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(54) **FATTY ACID DERIVATIVES OF BILE ACIDS AND BILE ACID DERIVATIVES**
FETTSÄUREDERRIVATE VON GALLENSÄURE UND GALLENSÄUREDERRIVATEN
DERIVES D'ACIDES GRAS D'ACIDES BILIAIRES ET DERIVES D'ACIDE BILIAIRE

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- (73) Proprietor: **Galmed International Ltd. B'Kara BKR 08 (MT)**
- (72) Inventor: **GILAT, Tuvia 64235 Tel Aviv (IL)**
- (74) Representative: **Weber, Thomas, Dr. et al Patentanwälte von Kreisler-Selting-Werner, Deichmannhaus am Dom 50667 Köln (DE)**
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US-A- 4 439 366 US-A- 4 440 688
- **D. KRITCHEVSKY ET AL: "Novel Derivatives of 3.alpha.,7.alpha.-Dihydroxy-5.beta.-cholan-24-oic-Acid (Chenodeoxycholic Acid) and 3.alpha.,7.beta.-Dihydroxy-5.beta.-cholan-24-oic Acid (Ursodeoxycholic Acid)" STEROIDS: STRUCTURE, FUNCTION, AND REGULATION., vol. 47, no. 1, 1986, pages 41-48, XP002105331 MA US**
 - **T. SUGAI ET AL: "Efficient Lipase-Catalyzed Preparation of Long Chain Fatty Acid Esters of Bile Acids: Biological Activity and Synthetic Application of the Products" BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY., vol. 60, no. 12, December 1996, pages 2059-2063, XP002105332 TOKYO JP**
 - **M. KELSEY ET AL: "Characterization of Microbial Metabolites of Sulfolithocholic Acid by High Performance Liquid Chromatography" JOURNAL OF STEROID BIOCHEMISTRY, vol. 14, no. 2, 1981, pages 205-211, XP002105333**
 - **M. KELSEY ET AL: "The Identification of Microbial Metabolites of Sulfolithocholic Acid" JOURNAL OF LIPID RESEARCH, vol. 21, no. 6, 1980, pages 751-759, XP002105334**
 - **KRAMER W ET AL: "Modified bile acids as carriers for peptides and drugs" JOURNAL OF CONTROLLED RELEASE, vol. 46, no. 1-2, 5 May 1997, page 17-30 XP004096309**
 - **CHEMICAL ABSTRACTS, vol. 124, no. 1, 1 January 1996 Columbus, Ohio, US; abstract no. 6191, WANG, WEI ET AL: "Effect of free fatty acid on cholesterol nucleation in model bile" page 667; column 2; XP002105336 & ZHONGHUA YIXUE ZAZHI (1995), 75(5), 291-4 CODEN: CHHTAT;ISSN: 0376-2491,**
 - **L. SLIEDREGT ET AL: "Design and Synthesis of Novel Amphiphilic Dendritic Galactosides for Selective Targetting of Liposomes to the Hepatic Asialoglycoprotein Receptor" JOURNAL OF MEDICINAL CHEMISTRY., vol. 42, no. 4, February 1999, pages 609-618, XP002105335 WASHINGTON US**

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- RUMP E T ET AL: "PREPARATION OF CONJUGATES OF OLIGODEOXYNICLEOTIDES AND LIPID STRUCTURES AND THEIR INTERACTION WITH LOW-DENSITY LIPOPROTEIN" BIOCONJUGATE CHEMISTRY, vol. 9, no. 3, 1 May 1998, pages 341-349, XP000750902
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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

[0001] The present invention relates to bile acid or bile salt fatty acid conjugates (hereinafter called "BAFAC"), to their use in dissolving cholesterol gallstones in bile, preventing their occurrence or recurrence and to their use for making medicaments for reducing or preventing arteriosclerosis.

[0002] It should be noted that the terms bile acids and bile salts are similar and are used interchangeably.

[0003] Gallstones are found in about 15% of people in most industrialized countries. Most gallstones are cholesterol gallstones, i.e. cholesterol being their main component. Thus, cholesterol gallstones represent a major health problem. Bile is often supersaturated with cholesterol which tends to crystallize. The prevention of cholesterol crystallization in bile will prevent the formation of cholesterol gallstones or their recurrence after procedures such as lithotripsy, dissolution, or stone extraction. The residence time of newly secreted bile in the gallbladder is short - less than 12-24 hours. The prevention of cholesterol crystallization in bile during such a period could prevent gallstone formation.

[0004] It has been proven that cholesterol gallstones can be dissolved medically and their recurrence prevented using certain bile salts such as chenodeoxycholic or ursodeoxycholic acid. Bile salt therapy is, however, of low efficacy, is very time consuming and has been largely abandoned. More effective therapies are thus required.

[0005] Recent work has demonstrated the major role played by phospholipids in cholesterol solubilization in bile. (T. Gilat et. al. *Biochimica et Biophysica Acta* 1286, (1996), 95-115; Y. Ringel et. al. *Biochimica et Biophysica Acta* 1390, (1998), 293-300.); and J. *Hepatology*, 28, (1998), 1008-1014.) Phospholipids are a major or sole component of cholesterol solubilizing lipid aggregates in bile. It has been demonstrated that the stepwise addition of phospholipids to bile will progressively prolong the nucleation time of the cholesterol in bile. (Z. Halpern et. al. *Gut* 34 (1993) 110 - 115).

[0006] Major differences between certain phospholipid molecular species in their cholesterol crystallization inhibiting potency in human or model biles have been demonstrated. Phospholipids differ from one another mainly in the fatty acids present in the stereospecific number sn-1 and/or sn-2 positions and in their head groups. It has been demonstrated that major prolongations in the nucleation time and major reductions in the cholesterol crystal growth rate and in the total cholesterol crystal mass are achieved with changes in phospholipid molecular species without changing the absolute or relative amounts of phospholipids. Cholesterol crystallization was markedly delayed when the sn-2 fatty acid was saturated, when the head group was serine instead of choline, etc. (Y. Ringel et. al. above).

[0007] It has also been shown that various phospholipid components by themselves (without the whole

phospholipid molecule), e.g. saturated fatty acids such as palmitic acid or stearic acid; or phosphatidyl glycerol have strong cholesterol crystallization inhibiting activity.

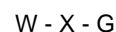
[0008] Thus, enriching human bile with phospholipids in general, or specific phospholipids or their components, such as fatty acids would markedly retard cholesterol crystallization in bile and achieve the desired result.

[0009] The problem was how to enrich human bile in vivo with phospholipids or their components. When bile salts are fed to humans they are very efficiently absorbed, taken up by the liver and excreted into bile. This also applies to synthetic bile salt analogues. There are specific and very efficient transport mechanisms in the body for these purposes. Thus, when ursodeoxycholic acid (which is normally present in human bile in minute amounts) is fed regularly it is absorbed and secreted into bile and eventually constitutes 30-50% of biliary bile acids. However, as indicated above bile salt therapy for the dissolution of cholesterol gallstones is not satisfactory.

[0010] Phospholipids and their components are well absorbed and taken up by the liver. Phospholipid secretion into bile is, however, tightly regulated by the liver and only limited amounts and species of phospholipids are secreted into bile in association with the secretion of bile salts and cholesterol. There is at present no efficient method to modulate, quantitatively or qualitatively, human biliary phospholipid compositions to any considerable degree. When dietary phospholipids reach the liver they may be metabolized, secreted into the blood or stored in the liver. Only small amounts and predetermined species are secreted into bile with minimal possibilities for modulation.

[0011] It has therefore been desirable to find a satisfactory method for the transport of phospholipids or one of their components into bile which would improve the solubilization of biliary cholesterol and prevent the formation of cholesterol gallstones or dissolve existing gallstones.

