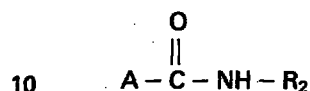


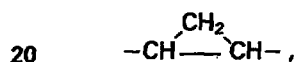
SPECIFICATION

Long-chain fatty acid amides and cyclopropano analogues thereof, their production and their use as anti-atherosclerotic agents

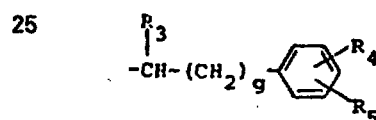
5 The present invention provides a compound of formula I,



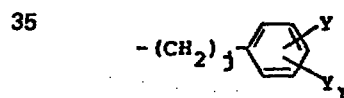
15 in which A is (i) the residue of a long-chain, unsaturated fatty acid (minus the carboxylic acid function), which residue has 7 to 23 carbon atoms and possesses from 1 to 4 ethylenically unsaturated groupings, or (ii) a corresponding residue in which each ethylene (-CH=CH-) grouping is replaced by a cyclopropanyl group



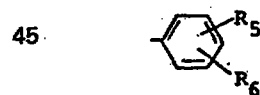
and R₂ is a radical of formula II, III or IV



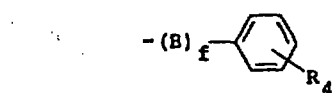
30 in which g is 0 or 1,
R₄ is hydrogen, fluorine, chlorine, bromine or C₁₋₃ alkyl or alkoxy,
R₅ is hydrogen, fluorine, chlorine, or C₁₋₃ alkyl or alkoxy, and
R₃ is hydrogen, C₁₋₈ alkyl or a radical of formula VI,



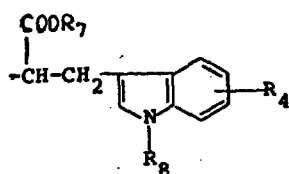
40 in which j is 0 or 1,
Y is hydrogen, fluorine, chlorine, bromine or C₁₋₃ alkyl or alkoxy,
and Y₁ is hydrogen, fluorine, chlorine or C₁₋₃ alkyl or alkoxy;



50 in which R₅ is as defined above,
and R₆ is hydrogen, fluorine, chlorine, bromine C₁₋₃ alkyl or alkoxy, or a group of formula VII,



in which B is -CH₂- or -O-,
f is 0 or 1, and
R₄ is as defined above;



IV

in which R_4 is as defined above,

R_7 is C_{1-8} alkyl or benzyl, and

R_8 is hydrogen, C_{1-8} alkyl or benzyl

provided that when R_2 is a radical of formula II or III then A is as defined under (ii).

In one group of compounds, in accordance with the invention, A is as defined under (i) (A then being A'), i.e. a residue of a long-chain fatty acid, such residue having 7-23 carbon atoms and having 1 to 4 ethylenically unsaturated positions. These residues are thus derived from long chain fatty acids of formula A'-COOH

having 8 to 24 carbon atoms and 1 to 4 ethylenically unsaturated positions. These acids are preferably unbranched and are preferably of the natural fatty acid order, i.e. those containing an even number of carbon atoms. A' is thus preferably unbranched and preferably contains an odd number of carbon atoms.

Particularly preferred residues A' include those of formula IX (Residues A'₁) and X (Residues A'₂):-



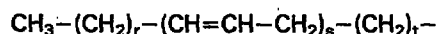
IX

in which n is 1 to 10,

o is 1 to 4, and

p is 3 to 9,

provided that $n + 2 \times o + p$ does not exceed 22,



X

in which r is 1 to 4,

s is 2 to 4, and

t is 1 to 7,

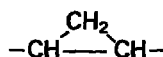
provided that $r + 3 \times s + t$ does not exceed 22.

Preferred residues of formula IX are those in which $n + 2 \times o + p$ is an even number, particularly those where $n = 5$ or 7 , $o = 1$ and $p = 7$. Preferred residues of formula X are those where r is 1 or 4, s is 2 to 4, and t is 2 or 6.

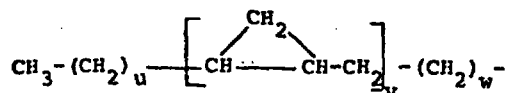
It will be appreciated that the compounds in which A is A' occur in isomeric forms due to the presence of one or more unsaturated positions. While the invention is not intended to be limited to any particular isomeric form, the compounds in which the hydrogen atoms on each pair of ethylenically unsaturated carbon atoms are in *cis*-configuration are preferred.

