent was removed by addition of ethyl acetate (20 ml) and 1 N sulfuric acid (20 ml). After filtration through a celite pad, the filtrate was transferred to a separating funnel. The
aqueous layer was extracted with ethyl acetate (20 ml), and the combined organic phases were washed with water (2x10 ml) and brine (10 ml), dried (MgSO₄), and evaporated to leave 1.33 g of an oily residue which was purified by column chromatography on silica gel. Elution with 20% ethyl acetate in petroleum ether gave 1.12 g of the pure title compound which was crystallized from ether-hexane, mp 95-97°C.

Anal. Calculated for C₁₃H₂₈O, 0.5 H₂O: C 83.08, H 11.39
Found: C 82.95, H 11.14

**Example 20**

**Protosta-2,17(20)Z,24-triene**

To an icecold solution of 3β-hydroxyprotosta-17(20)Z,24-diene (1.28 g, 3 mmol) in pyridine (15 ml) was added triflic anhydride (0.74 ml, 4.5 mmol), and the mixture was stirred at 0-5°C for two hours and then kept in the refrigerator overnight. Methanol (3 ml) was added, and the mixture was stirred for 15 minutes, diluted with ethyl acetate (100 ml), washed with 2 N hydrochloric acid (100 ml), water (2x20 ml) and brine (10 ml), dried (MgSO₄), and evaporated to leave an orange oil. Purification by column chromatography on silica gel (eluent: petroleum ether) gave 0.84 g (68.5%) of the title compound, mp 92-94°C (from ether-methanol).

Anal. Calculated for C₁₃H₂₈O: C 88.16, H 11.84
Found: C 88.08, H 11.76

1H NMR δ 0.78 (s,3H), 0.90 (s,3H), 0.92 (s,3H), 0.94 (s,3H), 1.11 (s,3H), 1.58 (bs,3H), 1.59 (s,3H), 1.69 (s,3H), 1.10-2.40 (m,21H), 5.12 (m,1H), 5.44 (m,2H)

**Example 21**

**Epoxidation of 3β-Hydroxyprotosta-17(20)Z,24-diene**

To a stirred solution of 3β-hydroxyprotosta-17(20)Z,24-diene (2.56 g, 6 mmol) in dichloromethane (30 ml) was added dropwise at 0-5°C 80% m-chloroperbenzoic acid (1.29 g, 6 mmol) dissolved in dichloromethane (30 ml).
After the addition was finished (15 minutes), the mixture was stirred at room temperature for another 30 minutes and evaporated. The residue was redissolved in ether (60 ml), and washed with 0.5 M sodium hydrogen carbonate (6 x 15 ml) and water (2 x 10 ml), dried (MgSO₄), and evaporated to give 2.78 g of a colourless foam which, in addition to minor amounts of the starting material, consisted of four more polar compounds, as revealed by TLC (petroleum ether - ethyl acetate 70:30; Rf values given below). These could be separated by column chromatography on silica gel eluting with 5% to 20% ethyl acetate in petroleum ether.

A. 17β,20β-Epoxy-3β-hydroxyprostost-24-ene
Yield: 0.91 g (34.2%); crystals from ether-hexane, mp 113-115°C; Rf 0.42.

1H NMR δ 0.79 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.01 (s, 3H), 1.13 (s, 3H), 1.23 (s, 3H), 1.62 (bs, 3H), 1.69 (bs, 3H), 1.10-1.80 (m, 19H), 1.90-2.15 (m, 5H), 3.24 (m, 1H)
5.10 (m, 1H)

B. 17α,20α-Epoxy-3β-hydroxyprostost-24-ene
Yield: 1.07 g (40.3%); crystals from ether-hexane, mp 110-112°C; Rf 0.32.

1H NMR δ 0.79 (s, 3H), 0.89 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.15 (s, 3H), 1.22 (s, 3H), 1.62 (bs, 3H), 1.69 (bs, 3H), 0.75-2.35 (m, 24H), 3.24 (dd,1H), 5.09 (m, 1H)