[0012] From IL-A-95668 (EP-A-0417725) and corresponding U.S. Specifications there are known bile acid derivatives of general formula I:



in which G is a bile acid radical, W is an active compound moiety of a medicament and X is either a direct bond or a bonding member between said bile acid radical and the active compound. In said specifications a long list of substituents is given but it does not mention specifically W as standing for a fatty acid radical, neither for a saturated one nor for an unsaturated one, i.e. said specifications do not mention anything about BAFAC.

[0013] Moreover, among all the objects of said compounds there cannot be found even a hint that any of said compounds may be utilized to enhance the solubi-

lization of biliary cholesterol, to prevent the formation of cholesterol gallstones, to dissolve existing cholesterol gallstones, to reduce or prevent arteriosclerosis.

[0014] US-A-4,439,366 discloses 7-acyl-chenodeoxycholic acid derivatives in which the acyl residue derives from a saturated or unsaturated, linear C₃₋₁₈ carboxylic acid for the treatment of biliary calculus. Explicitly disclosed are derivatives containing acyl-groups derived from butyric, caprylic, lauric, palmitic, stearic, oleic, linoleic, and arachidonic acid.

[0015] US-A-4,440,688 discloses 7-acyl-chenodeoxycholic acid derivated in which the acyl residue derives from a saturated or unsaturated, linear C₃₋₁₈ carboxylic acid. Explicitly disclosed are derivates containing acyl-groups derived from butyric, caprylic, lauric, palmitic, stearic, oleic, linoleic, and arachidonic acid. Bile acid derivatives containing two fatty acyl residues are disclosed.

[0016] D.KRICHEVSKY ET AL: "Novel Derivatives of 3.alpha.,7.alpha.-Dihydroxy-5.beta.-cholan-24-oic-Acid (Chenodeoxycholic Acid) and 3.alpha.,7.beta.-Dihydroxy-5.beta.-cholan-24-oic Acid (Ursodeoxycholic Acid)" STEROIDS: STRUCTURE, FUNCTION, AND REGULATION., vol. 47, no.1, 1986, pages 41-48 discloses 7-acylcheno- and ursodeoxycholic acids and additionally methyl 3,7-diacyl cheno- and ursodeoxycholates. The acyl residues are derived from butyric, caprylic, lauric, palmitic, oleic, and linoleic acid.

[0017] M. KELSEY ET AL.: "The Identification of Microbial Metabolites of Sulfolithocholic Acid" JOURNAL OF LIPID RESEARCH, vol. 21, no. 6, 1980, pages 751 - 759 discloses 3β-fatty acyl-derivatives of isolithocholic acid (ILA), namely palmitoyl, palmitoleyl, stearyl, and oleyl esters of ILA.

[0018] M. KELSEY ET AL: "Characterization of Microbial Metabolites of Sulfolithocholic Acid by High Performance Liquid Chromatography" JOURNAL OF STEROID BIOCHEMISTRY, vol. 14, no. 2, 1981, pages 205-211 discloses 3β-palmitoyl-5β-cholan-24-oic acid.

[0019] It has now been found that bile acids or salts conjugated with fatty acids (saturated or unsaturated) via a connecting bond X (covered by the term BAFAC) can serve as vehicles to transport the fatty acids into the bile using the very efficient entero-hepatic circulation of bile acids and salts: It has also been shown that BAFAC are absorbed from the intestine, taken up by the liver and secreted into bile. Said BAFAC improved cholesterol solubilization in bile and markedly retarded its crystallization. Said BAFAC are therefore useful agents for the prevention of the formation or recurrence of cholesterol gallstones and for the dissolution of cholesterol gallstones.

[0020] The administration of BAFAC has also an inhibiting effect on cholesterol crystallization in the vascular tree. In the physiologic situation ingested bile acids or salts are absorbed in the intestine, transported via the portal vein to the liver and excreted via the bile into the intestine. They thus recirculate in the entero-hepatic cir-

ulation, with only minute amounts reaching the systemic circulation (the vascular tree). The BAFAC behave more like lipids, which after intestinal absorption are transported via the lymph to the systemic circulation.

5 The BAFAC were shown to be transported both via the lymph and via the portal vein. By both routes they are taken up by the liver and secreted into the bile. At each entero-hepatic circulation they are excreted into the intestine, are again partly reabsorbed via the lymph and recirculated into the vascular tree prior to liver uptake. 10 As there are daily 10-12 cycles of entero-hepatic circulation the net effect will be recirculation of the BAFAC in the vascular tree.

[0021] Administration of BAFAC orally in divided doses in the course of the day will enhance this effect. The inhibiting effect of BAFAC on cholesterol crystallization has been proven. Thus, also their value in reducing and/or preventing cholesterol crystallization in the vascular tree, i.e. in arteriosclerosis.

20 **[0022]** In detail the present invention relates to a bile acid or bile salt fatty acid conjugates of general formula II

W-X-G

(II)

25 in which G is a bile acid or bile salt radical, W stands for one or two saturated fatty acid radicals containing 6 to 26 carbon atoms and X is an -NH- bonding member between said bile acid or bile salt radical and the fatty acid radical(s). Preferred embodiments become evident from the dependent claims.

[0023] As suitable bile acids there may be mentioned, cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid and derivatives and analogues thereof. The bile acids utilized may be unconjugated or, as in bile, be conjugated with glycine, taurine or a suitable amino acid. These possibilities are within the definition of a bile acid and thus within the scope of the present invention. The conjugation with the fatty acid radical is mostly performed at position 3 of the nucleus depending on the bile acid being used. It is also possible to perform the conjugation with the fatty acid radical at different positions, e.g. 6, 7, 12 and 24. When the bile acid is conjugated with glycine or taurine the conjugation with the fatty acid radical cannot be performed in position 24. The conjugation between the fatty acid radical and the bile acid can be in the α or the β configuration.

[0024] The bonding member X is a -NH- group.

[0025] Preferred fatty acids are saturated ones which 50 have suitably 6 - 26 carbon atoms, advantageously those having 14 to 22 carbon atoms. Preferred saturated fatty acids are behenic acid, arachidic acid, stearic acid, palmitic acid and myristic acid.

[0026] When W stands for two fatty acids they are suitably conjugated at positions 3 and 7.

[0027] The present invention also consists in a pharmaceutical composition enabling the dissolution of cholesterol gallstones in bile and preventing the formation

thereof; and enabling the prevention and/or reduction of arteriosclerosis, comprising as active ingredient a bile acid fatty acid derivative of general formula II.

[0028] Said composition may have the form of a tablet, a capsule, a solution, an emulsion, etc.

[0029] Said composition may comprise additional compounds such as carriers, solvents, emulgators, enhancers of absorption, inhibitors of cholesterol synthesis or secretion into the bile, etc. Said composition should advantageously comprise 0.1 - 1.5 g of the active ingredient.

[0030] The composition is suitably ingested once daily preferably at bedtime. It may also be ingested in divided doses during the day.

[0031] The present invention also consists in the use of a bile acid fatty acid derivative of general formula II or of a pharmaceutical composition comprising same for the manufacture of a medicament for the dissolution of cholesterol gallstones in bile and for the prevention of the formation thereof.

[0032] The present invention also consists in the use of a bile acid fatty acid derivative of general formula II or of a pharmaceutical composition comprising same for the manufacture of a medicament for the prevention and/or reduction of arteriosclerosis.

[0033] Cholesterol gallstones in bile can be dissolved and the formation thereof can be prevented by administering a bile acid fatty acid derivative of general formula II or a pharmaceutical composition comprising same.

[0034] Arteriosclerosis can be prevented and/or reduced by administering a bile acid fatty acid derivative of general formula II or a pharmaceutical composition comprising same.

[0035] The present invention will now be illustrated with reference to the accompanying Examples and drawings without being limited by them.

[0036] In said drawings:

Fig. 1 shows crystal observation time. Model bile solution. Effects of palmitoylamido-cholate (PalC).