Particularly preferred radicals A' include those derived from the following acids:- palmitoleic, oleic, petroselinic, vaccenic, punicic (or eleostearic), parinaric, gadoleic, cetoleic, linoleic, linolenic and arachidonic acids, more particularly, oleic, linoleic, linolenic, arachidonic and palmitoleic acid.

In another group of compounds in accordance with the invention, A is as defined under (ii) (A then being A''), i.e. cyclopropanyl derivatives of A' residues in which each ethylene moiety $-CH=CH-$ is replaced by the cyclopropanyl moiety



It is preferred that A'' residues are unbranched [apart from the cyclopropanyl radical(s)]. Particular residues A'' are those of formula XI (A'' then being A''₁).



XI

in which u is 1 to 15,

v is 1 or 2, and

w is 1 to 13,

provided that when v is 1, then $u + w$ is 3 to 19, and that when v is 2, then $u + w$ is 2 to 16.

Particular preferred A_1'' residues of formula XI are as follows:-

- (i) when v is 1, then $u + w = 7$ to 19,
when v is 2, then $u + w = 4$ to 16;
- 5 (ii) when v is 1, then $u + w =$ an odd number, 5
when v is 2, then $u + w =$ an even number;
- (iii) when v is 1, then $u = 5$ or 7 and $w = 6$,
when v is 2, then $u = 4$ and $w = 6$.

In view of these preferences, it will be appreciated that the preferred radicals A'' are derived from mono- or
10 di-unsaturated fatty acids of the type found in nature, e.g. palmitoleic or oleic acid ($v = 1$) and linoleic acid 10
($v = 2$).

It will also be appreciated that the compounds in which A is A'' exist in isomeric forms. While the invention
is not intended to be limited to any particular isomeric form, it is preferred that each pair of hydrogen atoms
attached to the tertiary carbon atoms of the cyclopropyl moieties be in *cis*-configuration.

15 Since residues A'' having a single cyclopropyl unit have a lesser number of asymmetric carbon atoms than 15
those with a plurality, the former are generally easier to refine and are therefore preferred where ease of
purification is an important factor.

In one group of compounds (hereinafter compounds Ia), A is A' and R_2 is a radical of formula IV. When R_7
is alkyl it is preferably unbranched and is more preferably ethyl. When R_4 is alkyl, it is preferably methyl,
20 when it is alkoxy, it is preferably methoxy, and when it is halogen it is preferably fluorine or chlorine. When 20
 R_4 is other than hydrogen, it is preferably in the 5-position of the indole nucleus. R_4 is preferably hydrogen,
however. R_8 is also preferably hydrogen.

In further groups of compounds, A is A' and R_2 is a radical of formula II (compound Ib);
or R_2 is a radical of formula III (compound Ic);
25 or R_2 is a radical of formula IV (compounds Id); 25

The preferred situations for these compounds are the same as those given above for the compounds in
which A is A' .

Compounds Ia) are preferred.

The invention also provides a process for the production of the compounds of formula I, comprising
30 acylating a compound of formula XII, 30



35 in which R_2 is as defined above, with an acid of formula XIII, 35



40 in which A is as defined above, or a reactive derivative thereof. 40

The acylation may be carried out in manner conventional for converting an amine function into its

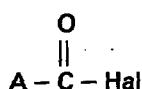
corresponding amide. For example, the process may be effected using the mixed anhydride technique in
45 which the compound of formula XII is treated with a compound of formula XIIIa, 45



in which A is as defined above, and

55 R_{12} is $n\text{-C}_{1-6}$ alkyl. 55

This embodiment may suitably be effected at a temperature of from -10° to $+35^\circ\text{C}$, and in an inert organic
60 medium, e.g. a chlorinated hydrocarbon, such as methylene chloride. Alternatively, the compound of 60
formula XII may be treated with an acyl halide of formula XIIIb,



XIIlb

5

in which A is as defined above, and

Hal is chlorine or bromine. This embodiment is suitably effected at a temperature of from 10° to 50°C, preferably 20° to 30°C, in an inert medium and in the presence of an acid acceptor.

In the acylation process, the compound of formula XII may be employed in the form of an acid addition salt and the reagents may be used in excess, when they are liquid under the reaction conditions, to serve as a reaction medium.