C. 17β,20β: 24,25-Diepoxy-3β-hydroxyprostostane
Yield: 0.24 g (8.7%); crystals from ether-hexane, mp 89-95°C; Rf 0.22.

1H NMR δ 0.79 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.01 (s, 3H), 1.12 (s, 3H), 1.23-1.25 (s, 3H), 1.28 (s, 3H), 1.32 (s, 3H), 1.10-2.10 (m, 24H), 2.71 (m, 1H), 3.24 (dd, 1H)

D. 17α,20α: 24,25-Diepoxy-3β-hydroxyprostostane
Yield: 0.17 g (6.2%); crystals from ether-hexane, mp 134-140°C; Rf 0.16.
$^1$H NMR δ 0.80 (s,3H), 0.88 (s,3H), 0.93-0.94 (s,3H), 0.99 (s,3H), 1.16 (s,3H), 1.21-1.23 (s,3H), 1.29 (s,3H), 1.32 (s,3H), 1.10-2.07 (m,23H), 2.29 (dd,1H), 2.72 (t,1H), 3.25 (dd,1H)

**Example 22**

**Epoxidation of Methyl 3β-Hydroxyprotosta-17(20)Z,24-dien-29-oate**

By following the procedure described in Example 21 but substituting 3β-hydroxyprotosta-17(20)Z,24-dien-29-oate for the 3β-hydroxyprotosta-17(20)Z,24-diene, the following five epoxy derivatives were prepared and separated by column chromatography on silica gel eluting with 10% to 60% ether in petroleum ether. Rf values of the new compounds, as determined by TLC in petroleum ether - ether 40:60, are given below.

**A. Methyl 17β,20β-Epoxy-3β-hydroxyprotost-24-en-29-oate**

Yield: 1.70 g (34.9%); crystals from ether, mp 149-152°C; Rf 0.46.

$^1$H NMR δ 0.78 (s,3H), 1.01 (s,3H), 1.12 (s,3H), 1.23 (s,3H), 1.39 (s,3H), 1.62 (bs,3H), 1.69 (bs,3H), 1.10-2.15 (m,23H), 3.10 (m,1H), 3.65 (s,3H), 3.73 (d,1H), 5.09 (m,1H)

**B. Methyl 24,25-Epoxy-3β-hydroxyprotost-17(20)Z-en-29-oate**

Yield: 0.50 g (10.3%); colourless foam; Rf 0.40.

$^1$H NMR δ 0.79 (s,3H), 1.10 (s,3H), 1.14 (dd,1H), 1.30 (s,3H), 1.42 (s,3H), 1.59 (m,3H), 1.61 (bs,3H), 1.69 (bs,3H), 1.20-1.75 (m,11H), 1.85-2.27 (m,10H), 2.30 (bd,1H), 4.12 (d,1H, J=6.1Hz), 4.42 (d,1H, J=6.1Hz), 4.65 (dd,1H), 5.12 (m,1H)

**C. Methyl 17α,20α-Epoxy-3β-hydroxyprotost-24-en-29-oate**

Yield: 1.26 g (25.9%); colourless foam; Rf 0.36.

$^1$H NMR δ 0.78 (s,3H), 0.90 (s,3H), 1.15 (s,3H), 1.22 (s,3H), 1.40 (s,3H), 1.62 (bs,3H), 1.69 (bs,3H), 1.10-2.30 (m,23H), 3.10 (m,1H), 3.65 (s,3H), 3.70 (d,1H), 5.08 (m,1H)
D. Methyl 17β,20β,24,25-Diepoxy-3β-hydroxyprotostan-29-oate
Yield: 0.45 g (9.0%); crystals from ether, mp 185-188°C; Rf 0.28.