A-Control solution. B,C-Replacement of 10% and 20% of Na taurocholate (NaTC) by equimolar amounts of PalC, respectively. D-Replacement of 20% of phospholids by PalC. E F addition of 10 mM and 20mM PalC to the solution, respectively;

Fig. 2 shows cholesterol crystal mass. Model bile solution. Effects of PalC. A, B, C, D, E and F - as in Fig. 1;

Fig. 3 shows crystal growth rate. Model bile solution. Effects of PalC. A, B, C, D, E and F - as in Fig. 1;

Fig. 4 shows thin layer chromatography. A-PalC standards in pure solution (left) and in hamsters bile. B-Hamster bile of control animals (left) and bile of PalC fed hamsters;

Fig. 5A shows steps in the conjugation of cholic acid (at C-3) with: behenic acid(C-22), arachidic acid (C-20), stearic acid (C-18), palmitic acid (C-16), myristic acid (C-14), lauric acid (C-12) and caproic

acid (C-6);

Fig. 5B shows stages in the synthesis of glycine conjugated stearyl-cholate;

Fig. 5C shows conjugation of oleoyl-cholate reference example;

Fig. 5D shows conjugation of cholic acid with two molecules of stearic acid at positions C-3 and C-7 of the bile acid nucleus;

Fig. 6 shows cholesterol crystal mass. Model bile solution. Effects of myristic (C-14), palmitic (C-16), stearic (C-18) and arachidic (C-20) acids conjugated with cholic acid (at C-3). The test compounds replaced 20 mole% of the NaTC in the control solution.

Fig. 7 shows nucleation time. Model bile solution. Effects of the compounds used in Fig. 6.

Fig. 8 shows cholesterol crystal mass of enriched human bile after 22 days of incubation. Effects of 5 mM palmitoyl (C-16) cholate, stearyl (C-18) cholate and arachidyl (C-20) cholate added to the bile in comparison with the control bile and bile with added 5mM cholic acid.

Fig. 9 shows nucleation time, model biles. Effects of replacement of 20 mole % of NaTC with equimolar amounts of caproyl (C-6) cholate, lauryl (C-12) cholate, stearyl (C-18) cholate, arachidyl (C-20) cholate and di-stearyl ursodeoxycholate in comparison with the model bile and without replacement of 20% of NaTC with cholic acid; and

Fig. 10 shows stearyl (C-18) cholate levels in hamsters 1, 2 and 3 hours after ingestion of 30 mg. Concentrations in heart blood, portal blood and gallbladder bile.

Example I

3 β -Behenylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic acid (Fig 5A-3)

[0037]

(a) 1.15 g of 3 β -amino-7 α ,12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-1) [Fr Patent 1017756 Dec. 18 1952, Chem. Abstr. 52:1293c] were dissolved in 30 ml dry dimethyl formamide and treated with 15 ml triethyl amine under stirring. 1.13 g of behenoyl chloride in 10 ml dimethyl formamide were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.8 g of 3 β -behenylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic of the methyl ester (Fig 5A-2).

¹H-NMR (CDCl₃) δ ,ppm: 0.69 (s,CH₃-18),0.88 (t, J=1Hz,CH₃-23),0.95 (s,CH₃-19), 0.99 (d,J=3Hz,

CH₃-21), 1.25, 1.14 [s, CH₂]₂₀, 2.14 (t, J=5Hz, CH₃-behenyl), 3.67 (s-COOCH₃), 3.91 (d, J=1.5 Hz, CH-7), 3.96 (s, J=4Hz, CH-12), 3.99 (m, CH-3), 5.60 (d, J=4.5 Hz, -CH₂CO-).

(b) The above methyl ester, 0.45 g, was dissolved in 20ml methanol, treated with 2ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted acid chloride, resulting in a white precipitate which was washed with water, to give 0.41g of the pure 3β-behenylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-3).

Example II

3β-arachidylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-5)

[0038]

(a) 1.0 g of 3β-amino-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-1) [see Example I] were dissolved in 30 ml dry dimethylformamide and treated with 15 ml triethyl amine under stirring. 1.0g of arachidoyl chloride in 10 ml dimethylformamide were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water, extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.6 g 3β-arachidylamido-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-4).

¹H-NMR (CDCl₃) δ, ppm: 0.70 (s, CH₃-18), 0.88 (t, J=6Hz, CH₃-23), 0.95 (s, CH₃-19), 0.99 (d, J=3.Hz, CH₃-21), 1.25, 1.14 [(s, CH₂)₁₈], 2.14 (t, J=5Hz, CH₃-arachidyl), 3.67 (s-COOCH₃), 3.91 (d, J=1.5 Hz, CH-7), 3.96 (t, J=4Hz, CH-12), 4.4 (m, CH-3), 5.60 (d, J=4.5 Hz, -CH₂CONH).

(b) 0.5 g 3β-arachidylamido-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-4) were dissolved in 20ml methanol, treated with 2ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted hydrogen chloride, resulting in a white precipitate which was washed with water, to give 0.7 g of the pure 3β-arachidylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-5).

Example III

3β-stearyl-amido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-7)

[0039]

Method 1

(a) 1.15 g 3β-amino-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-1) [see Example I] were dissolved in 30 ml dry dimethylformamide and treated with 15 ml triethyl amine under stirring. 1.13g of stearoyl chloride in 10 ml dimethyl formamide were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.68 g 3β-stearyl-amido-7α,12α-dihydroxy-5β-cholan-24 oic methylester (Fig 5A-6). ¹H-NMR (CDCl₃) δ, ppm: 0.69 (s, CH₃-18), 0.88 (t, J=1Hz, CH₃-23), 0.95 (s, CH₃-19), 0.99 (t, J=3.Hz, CH₃-21), 1.25, 1.44 [(s, CH₂)₁₆], 2.14 (t, J=5Hz, CH₃-stearyl), 3.67 (s-COOCH₃), 3.91 (d, J=1.5 Hz, CH-7), 3.99 (m, CH-3), 4.4 (m, CH-3), 5.60 (d, J=4.5 Hz, -CH₂CONH).

(b) 0.45 g 3β-stearyl-amido-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-6) were dissolved in 20 ml methanol, treated with 2 ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10 ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted hydrogen chloride, resulting in a white precipitate which was washed with water, to give 0.41 g of the 3β-stearyl-amido 7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-7). mp 63-65°C.

Method 2

2.5 g 3β-amino-7α,12α-dihydroxy-5β-cholanoic-24-oic acid (prepared according to Kramer et al., J. of Lipid Research 24, 910, 1983) were dissolved in acetonitril and added to a stirred solution of 1.2 g stearic acid and 3.6 g N-hydroxy succinamide in the same solvent. After 8 h the precipitate was filtered, washed with the solvent and evaporated to dryness. The residue was added to a solution of 1.2 g of stearic acid in 10 ml N-methyl morpholine and N,N'-dimethyl formamide (1:3). After being kept at room temperature overnight, the solution was diluted with water, extracted with ethyl acetate, to give 0.6 g of the acid (Fig 5A-7), identical to that of Method 1.

Method 3

A solution of 6 g stearoyl chloride was added dropwise to a stirred solution of 1.6 g of the amine (Fig 5B-18) in toluene at 0°, and left at the same temperature for 1 h. The resulting solution was heated at 50° for another hour, acidified with 3N-hydrochloric acid, and then filtered. The solid material was washed with water and dried at 45°, to give the acid (Fig 5A-7), identical with that described above.

Example IV

3β-palmitylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-9)

[0040]Method 1

(a) 1.0 g of the 3β-amino-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-1) [See Example I] were dissolved in 30 ml dry dimethylformamide and treated with 15 ml triethylamine under stirring. 0.8 g of palmitoyl chloride in 10 ml dimethylformamide were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.5 g 3β-palmitylamido-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-8).

¹H-NMR (CDCl₃) δ,ppm: 0.66 (s,CH₃-11),0.88 (t,J=1Hz,CH₃-23),0.91 (s,CH₃-19), 0.96 (d,J=3.5Hz,CH₃-21),1.22, 1.14 [(s,CH₂)₁₄] 2.13 (t,J=5Hz, CH₃-palmityl), 3.67 (s-COOCH₃), 3.82 (d,J=1.5 Hz-CH₇), 3.96 (s,J=3.9 Hz, CH-12), 4.09 (m,CH-3), 5.63 (d,J=4.5 Hz,-CH₂CONH).