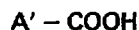
It will be appreciated that the acylation process of the invention does not affect the isomeric form of the starting materials so that the products will result in the same isomeric form as the starting materials.

The resulting compounds of formula I may be isolated and purified using conventional techniques.

The compounds of formula XII are either known or may be produced in conventional manner from available materials.

Compounds of formula XIIIc,

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XIIIc

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in which A' is as defined above,

are also known or may be produced in conventional manner from available materials.

The compounds of formula XIId,



XIId

30

in which A'' is as defined above,

are also conventional and may, for example, be produced by converting the ethylenically unsaturated positions in corresponding compounds of formula XIIIc into cyclopropanyl groups. This may be effected in conventional manner, for example by reaction with methylene diiodide by the method of Simmons-Smith [J.A.C.S. 81, 4256 (1959)]. For preparing compounds having a single cyclopropanyl group, the starting acids may be in *cis* or *trans* form and the product will have the same form. Likewise, mixtures of *cis* and *trans* forms will lead to corresponding mixtures of the product. When a cyclopropane acid (or derivative) is reacted with a compound of formula XII, having asymmetric carbon atoms, it will be appreciated that the resulting compound of formula I will be obtained as a mixture of diastereo-isomers, which may, if desired, be separated in known manner, by e.g. chromatography or crystallisation. Alternatively, if desired, the starting cyclopropane acid may be resolved into its antipodes and then reacted to give the corresponding isomeric product in relatively pure form. Similarly, for preparing cyclopropane acids having 2 or more cyclopropanyl units, the starting olefinic acids have a corresponding number of double bonds and the Simmons-Smith reaction leads to a mixture of diastereoisomers, which may, if desired, be separated before further reaction.

The compounds of formula XIIIa and XIIIb can be produced from the free acids in conventional manner, if convenient *in situ*.

The compounds of formula I of this invention possess pharmacological activity. In particular, the compounds of formula I are indicated for use in controlling the cholesterol ester content of mammalian arterial walls and are therefore particularly indicated for use as anti-atherosclerotic agents, i.e. agents useful in the prophylactic treatment of atherosclerosis and in the controlling of atherosclerotic conditions due to cholesterol ester accumulation in the arterial walls. Such ability of the compounds of the formula I is indicated by known test procedures in which the total cholesterol ester content of cultured cells is shown to be reduced by a test compound, as compared to untreated cells, and carried out, for example by the following procedures:

A) Cell culture

Rhesus monkey smooth muscle cells (from the arterial, e.g. aorta, wall) obtained by the method of K. Fisher-Dzoga et al (Experimental and Molecular Pathology 18, 162-176 (1973)) are routinely grown in 75 cm² tissue culture flasks using Minimum Essential Medium (Eagle) supplemented with 10% fetal bovine serum. For testing, a 75 cm² flask with a near confluent cell growth is selected. The cells are removed from the flask surface by mild enzymatic treatment with pronase. After centrifugation and decanting the enzyme solution, the cell pellet is resuspended in an appropriate volume of media for seeding the desired number of 60 mm tissue culture dishes. Five (5) ml of the diluted cell suspension are pipetted into each dish. After seeding, the dishes are labelled with the cell type, date and flask number of origin and incubated at 37°C in approximately

5% CO₂ atmosphere in a high humidity incubator. When the cultures are confluent, the actual drug testing is begun. Test compounds are routinely solubilised in 100% ethanol. An equivalent amount of ethanol is added to control groups as well. The tissue culture dishes are randomly divided into groups. To one group, hyperlipemic rabbit serum (HRS) is added at 5% by volume (control). To the remaining groups, 5% HRS and 1 mg per 100 ml of media of the test compound are added. The dishes are returned to the incubator for an additional 24 hours. All operations through to the final incubation are performed using sterile technique in a laminar flow hood. After the incubation period, the dishes are microscopically observed with the Zeiss Axiomat with phase contrast optics and the conditions of the cultures are recorded; especially in regard to the size, number and configurations of cytoplasmic inclusions and to cellular morphology. The media is removed from the cultures and 0.9% sodium chloride solution is added. The cells are removed from the flasks with the aid of a rubber policeman and transferred to a conical graduated centrifuge tube. The cells are washed three times by suspending in an isotonic salt solution, centrifuging at 800 × g for 10 minutes and aspirating the supernatant fluid.