$^1$H NMR δ 0.78 (s,3H), 1.01 (s,3H), 1.11 (s,3H), 1.25
(s,3H), 1.27 (s,3H), 1.32 (s,3H), 1.39 (s,3H), 1.10-2.06
(m,23H), 2.71 (m,1H), 3.10 (m,1H), 3.65 (s,3H), 3.73 (d,1H)

E. Methyl 17α,20α,24,25-Diepoxy-3β-hydroxyprotostan-29-oate
Yield: 0.29 g (5.8%); colourless foam; Rf 0.19

$^1$H NMR δ 0.78 (s,3H), 0.88 (s,3H), 1.15 (s,3H), 1.22
(s,3H), 1.28 (s,3H), 1.32 (s,3H), 1.40 (s,3H), 1.15-2.05
(m,22H), 2.27 (m,1H), 2.71 (t,1H), 3.11 (m,1H), 3.65
(s,3H), 3.72 (d,1H)

Example 23
3β-Mesyloxyprotosta-17(20)Z,24-diene

To an icecold solution of 3β-hydroxyprotosta-
17(20)Z,24-diene (0.43 g, 1.0 mmol) in pyridine (25 ml) was
added methanesulfonyl chloride (0.26 ml, 1.5 mmol) with
stirring. The mixture was stirred at 0-5°C for one hour and
then kept in the refrigerator overnight. After addition of
methanol (0.15 ml) and stirring for 10 minutes at 0-5°C,
the mixture was poured onto icecold water (5 ml). Ethyl
acetate (10 ml) and 4 N aqueous hydrochloric acid (10 ml)
were added with stirring, and the mixture was transferred
to a separating funnel. The aqueous layer (pH 2) was separ-
ated and the organic phase washed with water (2 x 5 ml) and
brine (5 ml), dried (MgSO$_4$) and evaporated. The resulting
oil was crystallized from ether-hexane to give 0.39 g
(77.3%) of the title compound, mp 99-102°C.

$^1$H NMR δ 0.75 (s,3H), 0.87 (s,3H), 0.97 (s,3H), 1.03
(s,3H), 1.12 (s,3H), 1.58 (m,3H), 1.60 (bs, 3H), 1.68 (bs,
3H), 1.05-2.37 (m,23H), 3.02 (s,3H), 4.37 (dd,1H), 5.11
(m,1H)
Example 24

3β,29-Epoxyprotosta-17(20)Z,24-diene

To a stirred solution of 3β,29-dihydroxyprotosta-17(20)Z,24-diene,29-monotosylate (0.60 g, 1.0 mmol) in dry tetrahydrofuran (8 ml) was added dropwise (5 minutes) 1 M lithium triethylhydroborate in tetrahydrofuran (4 ml), and the mixture was stirred for 45 minutes. A few drops of water, followed by 2 N sodium hydroxide (2 ml) and 30% aqueous hydrogen peroxide, were added, and the mixture was stirred for a further 30 minutes. After addition of water (20 ml), the product was extracted with ethyl acetate (20 + 10 ml). The combined organic phases were washed with water (2 x 5 ml) and brine (10 ml), dried (MgSO₄) and evaporated. The residual oil was purified by column chromatography on silica gel (eluting with 5% ethyl acetate in petroleum ether) to afford 0.38 g (89.5%) of the title compound which was crystallized from ether-methanol, mp 83-85°C.

1H NMR δ 0.79 (s,3H), 1.10 (s,3H), 1.14 (dd,1H), 1.30 (s,3H), 1.42 (s,3H), 1.59 (m,3H), 1.61 (bs,3H), 1.69 (bs,3H), 1.20-1.75 (m,11H), 1.85-2.27 (m,10H), 2.30 (bd,1H), 4.12 (d,1H, J=6.1 Hz), 4.42 (d,1H, J=6.1 Hz), 4.65 (dd,1H), 5.12 (m,1H)

EIMS: calcd for C₃₀H₄₈O (M⁺) 424.4, found 424.3

Example 25

3β,20(R)-Dihydroxyprotosta-16,24-diene

A. From 17β,20β-Epoxy-3β-hydroxyprotost-24-ene by Lithium Aluminium Hydride Treatment / Acid Hydrolysis.

17β,20β-Epoxy-3β-hydroxyprotost-24-ene (111 mg, 0.25 mmol) was added to a solution of lithium aluminium hydride (57 mg, 1.50 mmol) in dry ether (5 ml), and the mixture was refluxed for 3.5 hours. Ethyl acetate (5 ml) and 2 N sulfuric acid (2.5 ml) were added, and the mixture was kept at room temperature for 2 days. The aqueous layer was separated and extracted with ethyl acetate (5 ml), and the combined organic phases were washed with 1 M sodium hydrogen carbonate (2 x 5 ml), water (2 x 5 ml) and brine (5 ml),
dried (MgSO₄) and evaporated. The oily residue (106 mg) was purified by column chromatography on silica gel (eluting with 15% to 25% ethyl acetate in pentane) to afford 15 mg (13.6%) of the desired compound as a colourless oil.