(b) The above methyl ester, 0.45 g, was dissolved in 20 ml methanol, treated with 2 ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10 ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted hydrogen chloride, resulting in a white precipitate which was washed with water, to give 0.4g of the pure 3β-palmitylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig. 5A-9).

Method 2

2.5 g 3β-amino-7α,12α-dihydroxy-5β-cholanoic-24-oic acid (Fig 5B-18) (prepared according to Kramer et al., J. of Lipid Research 24, 910, 1983) were dissolved in acetonitrile and added to a stirred

solution of 1.2 g palmitic acid and 3.6 g N-hydroxy succinamide in the same solvent. After 8 h the precipitate was filtered, washed with the solvent and evaporated to dryness. The residue was added to a solution of 1.2 g of palmitic acid in 10 ml N-methyl morpholine and N,N'-dimethylformamide (1:3). After being kept at room temperature overnight, the solution was diluted with water, extracted with ethyl acetate, to give 0.6 g of the acid (Fig 5A-9), identical to that of Method 1.

Example V

3β-myristylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-11)

[0041]

(a) 0.5 g of 3β-amino-7α,12α-dihydroxy-5β-cholan-24-oic methylester (1) [See Example I] were dissolved in 30 ml dry dimethylformamide and treated with 15 ml triethylamine under stirring. 0.4 g myristoyl chloride in 10 ml dimethylformamide were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water, extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.4 g 3β-myristylamido-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-10).

¹H-NMR (CDCl₃) δ,ppm: 0.69 (s,CH₃-18),0.88 (t,J=1Hz,CH₃-23),0.95 (s,CH₃-19), 0.99 (d,J=3.Hz, CH₃-21), 1.25 [(s,CH₂)₁₂], 2.14 (t,J=5Hz, CH₃-myristyl),3.67 (s-COOCH₃), 3.91 (d,J=1.5 Hz, CH-7), 3.99 (d,J=4Hz,CH-12), 4.4(m,CH-3), 5.60 (d,J=4.5 Hz,-CH₂CONH).

(b) 0.45 g 3β-myristylamide-7α,12α-dihydroxy-5β-cholan-24-oic methylester (Fig 5A-10) were dissolved in 20ml methanol, treated with 2ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10 ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted hydrogen chloride, resulting in a white precipitate which was washed with water, to give 0.26 g of pure acid (Fig 5A-11).

Example VI

3β-laurylamido-7α,12α-dihydroxy-5β-cholan-24-oic acid (Fig 5A-13)

[0042]

(a) 0.6 g of 3β-amino-7α,12α-dihydroxy-5β-cholan-

24-oic methylester (Fig 5A-1) were dissolved in 30 ml dry dimethyl formamide and treated with 15ml triethyl amine under stirring. 0.6 g of lauryl chloride in 10 ml dimethyl formamide were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.5 g 3 β -laurylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-12).

¹H-NMR (CDCl₃) δ ,ppm:0.67 (s,CH₃-18),0.89 (t, J=1Hz,CH₃-23),0.94 (s,CH₃-19), 0.99 (d,J=3.Hz, CH₃-21),1.25 [(s,CH₂)₁₀], 2.14 (t,J=5Hz, CH₃-lauryl), 3.67 (s-COOCH₃), 3.91 (d,J=1.5Hz, CH-7), 3.99 (t,J=3.95Hz, CH-12), 4.4 (m,CH-3), 5.60 (d,J=4.5 Hz-CH₂CONH).

(b) 0.45 g 3 β -laurylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-12) were dissolved in 20 ml methanol, treated with 2ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10 ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted hydrogen chloride, resulting in a white precipitate which was washed with water, to give 0.41g of the pure acid (Fig 5A-13), mp.82-88.

Example VII

3 β -caprylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic acid (Fig 5A-15)

[0043]

(a) 1.0g of 3 β -amino-7 α ,12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-1) [see Example I] were dissolved in 30 ml dry methylene chloride and treated with 15 ml triethyl amine under stirring. 1.2 g of caproyl chloride acid in 10 ml methylene chloride were added dropwise to the resulting solution, and the stirring was continued overnight. The reaction mixture was poured into water extracted with methylene chloride, the organic fraction was then dried over sodium sulfate, evaporated to dryness and chromatographed over silica gel with a mixture of ethyl acetate and hexane (6:4 and 8:2), to give 0.7 g 3 β -caprylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-14).

¹H-NMR (CDCl₃) δ ,ppm:0.69 (s,CH₃-18),0.88 (t, J=1Hz,CH₃-23),0.95 (s,CH₃-19), 0.99 (t,J=3.Hz, CH₃-21),1.25 [(s,CH₂)₄], 2.14 (t,J=5Hz, CH₃-capryl), 3.67 (s-COOCH₃), 3.91 (d,J=1.5Hz, CH-7), 3.99 (t,J=4HzCH-12), 4.4 (m,CH-3); 5.60 (d,J=4.5

Hz-CH₂CONH).

(b) 0.5 g 3 β -caprylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-14) were dissolved in 20ml methanol, treated with 2ml 1N sodium hydroxide and left for 24 h at room temperature. The methanol was then distilled off, 10ml water were added and the reaction mixture was extracted with ethyl acetate. The water fraction was then acidified with diluted hydrogen chloride, resulting in a white precipitate which was washed with water, to give 0.4 g of pure acid (Fig 5A-15).

Example VIII

N-(-Carboxymethyl)-3 β -stearylamido-7 α ,12 α -dihydroxy-5 β -cholan-24 amide (Fig 5B-17)

[0044]

(a) 0.5 g 3 β -stearylamido-7 α ,12 α -dihydroxy-5 β -cholinoic acid (Fig 5A-7) were dissolved in 25 ml dry 1,4-dioxane and were cooled to -10°. The stirred solution was treated with 0.5 ml triethylamine, then with 0.085 ml chloroethyl formate and stirred at the same temperature for 15 min. The solution was left to reach room temperature, treated with 0.1 ml triethylamine and with 14 g ethyl glycine hydrochloride, and left overnight. The reaction mixture was poured into water, extracted with ethyl acetate and washed with water. The extract was evaporated to dryness and chromatographed on silica gel, using a mixture of ethyl acetate : hexane 60:40, pure ethyl acetate and then ethyl acetate:methanol 9:1, to give 0.27 g of the product (Fig 5B-16).

(b) 0.27 g of the above compound were dissolved in 20 ml methanol and treated with 2 ml sodium hydroxide 1N. After 24 h the methanol was evaporated till dryness, dissolved in water and extracted with ethyl acetate. The aqueous fraction was acidified with HCL 1N. The precipitate obtained was washed with water and dried, to give 0.24g of the dry material (Fig 5B-17).

Example IX (reference example)

3 β -oleylamido-7 α 12 α -dihydroxy-5 β -cholan-24-oic acid (Fig.5C-20)

[0045]

(a) 1.6 g 3 β -amino-7 α 12 α -dihydroxy-5 β -cholan-24-oic methylester (Fig 5A-1) were dissolved in 30 ml dry dimethyl formamide and treated with 3 ml triethyl amine under stirring. A solution of 1.38 g oleyl chloride in 10 ml dry DMF was added dropwise, and the resulting solution was left at room temperature

overnight. The reaction mixture was poured into water, extracted with ethyl acetate, the organic fraction was purified by washing with diluted hydrochloric acid, sodium bicarbonate and then with water. Evaporation to dryness in vacuum resulted in 3.1 g which were chromatographed over silica gel, using a mixture of ethyl acetate/hexane (4:6 and 10:8) to give 1.8 g of the methyl ester. (Fig.5C-19).