B) Cell extraction procedure

An appropriate volume of isopropyl alcohol (about 1 ml/mg protein) is then added to the cell pellet and the sample sonicated with a micro probe (140 × 3 mm) for 10 seconds with a "LO" setting of 50 on a Bronwell Biosonik IV. After centrifugation for 15 minutes at 800 × g, the clear supernatant is decanted and an aliquot taken for cholesterol analysis.

The residue is dissolved in 0.1N sodium hydroxide and an aliquot taken for protein determination by the method of Lowry, et al. (J. Biol. Chem. 193, 265; 1951).

C) Assay

Free cholesterol: The isopropyl alcoholic solutions of standards, samples and blank (isopropyl alcohol alone) are treated in a similar manner. An aliquot of 0.4 ml of free reagent (Reagent A, Table 1 below) is added to a 10 × 75 mm disposable glass test tube to which 20 µl of the isopropyl alcoholic solution is added and mixed. After standing at room temperature for approximately 5 minutes, 0.8 ml of 0.5N sodium hydroxide (Reagent C, Table 1) is added and mixed. The fluorescence is measured with an Aminco-Bowman spectrophotofluorometer with an excitation wavelength of 325 nm and emission wavelength of 415 nm. A 1 cm light patch cuvette is used with a xenon lamp, an IP28 photomultiplier tube and 2 mm slits.

Total cholesterol: The same procedure described above for free cholesterol is followed for total cholesterol except that the total reagent (Reagent B, Table 1) is used instead of the free reagent and the samples are incubated for 20 minutes at 37° before the addition of the 0.5N sodium hydroxide solution (Reagent C, Table 1).

Alternatively, the assay for cholesterol, i.e. Step C (above) obtained from Steps A and B, may be carried out by the method of Ishikawa et al (J. Lipid Res. 15, 286; 1974).

The amount of cholesterol ester is found by subtracting the amount of free cholesterol from the total cholesterol content of the cells determined by the assay. A finding of a lower amount of cholesterol ester in the group of cells to which test compound was added, as compared to the control group (untreated) shows that the test compound is active in reducing the cholesterol ester in the cells.

TABLE 1

Composition of Reagents for Cholesterol Determination			
A. Free Cholesterol Reagent			
Sodium phosphate buffer pH 7.0	.05	M	
Cholesterol oxidase	.08	U/ml	
Horseradish peroxidase	30.	U/ml	
p-Hydroxyphenylacetic acid	.15	mg/ml	
B. Total Cholesterol Reagent			
Sodium phosphate buffer pH 7.0	.05	M	
Cholesterol ester hydrolase	.08	U/ml	
Cholesterol oxidase	.08	U/ml	
Horseradish peroxidase	30.	U/ml	
Sodium taurocholate	5.	mM	
Carbowax-6000	.17	mM	
p-Hydroxyphenylacetic acid	.15	mg/ml	
C. Sodium Hydroxide Solution			
	.5N		

For the above-mentioned use, an indicated suitable daily dosage is from about 100 mg to about 5,000 mg, suitably administered in divided doses of from 25 to 2,500 mg, two to four times daily, or in retard form.

The compounds may be admixed with conventional pharmaceutically acceptable diluents and carriers, and, optionally, other excipients, and administered in such forms as tablets or capsules.

The compounds are preferably administered orally.

The following Examples illustrate the invention.

5

EXAMPLE 1: α -[(1-Oxo-9,12-cis,cis-octadecadienylamino)]-1H-indole-3-propanoic acid ethyl ester

To a solution of 7.0 g of linoleic acid in 150 ml methylene chloride cooled to -20° is added 2.5 g triethylamine and then 2.7 g ethyl chloroformate. The reaction mixture is stirred for two hours and gradually allowed to warm to room temperature. There is then added 2.5 g triethylamine followed by 6.7 g of DL

10 tryptophan ethyl ester hydrochloride and the reaction mixture is stirred for 18 hours. The reaction mixture is then extracted several times with 2N aqueous sodium hydroxide washed with saturated aq. sodium chloride solution, and the organic phase dried over anhydrous sodium sulphate, filtered and the filtrate evaporated i.v. to dryness. The residue is then filtered over silica gel using chloroform as the eluant to obtain the title product in solvent, which is then evaporated to yield the title product as a waxy solid.