$^1$H NMR δ 0.79 (s, 3H), 1.10 (s, 3H), 1.14 (dd, 1H), 1.30 (s, 3H), 1.42 (s, 3H), 1.59 (m, 3H), 1.61 (bs, 3H), 1.69 (bs, 3H), 1.20-1.75 (m, 11H), 1.85-2.27 (m, 10H), 2.30 (bd, 1H), 4.12 (d, 1H, J=6.1Hz), 4.42 (d, 1H, J=6.1Hz), 4.65 (dd, 1H), 5.12 (m, 1H)

EIMS: calcd for C₃₀H₄₈O (M⁺-H₂O) 424.3705, found 424.356.

B. From 17β,20β-Epoxy-3β-hydroxyprogost-24-ene by Treatment with Aluminium Chloride

A solution of 17β,20-epoxy-3β-hydroxyprogost-24-ene (0.44 g, 1.0 mmol) and triethylamine (0.21 ml, 1.5 mmol) in dry tetrahydrofuran (8 ml) added to a dried 50 ml 2-necked round-bottomed flask equipped with a magnetic stirring bar and a septum cap. The flask was cooled to 0°C, 0.375 M aluminium chloride (anhydrous) in dry tetrahydrofuran (2 ml, 0.75 mmol) was added by syringe, and the mixture was stirred for 10 minutes at 0-5°C and then for 5 hours at room temperature. The reaction mixture was poured into cold water (40 ml), and the product was extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with water (2 x 10 ml) and brine (10 ml), dried (MgSO₄) and evaporated to yield 0.58 g of the crude product. Crystallization from ether-hexane gave 0.33 g (77.7%) of the pure compound, mp 151-154°C.

Example 26
3β,20(S)-Dihydroxyprogost-16,24-diene

17β,20-Epoxy-3β-hydroxyprogost-24-ene (221 mg, 0.5 mmol) and triethylamine (0.105 ml, 0.75 mmol) were dissolved in dry tetrahydrofuran (4 ml) in a dried 10 ml 2-necked round-bottomed flask equipped with a magnetic stirring bar and a septum inlet. After establishing an argon
atmosphere, the flask was cooled to 0°C, and 0.375 M aluminium chloride (anhydrous) in dry tetrahydrofuran (0.4 ml, 0.15 mmol) was added by syringe with stirring. The reaction was stirred for 5 minutes at 0-5°C and then for 42 hours at room temperature. The mixture was poured into icecold water (25 ml) and extracted with ethyl acetate (20 + 10 ml), and the combined organic phases were washed with water (2 x 10 ml) and brine (10 ml), dried (MgSO₄) and evaporated to afford 232 mg of crude product. Purification by column chromatography on silica gel eluting with 15% to 25% of ethyl acetate in petroleum ether gave, in addition to 106 mg of unreacted starting material, 68 mg (30.7%) of the title compound which was crystallized from ether-hexane, mp 122-128°C.

1H NMR δ 0.79 (s,3H), 0.92 (s,3H), 0.99 (s,6H), 1.19 (s,3H), 1.28 (s,3H), 1.60 (bs, 3H), 1.68 (bs,3H), 1.00-2.00 (m,21H), 2.22 (bd,1H), 2.71 (m,1H), 3.23 (dd,1H), 5.11 (m,1H), 5.44 (m,1H)