(b) A solution of 1.2 g methyl ester in 20 ml methanol was treated at room temperature with a solution of 5 ml sodium hydroxide 1N and kept at room temperature for 48 hours and then evaporated to dryness. The residue was dissolved in 20 ml water and extracted with 25 ml ethyl acetate 3 times. The water extract was acidified with a hydrochloric solution to give a precipitate which was filtered. This residue was chromatographed on silica gel with a mixture of ethyl acetate: hexane : acetic acid (10:4:0.3), to give 0.3 g of 3 β -oleylamido-7 α -12 α -dihydroxy-5 β -cholan-24-oic acid (Fig.5C-20)

Example X

3 β ,7 α -distearyl-amido-5 β -ursodeoxycholan-24-oic acid (Fig.5D-26)

[0046]

(a) 20 g ursodesoxy-cholan-24-oic acid were dissolved in 200 ml abs.methanol, treated with 1 ml conc. sulfuric acid and stirred for 24 hrs. Most of the solvent was distilled off and the residue was poured into water and extracted with methylene chloride. The organic extract was washed with a solution of sodium bicarbonate and of sodium chloride, and evaporated to dryness resulting in 19.5 g of the 3 α ,7 β -dihydroxy-5 β -ursodeoxycholan-24-oic acid methyl ester. (Fig.5D-21)

¹H-NMR (CDCl₃) δ ,ppm:0.66 (s,CH₃-18), 0.90 (t, J=1Hz,CH₃-23), 0.93 (s,CH₃-19), 0.94 (d,J=3Hz, CH₃-21), 3.58 (m,CH-3, CH-7), 3.65 (s-COOCH₃).

(b) 4.06 g of the methyl ester (Fig.5D-21) were dissolved in 30 ml dry pyridine and cooled to 0°C. The reaction mixture was stirred and treated dropwise for 15 min. with a solution of 1.49 g methane-sulfonyl chloride in 5 ml pyridine. After being left standing for 3 hrs. at the same temperature, the reaction mixture was poured on ice and water, and then extracted with ethyl acetate. The organic phase was washed with hydrochloric acid, sodium bicarbonate and sodium chloride solution, filtered and evaporated in vacuum. The residue consisting of 4 compounds was chromatographed over a silica column using as eluant a mixture of ethyl acetate and hexane. The less polar compound, 5.3 g, was the desired 3 α ,7 β -dimesyl-5 β -ursodeoxycholan-24-oic acid

24-oic methyl ester. (Fig.5D-22).

¹H-NMR (CDCl₃) δ ,ppm:0.65 (s,CH₃-18),0.90 (d, J=4Hz,CH₃-23), 0.97 (s,CH₃-19), 1.2 (t,J=3Hz, CH₃-21), 2.97 (s,CH₃SO₂) 2.98 (s,CH₃SO₂), 3.64 (s,CH₃SO₂) 4.09 (q,J=12Hz,H-7), 4.62 (m,H-7)

(c) 5.65 g of the dimesyl derivative were dissolved in 50 ml dry DMF, treated with dry sodium azide and heated to 130° for 2 hrs. The reaction mixture was cooled, poured into ice water and extracted with ethyl acetate. The extract was then washed with a solution of sodium acetate and sodium chloride, filtered and evaporated to dryness, resulting in 4.6 g of the 3 β ,7 α -diazido-5 β -ursodeoxycholan-24-oic acid methyl ester (Fig.5D-23)

(d) 4.5 g of the diazido compound (Fig.5D-23) were dissolved in 120 ml methanol to which 150 mg of 5% palladium on carbon were added and hydrogenated at atmospheric pressure for 4 days. The hydrogenation was repeated with additional 150 mg of 5% palladium on carbon. The hydrogenated mixture was filtered and evaporated in vacuum to give 3 g of the 3 β ,7 α -diamino-5 β -ursodeoxycholan-24-oic acid methyl ester (Fig.5D-24).

¹H-NMR (CDCl₃) δ ,ppm:0.65 (s,CH₃-18), 0.92 (d, J=4Hz,CH₃-23), 0.96 (s,CH₃-19), 1.2 (t, J=3Hz, CH₃-21), 3.68 (s,COOCH₃), 3.72,3.95 (m,2H-7,3)

(e) 1.47 g of the 3 β ,7 α -diamino-5 β -ursodeoxycholan-24-oic acid methyl ester (Fig.5D-24) were dissolved in 50 ml of a dry 1:1 mixture of DMSO and DMF, treated with 2 ml triethylamine and 30 mg dimethylamino pyridine and 5.1 g stearic anhydride. The reaction mixture was heated to 50°, stirred for 18 hrs, poured into ice-water and extracted 3 times with ethyl acetate. The organic phase was washed with hydrochloric acid, sodium bicarbonate and sodium chloride solution. After evaporating of the organic solvent 2.05 g of an oily residue were obtained. Separation on silica gel using ethyl acetate: hexane as an eluant (1:4) resulted in a number of fractions, one of which, 80 mg, was the desired 3 β ,7 α -distearyl-amido-5 β -ursodeoxycholan-24-methylester (Fig.5D-25), according to its MS and ¹H-NMR

MS FAB: MH+ 937 (MW) 936)

¹H-NMR (CDCl₃) δ ,ppm:0.66 (s,CH₃-18), 0.86 (d, J=4Hz,CH₃-23), 0.96 (s,CH₃-19), 1.2 (t,J=3Hz, CH₃-21), 1.26[s,(CH₂)₁₆], 3.64 (s,COOCH₃), 3.05 (d J=7.Hz, H-7) 5.75 (m,H-3)

(f) 78 mg of the methylester (Fig.5D-25) were dissolved in 20 ml methanol, treated with 2 ml sodium hydroxide 1 N and left for 48 hrs at room temperature. The methanol was evaporated in vacuum, the residue was dissolved in 25 ml water, filtered and then acidified with diluted hydrochloric acid to give

a precipitate which consisted of 3 β ,7 α -distearylami-
do-5 β -ursodeoxycholan-24-oic acid (Fig.5D-26y).

Example XI

Materials and Methods

[0047] Cholesterol (Sigma, St. Louis, Mo.) was twice recrystallized from hot ethanol; Na-taurocholate (Na-TC; Sigma, St. Louis, Mo.) was twice recrystallized from ethanol and ether (J.L Pope, J. Lipid Res. 8, (1967) 146-147); egg yolk lecithin (EYL) (Avanti Polar Lipids, Alabaster, Al.) was used without further purification. All lipids used in this study were pure by TLC standard. The synthetic bile acid conjugate used in Examples XII to XIV was palmitylamido-7 α ,12 α -dihydroxy-5 β -cholan-24-oic acid (PalC) (prepared as described in Example IV).

1. Preparation of Biles

A. Model Bile

[0048] EYL, cholesterol and Na-TC mixtures were dissolved in CHCl₃/CH₃OH(2:1 v/v), dried under N₂ at room temperature, lyophilized overnight and kept at -20°C under argon until used. Model bile solutions were prepared by suspending the dried lipids in 150mM NaCl 1.5mM disodium EDTA, 50mM Tris-HCl pH 8.0 and incubating the suspension at 55°C for 1 hour. The solubilized model biles were incubated, in sealed vials under argon, at 37°C for the duration of the experiment. Aliquots from the models were examined daily.

[0049] All models were prepared in triplicate and were kept at the same conditions throughout the experimental period.

[0050] The composition of the model bile was:
cholesterol 15 mM, EYL 30 mM, Na-TC 150 mM.

[0051] One hundred per cent EYL was used for preparation of the control solution. The other investigated model bile solutions were prepared by adding or substituting (10-20%) of the EYL or Na-TC by the synthetic bile acid conjugate (PalC).

B. Native human gallbladder bile

[0052] Native human gallbladder bile was obtained from cholesterol gallstone patients at cholecystectomy. Pooled bile from several patients was cholesterol enriched by incubation with dried cholesterol or by mixing with a concentrated model bile prior to use in experiments in order to facilitate crystallization.