15

EXAMPLE 2:

In manner analogous to Example 1 and employing appropriate starting materials in approximately equivalent amounts, the following compounds may be obtained:-

- a) α -[(1-oxo-9-cis-octadecenylamino)]-1H-indole-3-propanoic acid, ethyl ester (waxy solid);
- 20 b) α -[(1-oxo-9,12,15-cis,cis,cis-octadecatrienylamino)]-1H-indole-3-propanoic acid, ethyl ester;
- c) α -[(1-oxo-9-cis-hexadecenylamino)]-1H-indole-3-propanoic acid, ethyl ester;
- d) α -[(1-oxo-5,8,11,14-cis,cis,cis-eicosatetraenyl-amino)]-1H-indole-3-propanoic acid, ethyl ester;
- e) 2-(1-oxo-9-cis-octadecenylamino)-3-(1-methylindolyl)-propanoic acid, ethyl ester;
- 25 f) 2-(1-oxo-9-cis-octadecenylamino)-3-(1H-5-methoxyindolyl)-propanoic acid, ethyl ester;
- g) 2-(1-oxo-9-cis-octadecenylamino)-3-(1H-5-chloroindolyl)-propanoic acid, ethyl ester;
- 25 h) 2-(1-oxo-9-cis-octadecenylamino)-3-(1-benzylindolyl)-propanoic acid, ethyl ester
- i) α -[(1-oxo-9,12-cis,cis-octadecadienylamino)]-1H-indole-3-propanoic acid, *n*-propyl ester;
- k) α -[(1-oxo-9,12-cis,cis-octadecadienylamino)]-1H-indole-3-propanoic acid, *n*-octyl ester; (m.p. $66-68^{\circ}\text{C}$)
- l) α -[(1-oxo-9,12-cis,cis-octadecadienylamino)]-1H-indole-3-propanoic acid, benzyl ester;
- 30 m) α -[(1-oxo-9-cis-octadecenylamino)]-1H-indole-3-propanoic acid, *n*-propyl ester;
- n) α -[(1-oxo-9-cis-octadecenylamino)]-1H-indole-3-propanoic acid, *n*-butyl ester;
- o) α -[(1-oxo-9-cis-octadecenylamino)]-1H-indole-3-propanoic acid, *n*-octyl ester;
- p) α -[(1-oxo-9-cis-octadecenylamino)]-1H-indole-3-propanoic acid, benzyl ester;
- q) N-(α -methylbenzyl)-cis-2-octyl-cyclopropanoetanamide;
- 35 [m.p. $44-46^{\circ}$] (produced from d(+)- α -methylbenzylamine);
- r) α -[(1-oxo-cis-2-octyl-cyclopropanoetylaminol)]-1H-indole-3-propanoic acid, ethyl ester;
- s) N-(α -methylbenzyl)-cis-2-octyl-cyclopropanoetanamide;
- (mixture of 2 racemates; m.p. $30-32^{\circ}$); (produced from d, 1- α -methylbenzylamine)
- 40 t) N-(*o*-methylphenyl)-cis-2-octyl-cyclopropanoetanamide;
- u) N-(α -(*p*-methylbenzyl)-benzyl)-cis-2-octyl-cyclopropanoetanamide;
- (m.p. $78-81^{\circ}\text{C}$)
- v) N-(α -methylbenzyl)-cis-2-hexyl-cyclopropanoetanamide;
- w) N-(α -methylbenzyl)-trans-2-octyl-cyclopropanoetanamide;
- 45 x) N-(α -methylbenzyl)-cis-2-tetradecylcyclopropanobutanamide;
- y) N-(α -(*p*-methylbenzyl)-*p*-methyl-phenylethyl)-cis-2-octyl-cyclopropanoetanamide;
- (m.p. $60-65^{\circ}\text{C}$)
- z) N-(α -methylbenzyl)-cis,cis-2-[(2-pentylcyclopropyl)-methyl]cyclopropanoetanamide; [mixtures of diastereoisomers - oil - produced from d(+)- α -methylbenzylamine];
- 50 aa) α -cis,cis-[(1-oxo-2-pentylcyclopropyl)-methyl]-cyclopropanoetanaminol)-1H-indole-3-propanoic acid, ethyl ester; (from DL-tryptophan ethyl ester, hydrochloride)
- ab) N-(α -methylbenzyl)-cis,cis-2-[2-pentylcyclopropyl)-methyl]-cyclopropanoetanamide (mixture of diastereoisomers - produced from (d,1)- α -methylbenzylamine);
- ac) N-(*o*-methylphenyl)-cis,cis-2-[(2-pentylcyclopropyl)-methyl]-cyclopropanoetanamide;
- 55 ad) N-(α -(benzyl)- β -(phenyl)ethyl)-cis-2-octylcyclopropanoetanamide [m.p. $40-45^{\circ}$];
- ae) 2-(1-oxo-9-cis-octadecenylamino)-3-(1H-5-fluoroindolyl)-propionic acid, ethyl ester (wax);