EIMS: calcd for C₃₀H₄₈O (M⁺-H₂O) 424.3705, found 424.36

Example 27
3β,20,24-Trihydroxyprogostane

3β-Hydroxyprogost-a-17(20)Z,24-diene (427 mg, 1.0 mmol) was dissolved in dry tetrahydrofuran (10 ml) in a dried 50 ml round-bottomed flask equipped with a magnetic stirring bar and a septum cap. After establishing an argon atmosphere, the stirred solution was cooled to 0°C and 1 M borane in tetrahydrofuran (12 ml) was added by syringe. The mixture was stirred for 5 minutes at 0-5°C and for 18 hours at room temperature. The flask was cooled to 0°C, and water (1 ml) was added, followed by 2 N sodium hydroxide (12 ml) and 30% hydrogen peroxide (3.6 ml). After stirring for 1 hour at room temperature, the reaction mixture was poured into water (40 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic extracts were washed with water (4 x 10 ml) and brine (20 ml), dried (MgSO₄) and evaporated
to yield 580 mg of a solid residue. Purification by column chromatography on silica gel (50% ethyl acetate in petroleum ether as eluant) gave 110 mg of a less polar product (A), characterized as a 4:1 mixture of two diastereomeric 3β,20,24-trihydroxyprotostanes, and 302 mg of a more polar product (B) representing an approximate 2:1 mixture of two other 3,20,24-trihydroxyprotostane diastereomers. The latter was crystallized from ether, mp 179-182°C.

Product (A)

$^1$H NMR: (A) δ 0.78 (s,3H), 0.91 (s,6H), 0.92 (d,6H), 0.98 (s,3H), 1.09 (s,3H), 1.21 (s,3H), 1.05-2.20 (m,28H), 3.23 (dd,1H), 3.33 (m,1H)

EIMS: calcd for C$_{30}$H$_{50}$O ($M^+\text{-2H}_2$O) 426.3862, found 426.38

Product (B)

$^1$H NMR: (B) δ 0.78 (s,3H), 0.83 (s,3H), 0.92 (s,3H), 0.92 (d,6H), 0.98 (s,3H), 1.11 (s,3H), 1.13 (s,3H), 1.10-2.00 (m,28H), 3.24 (dd,1H), 3.32 (m,1H)

EIMS: calcd for C$_{30}$H$_{50}$O ($M^+\text{-2H}_2$O) 426.3862, found 426.28

Example 28

3β,17-Dihydroxyprotostane

3β-Hydroxyprotostet-17(20)Z-ene (429 mg, 1.0 mmol) was added to a dried 50 ml round-bottomed flask equipped with a stirring bar and a septum cap and connected to an argon/vacuum line. The flask was evacuated and filled with argon, and dry tetrahydrofuran (10 ml) was added. The stirred solution was cooled to 0°C, and 1 M borane in tetrahydrofuran (6 ml) was added by syringe. The reaction was stirred for 5 minutes at 0°C and then for 18 hours at room temperature. The flask was cooled to 0-5°C, and water (1 ml) was added with stirring, followed by 2 N sodium hydroxide (6 ml) and 30% hydrogen peroxide (1.8 ml). After stirring for 1 hour at room temperature, the reaction
mixture was transferred to a separating funnel, diluted with water (40 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic extracts were washed with water (2 x 20 ml) and brine (20 ml), dried (MgSO$_4$) and evaporated to give 486 mg of a solid product. The residue was subjected to column chromatography on silica gel eluting with 15% to 25% ethyl acetate in petroleum ether to give, in addition to 221 mg of unreacted starting material, 75 mg of the title compound which crystallized from ether-hexane, mp 147-150°C.

$^1$H NMR $\delta$ 0.79 (s,3H), 0.83 (s,3H), 0.87 (d,3H), 0.87 (d,3H), 0.91 (s,3H), 0.96 (s,3H), 0.98 (s,3H), 1.16 (s,3H), 1.00-2.00 (m,29H), 3.25 (dd,1H)

EIMS: calcd for C$_{30}$H$_{52}$O ($M^+$-H$_2$O) 428.4018, found 428.30
WHAT WE CLAIM IS:

1. A compound of the formula II

wherein

- $R^1$ stands for hydrogen or methyl;
- $R^2$ is hydrogen, methyl, $\text{CH}_2\text{OH}$, $\text{CH}_2\text{OR}^3$, $\text{CHO}$, $\text{CH=CH}_2$, COOH or $\text{COOR}^4$;
- $R^3$ stands for straight or branched ($C_1$-$C_6$) alkyl, aralkyl or aryl, optionally substituted with halogen, hydroxy or carboxy; alkanesulfonyl or arenesulfonyl; ($C_1$-$C_4$) alkanoyl or aroyl, optionally substituted with halogen, hydroxy or carboxy;
- $R^4$ stands for straight or branched ($C_1$-$C_6$) alkyl, ($C_2$-$C_6$) alkenyl, ($C_2$-$C_6$) alkynyl, aralkyl, aryl, alkanoyloxy-alkyl or dialkylaminoethyl;

$Q^1$ and $Q^2$ are each independently hydrogen, hydroxy or a group $\text{OR}^3$; or, taken together, $Q^1$ and $Q^2$ stand for oxygen; or $Q^1$ ($Q^2$) and $R^1$ ($R^2$), when taken together, constitute a double bond that connects carbon atoms 3 and
4; or Q² and R², when taken together with carbon atoms 3 and 4, may form an oxetane ring;

X is hydrogen; or X and Q¹ (Q²), when taken together, form a double bond connecting carbon atoms 2 and 3;

the C24,25-bond is a double bond or a single bond;
and, additionally, one or more of the double bonds connecting carbon atoms 2 and 3, 3 and 4, 17 and 20, and/or 24 and 25 may optionally be epoxidized with formation of an oxirane ring or hydrated to give a carbon-carbon single bond where one of the carbon atoms is substituted with hydroxy,

with the proviso that when, at the same time, the C24,25-bond is a double bond, Q¹ is hydrogen, Q² is hydroxy, and R¹ is methyl, then R² cannot be methyl or hydroxymethyl;

and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof.

2. A compound according to claim 1 having the formula I and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof.
3. A compound according to claim 1 in which $Q^1$ is hydrogen, $Q^2$ is hydroxy, $R^1$ is methyl, $R^2$ is methyl, hydroxymethyl, carboxy, methoxycarbonyl or hydrogen, and where one or both of the double bonds connecting carbon atoms 17 and 20 and 24 and 25 are epoxidized with formation of an oxirane ring.

4. A compound according to claim 1 in which $Q^1$ is hydrogen, $Q^2$ is hydroxy, $R^1$ is methyl, $R^2$ is methyl, hydroxymethyl, carboxy, methoxycarbonyl or hydrogen, and where one or both of the double bonds connecting carbon atoms 17 and 20 and 24 and 25 are hydrated.

5. A compound according to claim 1 which is

a) $17\beta,20\beta$-Epoxy-3$\beta$-hydroxyprotost-24-ene,

b) $17\alpha,20\alpha$-Epoxy-3$\beta$-hydroxyprotost-24-ene,

c) $17\beta,20\beta;24,25$-Diepoxo-3$\beta$-hydroxyprotostane,

d) $17\alpha,20\alpha;24,25$-Diepoxo-3$\beta$-hydroxyprotostane,

e) Methyl $24,25$-Epoxy-3$\beta$-hydroxyprotost-17(20)$Z$-en-29-oate,

f) $3\beta,29$-Epoxyprotosta-17(20)$Z,24$-dien,

g) $3\beta,20(R)$-Dihydroxyprotosta-16,24-diene,

h) $3\beta,20(S)$-Dihydroxyprotosta-16,24-diene, or

i) $3\beta,20,24$-Trihydroxyprotostane.

6. A diastereoisomer of a compound according to claims 1, 3-4, 5c-5e and 5i in pure form; or a mixture of such diastereoisomers.
7. A method for producing a compound according to claim 1 in which

a) an alkyl ester of formula I is reacted with lithium aluminium hydride to form a protostanediol of formula 4;

\[ R = \text{alkyl} \]

b) the compound of formula 4 is treated with \( p \)-toluenesulfonyl chloride in the presence of a base (e.g. pyridine) to produce a mixture of tosylates of formulas 5a and 5b which were separated;

\[ 5a \quad R=\text{H} \quad 5b \quad R=\text{Ts} \]