2. Evaluation of cholesterol crystal formation and growth

2.1 Crystal observation time (COT) assay

[0053] COT (also called "Nucleation time") was determined as described by Holan et. al. in Gastroenterology 77, (1979) 611 - 617. Aliquots from each model bile were examined daily by polarized light microscopy. COT was defined as the initial time of detection of at least three cholesterol monohydrate crystals per microscopic field at 100 fold magnification.

2.2 Crystal growth rate (CGR) assay

[0054] Crystal growth was monitored spectrophotometrically using a microplate reader (SPECTRA-STL, Austria) (G.J. Somjen, et. al. J. Lipid Res. 38, (1977) 1048 - 1052). Aliquots (50 μ l) of lipid solutions were mixed and shaken vigorously with equivalent volumes of Na-taurodeoxycholate (200mM), in microplate wells. After 60 minutes at room temperature, the microplates were shaken again and the absorbance, at 405nm, in each well was measured. Each model was prepared in triplicate and sampled in duplicate for measurement.

[0055] The data were collected and analyzed by an IBM compatible personal computer, and the optical density (OD), averaged for triplicate preparations, was calculated. A graph describing the averaged OD changes for each solution was plotted. The slope in the steepest region of the curve was determined by a linear regression fit to at least three measurements and defined as the CGR. CGR and OD differences between day 0 and day 14 were calculated for each model.

2.3 Measurement of crystal mass

[0056] Chemical analyses of cholesterol were performed on each sample on the last day of the experiment (day 14), as previously described (G.J. Somjen see above). The samples were collected from the micro wells, centrifuged in an Airfuge (Beckman) at 70,000 rpm for 5 min. Separate determinations were performed on the total sample (before centrifugation) as well as on the supernatant solution. The amount of cholesterol in the precipitated pellets was calculated by subtracting the amounts in the supernatant solutions from the total. The crystalline character of the pellet was confirmed by polarized light microscopy. The crystal mass was also measured spectrophotometrically as the OD difference between day 0 and day 14 of the incubation.

3. Data Analysis

[0057] Each lipid dispersion was prepared in triplicate and duplicate aliquots were measured from all samples. Mean values of OD and standard errors were calculated. Crystals growth rates were calculated from linear re-

gression analysis of the crystal growth curves as explained above. Comparisons between the different model solutions were performed by one way analysis of variance.

Example XII

[0058] The effects of the bile salt fatty acid conjugate prepared in Example IV (PaIC hereinafter "test compound") on cholesterol crystallization kinetics in model and human biles.

[0059] Replacement and addition experiments were performed. The following results were obtained.

A. Model Bile

[0060]

a. In a model bile solution (composition: Na taurocholate 150mM cholesterol 15mM egg lecithin 30mM total lipids 10.3 gm/dl) when 20% of the Na taurocholate were replaced by the test compound (PaIC) the nucleation time was prolonged by 167%; the cholesterol crystal growth rate was reduced by 67% and the total cholesterol crystal mass after 14 days of incubation was reduced by 53%.

b. When the test compound was added to the whole model bile solution (at a concentration of 20% of bile salts) the effects were as follows:

[0061] The nucleation time was prolonged by 200%; the cholesterol crystal growth rate was reduced by 59% and the total crystal mass after 14 days of incubation was reduced by 51%.

B. Native human bile

[0062] When the test compound (at a concentration of 10mM) was incubated with pooled native human gallbladder bile from patients with cholesterol gallstones the results were as follows:

[0063] In the native human bile, from which cholesterol crystals were removed by prior ultracentrifugation for 2 hours, new cholesterol crystals were observed from day 2 of incubation (at 37°C). Crystal numbers increased progressively reaching a peak of more than 150 crystals per microscopic field on day 14. In the same bile incubated with 10mM of the test compound no cholesterol crystals were seen throughout the incubation period of 21 days.

EXAMPLE XIII

[0064] Nucleation studies of cholesterol crystals in model biles were performed as follows:

PaIC was added to the model bile in the following proportions (moles %)

Replacing NaTC by 10%, 20% ("B", "C");

Replacing PC by 20% ("D"); and

Adding 10%, 20% of total NaTC ("E", "F")

The results of the Experiments of Examples XII and XIII are summarized in accompanying Figs. 1 to 3 as follows (in all Figs. 1 to 3 "A" represents the control model bile without PaIC):

[0065] Fig. 1 illustrates the crystal observation time = nucleation time. The results are given as means of triplicates. The crystal observation time was prolonged by 167% in C and by 200% in F.

[0066] Fig. 2 illustrates the cholesterol crystal mass. The cholesterol mass on day 14 was reduced by 17% in B and by 53% in C. It was reduced by 51% in F.

[0067] Fig. 3 illustrates the crystal growth rate. The crystal growth rate was reduced by 70% in B and by 59% in F.

[0068] These experimental data confirmed that conjugates of bile salts and fatty acids prevent or retard cholesterol crystallization in model and human biles.

Example XIV

A. Animals

[0069] Male, 6-7 weeks old hamsters weighing 79-83 g were maintained in an animal house with ad libidum access to water and chow.

[0070] Test hamsters were given per os by a special syringe 10 -15 mg/animal/day of PaIC dissolved in 1ml of saline. Control animals were given an equivalent volume of saline alone. Both groups behaved normally in the course of the treatment. On the second day 4 hours after the application of the PaIC in saline to the respective animals they were killed by an overdose of chloroform vapors. The abdomens were opened, the bile ducts were tied up, the gallbladders were excised and rinsed twice in saline. They were then placed on the top of conical plastic tubes and incised. The bile was collected at the bottom of the tubes. 2 series of animals were examined;

I. 5 control and 5 test animals each receiving 10 mg PaIC/day;

II. 3 control and 9 test animals each receiving 15 mg PaIC day.

B. Biochemical procedures

[0071] Bile samples were centrifuged (Eppendorf centrifuge) for 5 min. at 2000 rpm. Debris was discarded and the supernatant was extracted according to the Folch procedure (chloroform : methanol 2 : 1). After partition with water, the lipid phase was analyzed by thin layer chromatography on silica gel 60 thin layer plates (Merck). The eluent was dichloromethane : methanol : acetic acid (100 : 5 : 1, v : v :v). Samples were compared

with a true standard, Reference front (Rf) about 0.2 after development with I₂ vapors.

C. Results

[0072] In series I 140 µl of control and 100 µl of test bile were obtained. In series II 95 µl of control and 240 µl of test bile were obtained. The control biles and the test biles of each series were separately pooled. The TLC analysis (shown in Fig. 4) of the extracted lipids demonstrated the presence of PalC in the bile of the test animals. This proves that the ingested PalC is absorbed and excreted into the bile.

[0073] Fig. 4 illustrates TLC comparisons of the Experiments performed in Example XIV:

A. illustrates PalC standards:

in pure solution (left) and in bile (right):

B. illustrates hamster biles:

from control hamsters (left); and from hamsters fed with PalC (right). A PalC band is seen in this column.

[0074] Figs. 5A, 5B, Fig. 5C and 5D illustrate the formulae of the compounds described in Examples I to X, respectively.

Example XV

Methods

[0075] The model bile solution had the following composition:

[0076] Cholesterol 15mM, EYL 30ml, NaTC 150 mL. It was prepared as described in Example XI. In the test solutions 20 mole percent of NaTC were replaced by an equimolar amount of each specific fatty acid/bile acid conjugate tested. The results obtained with the conjugates of saturated fatty acids of chain length C₁₄, C₁₆, C₁₈ and C₂₀, respectively, conjugated with cholic acid at position C₃ are shown in Figs. 6 and 7.

[0077] Fig. 6 shows the effect of these conjugates on the cholesterol crystal mass following 14 days of incubation of the control and test solutions. All the above conjugates reduced the final crystal mass in comparison with the control solution. The C₁₈ conjugate reduced it to 14% of the control; the C₂₀ conjugate reduced it to 38%.

[0078] A conjugate of C₂₂ tested in a different experiment showed a similar activity to that of C₂₀.