The compounds of formula I are also indicated for use as general agents for the lowering of serum cholesterol and cholesterol ester levels as indicated by oral administration at a dose of 200 mg/kg of the test compound per day for 9 weeks to rabbits in conjunction with a high cholesterol diet resulting in, compared to 60 controls, a reduction in cholesterol and cholesterol ester serum levels, as well as a lessened formation or 60 absence of arterial wall plaques.

For this use, the indicated dosage is the same as described above.



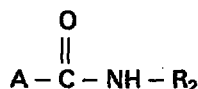
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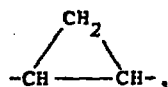
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CLAIMS

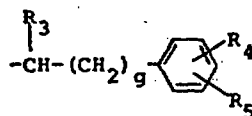
1. A compound of formula I



in which A is (i) the residue of a long-chain, unsaturated fatty acid (minus the carboxylic acid function), which residue has 7 to 23 carbon atoms and possesses from 1 to 4 ethylenically unsaturated groupings, or (ii) a corresponding residue in which each ethylene ($-\text{CH}=\text{CH}-$) grouping is replaced by a cyclopropanyl group



and R_2 is a radical of formula II, III or IV

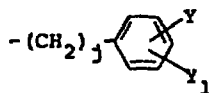


in which g is 0 or 1,

R_4 is hydrogen, fluorine, chlorine, bromine or C_{1-3} alkyl or alkoxy,

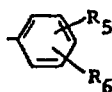
R_5 is hydrogen, fluorine, chlorine, or C_{1-3} alkyl or alkoxy, and

R_3 is hydrogen, C_{1-8} alkyl or a radical of formula VI,



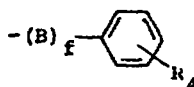
in which j is 0 or 1,

Y is hydrogen, fluorine, chlorine, bromine or C_{1-13} alkyl or alkoxy, and Y_1 is hydrogen, fluorine, chlorine or C_{1-3} alkyl or alkoxy;



in which R_5 is as defined above,

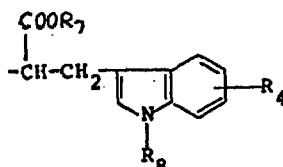
and R_6 is hydrogen, fluorine, chlorine, bromine C_{1-3} alkyl or alkoxy, or a group of formula VII,



in which B is $-\text{CH}_2-$ or $-\text{O}-$,

f is 0 or 1, and

R_4 is as defined above;





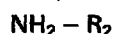
in which R_4 is as defined above,

R_7 is C_{1-8} alkyl or benzyl, and

R_8 is hydrogen, C_{1-8} alkyl or benzyl

provided that when R_2 is a radical of formula II or III then A is as defined under (ii).

- 5 2. A compound according to Claim 1, in which A is as defined under (i). 5
3. α -[(1-Oxo-9-*cis*-octadecenylamino)]-1H-indole-3-propanoic acid, ethyl ester.
4. A compound according to Claim 1, in which A is as defined under (ii).
5. A compound according to Claim 4, in which R_2 is a radical of formula II.
6. N-[α -(Benzyl)- β -(phenyl)ethyl]-*cis*-2-octyl-cyclopropanooctanamide.
- 10 7. A process for the production of a compound of formula I, stated in Claim 1, characterised by acylating a 10
compound of formula XII,



XII

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in which R_2 is defined above, with an acid of formula XIII,



XIII

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in which A is as defined above, or a reactive derivative thereof.

8. A pharmaceutical composition comprising a compound of any one of the preceding claims, in 25
association with a pharmaceutically acceptable diluent or carrier.
9. A compound of any one of Claims 1 to 7 for use as an anti-atherosclerotic agent. 25