c) the monotosylate of formula 5a is reduced with lithium aluminium hydride to form a protosterol of formula 6, or with lithium triethylhydroborate to give an oxetane of formula 24;
d) the compound of formula 6 is treated with an organic peracid (e.g. m-chloroperbenzoic acid) to form a mixture of epoxides which is separated to yield the desired 17\(\beta\),20\(\beta\)- and 17\(\alpha\),20\(\alpha\)-epoxy-3\(\beta\)-hydroxyprotost-24-enes in pure form and the corresponding 17\(\beta\),20\(\beta\);24,25- and 17\(\alpha\),20\(\alpha\);24,25-di-epoxy-3\(\beta\)-hydroxyprotostanes as C24 diastereomeric mixtures;

e) alternatively, the compound of formula 6 is subjected to hydroboration (e.g. with borane) followed by oxidation (e.g. with hydrogen peroxide/sodium hydroxide) to form a mixture of diastereomeric 3\(\beta\),20,24-trihydroxyprotostanes;

f) alternatively, the compound of formula 6 is subjected to oxymercuration (e.g. with mercury(II)acetate) followed by demercuration (e.g. with sodium borohydride) to form a mixture of diastereomeric trihydroxyprotostanes.

8. A pharmaceutical composition containing a compound according to claim 1, alone or together with pharmaceutically acceptable, non-toxic carriers and/or auxiliary agents, for use as a cholesterol-lowering and anti-atherosclerosis agent.
9. A pharmaceutical composition comprising a compound of formula II, in which the C24,25-bond is a double bond, 
Q^1 is hydrogen, Q^2 is hydroxy, R^1 is methyl, and R^2 is 
methyl or hydroxymethyl, alone or together with pharma-
aceutically acceptable, non-toxic carriers and/or auxiliary 
agents, for use as a cholesterol-lowering and anti-athero-
sclerosis agent.

10. A method of treating patients in need of treatment 
characterized in administering to said patients an effec-
tive amount of one or more compounds according to claim 1, 
if necessary together or concomitantly with one or more 
other therapeutically active components.

11. The use of a compound of claim 1 in the manufacture 
of a medicament for the treatment and prophylaxis of hyper-
cholesterolemia and atherosclerosis.
### A. CLASSIFICATION OF SUBJECT MATTER

| IPC 6 | C07J13/00 | A61K31/56 | C07J71/00 | C07J15/00 | C07J31/00 | C07J21/00 |

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

| IPC 6 | C07J | A61K |

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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<tbody>
<tr>
<td>X</td>
<td>US,A,3 598 811 (IMMER HANS U) 10 August 1971 see examples 7,9,10</td>
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<td>JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 113, no. 21, 9 October 1991, DC US, pages 8171-8172, XP0002017284 E. J. COREY ET AL: &quot;New Mechanistic and Stereocchemical Insights on the Biosynthesis of Sterols mfrom 2,3-Oxidosqualene&quot; see page 1871, compound 13</td>
<td>1,4,6</td>
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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

**Date of the actual completion of the international search**

30 October 1996

**Date of mailing of the international search report**

28.11.96

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016

**Authorized officer**

Watchorn, P
<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>TETRAHEDRON LETTERS, no. 13, 1969, OXFORD GB, pages 1023-1026, XP002017288 TETSUYASU HATTORI ET AL: &quot;3.beta.-Hydroxy-4.beta.-methylfusida-17(20)[16,21-cis], 24-diene[3.beta.-Hydroxy-protosta-17(20)[16,21-cis], 24-diene and a Related Triterpene Alcohol&quot; see page 1024; examples VI,VII</td>
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<td>TETRAHEDRON LETTERS, no. 46, 1968, OXFORD GB, pages 4769-4772, XP002017289 SHIGENOBU OKUDA ET AL: &quot;Isolation of 3.beta.-Hydroxy-4.beta.-hydroxymethylfusid a-17(20)[16,21-cis], 24-diene&quot; see page 4770; example VI</td>
<td>1,4,6</td>
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</table>
This International Search Report has not been established in respect of certain claims under Article 17(3)(a) for the following reasons:

1. **X** Claims Nos.: Claim 10
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: although claim 10 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compounds.

2. **☐** Claims Nos.: 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. **☐** Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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.

2. **☐** As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. **☐** As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. **☐** 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 claims Nos.:

**Remark on Protest**

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

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)
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