[0079] Fig. 7 shows the nucleation time (crystal observation time) of the various test solutions as compared to the control solution. Replacement of 20% of the bile salt (NaTC) by the specific conjugates resulted in a prolongation of the nucleation time with C₁₆, C₁₈ and C₂₀

conjugates. C₁₄ did not prolong the nucleation time. The C₂₀ conjugate prolonged the nucleation time by more than 360%.

5 Example XVI

[0080] Pooled human gallbladder bile obtained at operations was enriched with a concentrated lipid solution to enhance cholesterol crystallization. The final concentration in bile of the added lipids was NaTC 60mM, EYL 18.4mM and cholesterol 9.2mM. The enriched bile was ultracentrifuged at 50,000 rpm for 1 hour to remove cholesterol crystals and was then distributed into 5 vials. The first vial contained only enriched bile (control). To the other 4 vials the following solutions were added (at 5mM): cholic acid, C-16 (palmitoyl) cholate, C-18 (stearoyl) cholate and C-20 (arachidyl) cholate. Following 22 days of incubation at 37°C all biles were centrifuged in an airfuge at 70,000rpm for 5 minutes. The sediment was removed and its cholesterol content measured chemically. The results are shown in Fig. 8, as µ moles of cholesterol in the sedimented crystal mass. It is obvious that all three bile salt/fatty acid conjugates very markedly reduced cholesterol crystallization in comparison to the control bile with or without cholic acid.

Example XVII

[0081] A model bile solution was prepared as described in example XI, with the same lipid composition, and served as a control. In all other samples 20 mole% of the NaTC were replaced by equimolar amounts of: cholic acid, C₆ cholate, C₁₂ cholate, C₁₈ cholate, C₂₀ cholate (all these saturated fatty acids were conjugated at position C₃ of the cholate) and di-stearoyl ursodeoxycholate (with the stearic acid radicals conjugated in equal proportions at positions C₃ and C₇ of the bile acid).

[0082] All samples were incubated at 37°C in the same manner as described in example XI and the nucleation time was determined by periodic light microscopic observations. The results are shown in Fig. 9. The results proved that: 1) All conjugates (BAFAC) tested retarded cholesterol crystallization as compared to the control model bile and to equimolar amounts of cholic acid. 2) That BAFAC with longer fatty acid chains were more effective than those with shorter chains. 3) That the conjugate with 2 fatty acids (di-stearoyl ursodeoxycholate) was particularly effective.

50 Example XVIII

Absorption and Transport of Stearoyl-Cholate (C-18 cholate)

[0083] Female hamsters weighing 80-100 g were given a single dose of 30 mg of C-18 cholate by intragastric administration. Single animals were sacrificed at 1, 2,

and 3 hours after administration. Heart blood, portal blood and gallbladder bile were sampled. Two groups of animals (A and B) were studied in parallel. Stearoyl cholate levels were measured with a HPLC instrument (Kontron Switzerland) using a UV detector at 206 nm.

[0084] The results are shown in Fig. 10

[0085] In group A: Heart blood levels after 1, 2 and 3 hours were 99,7,2 μM , while portal blood levels were 68,99 and 133 μM , respectively. C-18 cholate levels in gallbladder bile were 540 and 270 μM at 2 and 3 hours, respectively. Results in group B were similar.

[0086] The data show: 1) That C-18 (stearoyl) cholate is absorbed from the intestine. 2) That it is transported both in the systemic circulation (via the lymph) and in the portal vein 3) That it is actively secreted into the bile and concentrated in it.

Example XIX

[0087] A model bile solution was prepared in the same manner as described in Example XI. It had the same lipid composition and served as control.

[0088] In the test solutions cholic acid, stearoyl (C - 18:0) cholate and oleoyl (C - 18:1) cholate were added in 20mM concentrations.

All samples were incubated at 37°C for 100 hrs. The difference in the optical density between 100 hrs. and 0 hrs (as described in Example XI) was used to measure the total crystal mass at 100 hrs. In comparison with the control solution (100%) the crystal mass with cholic acid was 114%, with stearoyl-cholate 62% and with oleoyl-cholate 55%.

[0089] These results prove that BAFAC with a saturated as well as with an unsaturated (oleic) acid both decrease cholesterol crystallization in comparison with the control bile and with equimolar amounts of cholic acid.

Claims

1. Bile acid or bile salt fatty acid conjugates of general formula II



in which G is a bile acid or bile salt radical, W stands for one or two saturated fatty acid radicals containing 6 to 26 carbon atoms and X is an -NH- bonding member between said bile acid or bile salt radical and the fatty acid radical(s).

2. Bile acid or bile salt fatty acid conjugates according to Claim 1, wherein the bile acids are selected among cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid and their derivatives and analogues thereof.

3. Bile acid or bile salt fatty acid conjugates according to Claim 1 or 2, wherein the bile acid is conjugated with glycine, taurine or a suitable amino acid.

4. Bile acid or bile salt fatty acid conjugates according to any of Claims 1 to 3, wherein the conjugation with the fatty acid radical is performed at position 3 of the bile acid nucleus.

5. Bile acid or bile salt fatty acid conjugates according to any of Claims 1 to 3, wherein the conjugation with the fatty acid radical is performed at a position selected among the 6, 7, 12 and 24 position of the bile acid nucleus.

6. Bile acid or bile salt fatty acid conjugates according to any of Claims 1 to 5, wherein the conjugation between the fatty acid radical and the bile acid is selected among the α - or the β -configuration.

7. Bile acid or bile salt fatty acid conjugates according to Claim 1 herein the saturated fatty acids have 14 to 22 carbon atoms.

8. Bile acid or bile salt fatty acid conjugates according to Claim 1 or 7, wherein the saturated fatty acid is selected among behenyllic acid, arachidylic acid, stearic acid, palmitic acid and myristylic acid.

9. 3 β -Behenylamido-7 α ,12 α -dihydroxy-5-cholan-24-oic acid.

10. 3 β -Arachidylamido-7 α ,12 α -dihydroxy-5-cholan-24-oic acid.

11. 3 β -Stearyl amido-7 α ,12 α -dihydroxy-5-cholan-24-oic acid.

12. 3 β -Palmitylamido-7 α ,12 α -dihydroxy-5-cholan-24-oic acid.

13. 3 β -Myristylamido-7 α ,12 α -dihydroxy-5-cholan-24-oic acid.

14. N-(Carboxymethyl)-3 β -stearyl amido-7 α ,12 α -dihydroxy-5 β -cholan-24-amide.

15. Bile acid or bile salt fatty conjugates according to any of Claims 1 to 4, 6 and 7, wherein W stands for two fatty acids which are conjugated at positions 3 and 7 of the bile nucleus.

16. A pharmaceutical composition enabling the dissolution of cholesterol gallstones in bile and for preventing the formation thereof and enabling the prevention and/or reduction of arteriosclerosis comprising as active ingredient a bile acid or bile salt fatty acid derivative of general formula II according

to any of Claims 1 to 15.

17. A pharmaceutical composition according to Claim 16 the form of which is selected among a tablet, a capsule, a solution and an emulsion.
18. A pharmaceutical composition according to Claim 16 or 17 comprising an additional compound selected among a carrier, a solvent, an emulgator, an enhancer of absorption and an inhibitor of cholesterol synthesis or secretion into the bile.
19. A pharmaceutical composition according to any of Claims 16 to 18 comprising 0.1-1.5 g of the active ingredient.
20. Use of a bile acid or bile salt fatty acid conjugate as defined in any of Claims 1 to 15 or of a pharmaceutical composition as defined in any of Claims 16 to 19 for the manufacture of a medicament for the dissolution of cholesterol gallstones in bile and for the prevention of the formation thereof.
21. Use of a bile add or bile salt fatty acid conjugate as defined in any of Claims 1 to 15 or of a pharmaceutical composition as defined in any of Claims 16 to 19 for the manufacture of a medicament for the prevention and/or reduction of arteriosclerosis.

Patentansprüche

1. Gallensäure- oder Gallensalz-Fettsäure-Konjugate der allgemeinen Formel II



wobei G ein Gallensäure- oder Gallensalzrest ist, W für ein oder zwei gesättigte Fettsäurereste mit 6 bis 26 Kohlenstoffatomen steht und X ein -NH-Bindungselement zwischen dem Gallensäure- oder Gallensalzrest und dem oder den Fettsäureresten ist.

2. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß Anspruch 1, wobei die Gallensäuren aus Cholsäure, Chenodesoxycholsäure, Ursodesoxycholsäure, Desoxycholsäure und ihren Derivaten und Analoga ausgewählt sind.
3. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß Anspruch 1 oder 2, wobei die Gallensäure mit Glycin, Taurin oder einer geeigneten Aminosäure konjugiert ist.
4. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß einem der Ansprüche 1 bis 3, wobei die Kon-

jugation mit dem Fettsäurerest an Position 3 des Gallensäurekerns erfolgt.

5. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß einem der Ansprüche 1 bis 3, wobei die Konjugation mit dem Fettsäurerest an einer Position erfolgt, die aus den Positionen 6, 7, 12 und 24 des Gallensäurekerns ausgewählt ist.
6. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß einem der Ansprüche 1 bis 5, wobei die Konjugation zwischen dem Fettsäurerest und der Gallensäure aus der α - oder β -Konfiguration ausgewählt ist.
7. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß Anspruch 1, wobei die gesättigten Fettsäuren 14 bis 22 Kohlenstoffatome aufweisen.
8. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß Anspruch 1 oder 7, wobei die gesättigte Fettsäure aus Behensäure, Arachinsäure, Stearinsäure, Palmitinsäure und Myristinsäure ausgewählt ist.
9. 3β -Behenylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-säure.
10. 3β -Arachidylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-säure.
11. 3β -Stearylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-säure.
12. 3β -Palmitylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-säure.
13. 3β -Myristylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-säure.
14. N-(Carboxymethyl)- 3β -stearylamido- $7\alpha,12\alpha$ -dihydroxy-5 β -cholan-24-amid.
15. Gallensäure- oder Gallensalz-Fettsäure-Konjugate gemäß einem der Ansprüche 1 bis 4, 6 und 7, wobei W für zwei Fettsäuren steht, die an die Positionen 3 und 7 des Gallensäurekerns konjugiert sind.
16. Pharmazeutische Zusammensetzung, die die Auflösung von Cholesterin-Gallensteinen in der Galle sowie die Prävention ihrer Bildung ermöglicht und die Prävention und/oder Reduktion von Arteriosklerose ermöglicht und als Wirkstoff ein Gallensäure- oder Gallensalz-Fettsäure-Derivat der allgemeinen Formel II gemäß einem der Ansprüche 1 bis 15 umfasst.
17. Pharmazeutische Zusammensetzung gemäß An-

spruch 16, deren Form aus einer Tablette, einer Kapsel, einer Lösung und einer Emulsion ausgewählt ist.

18. Pharmazeutische Zusammensetzung gemäß Anspruch 16 oder 17, die eine zusätzliche Verbindung umfasst, welche aus einem Träger, einem Lösungsmittel, einem Emulgator, einem Resorptionsverstärker und einem Inhibitor der Cholesterinsynthese oder der Sekretion von Cholesterin in die Galle ausgewählt ist.
19. Pharmazeutische Zusammensetzung gemäß einem der Ansprüche 16 bis 18, die 0,1 bis 1,5 g des Wirkstoffs umfasst.
20. Verwendung eines Gallensäure- oder Gallensalz-Fettsäure-Konjugats, wie es in einem der Ansprüche 1 bis 15 definiert ist, oder einer pharmazeutischen Zusammensetzung, wie es in einem der Ansprüche 16 bis 19 definiert ist, zur Herstellung eines Medikaments zur Auflösung von Cholesterin-Gallensteinen in der Galle und zur Prävention ihrer Bildung.
21. Verwendung eines Gallensäure- oder Gallensalz-Fettsäure-Konjugats, wie es in einem der Ansprüche 1 bis 15 definiert ist, oder einer pharmazeutischen Zusammensetzung, wie es in einem der Ansprüche 16 bis 19 definiert ist, zur Herstellung eines Medikaments zur Prävention und/oder Reduktion von Arteriosklerose.

Revendications

1. Conjugués d'acide biliaire ou d'acide gras de sel biliaire de formule générale II :



dans laquelle **G** est un acide biliaire ou un radical de sel biliaire, **W** représente un ou deux radicaux d'acide gras saturés contenant de 6 à 26 atomes de carbone et **X** est un élément de liaison **-NH-** entre ledit acide biliaire ou radical de sel biliaire et le ou les radicaux d'acide gras.

2. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon la revendication 1, caractérisés en ce que les acides biliaires sont choisis parmi l'acide cholique, l'acide chénodéoxycholique, l'acide ursodéoxycholique, l'acide déoxycholique et leurs dérivés et analogues.
3. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon la revendication 1 ou 2, **caractérisés en**

ce que l'acide biliaire est conjugué à la glycine, la taurine ou un acide aminé approprié.

4. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 à 3, **caractérisés en ce que** la conjugaison avec le radical d'acide gras est effectuée en position **3** du noyau de l'acide biliaire.
5. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 à 3, **caractérisés en ce que** la conjugaison avec le radical d'acide gras est effectuée dans une position choisie parmi les positions **6, 7, 12** et **24** du noyau de l'acide biliaire.
6. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 à 5, **caractérisés en ce que** la conjugaison entre le radical d'acide gras et l'acide biliaire est choisie parmi la configuration α ou β .
7. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon la revendication 1, **caractérisés en ce que** les acides gras saturés possèdent de **14** à **22** atomes de carbone.
8. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 ou 7, **caractérisés en ce que** l'acide gras saturé est choisi parmi l'acide behénylique, l'acide arachidylique, l'acide stéarique, l'acide palmitique et l'acide myristylique.
9. Acide 3β -behénylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-oïque.
10. Acide 3β -arachidylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-oïque.
11. Acide 3β -stéarylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-oïque.
12. Acide 3β -palmitylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-oïque.
13. Acide 3β -myristylamido- $7\alpha,12\alpha$ -dihydroxy-5-cholan-24-oïque.
14. N-(carboxyméthyl)- 3β -stéarylamido- $7\alpha,12\alpha$ -dihydroxy-5 β -cholan-24-amide.
15. Conjugués d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 à 4, 6 et 7, **caractérisés en ce que W** représente deux acides gras qui sont conjugués aux positions **3** et **7** du noyau biliaire.
16. Composition pharmaceutique permettant la disso-

- lution de calculs biliaires à cholestérol dans la bile et destinée à empêcher leur formation et à permettre la prévention et/ou la réduction de l'artériosclérose, comprenant comme ingrédient actif un dérivé d'acide biliaire ou d'acide gras de sel biliaire de formule générale II selon l'une des revendications 1 à 15. 5
- 17.** Composition pharmaceutique selon la revendication 16, dont la forme est choisie parmi un comprimé, une capsule, une solution et une émulsion. 10
- 18.** Composition pharmaceutique selon la revendication 16 ou 17, comprenant un composant supplémentaire choisi parmi un porteur, un solvant, un émulsifiant, un renforteur d'absorption et un inhibiteur de synthèse de cholestérol ou de sécrétion dans la bile. 15
- 19.** Composition pharmaceutique selon l'une des revendications 16 à 18, comprenant 0,1 à 1,5 g de l'ingrédient actif. 20
- 20.** Utilisation d'un conjugué d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 à 15 ou d'une composition pharmaceutique selon l'une des revendications 16 à 19 pour la fabrication d'un médicament pour la dissolution de calculs biliaires à cholestérol dans la bile et pour la prévention de leur formation. 25
30
- 21.** Utilisation d'un conjugué d'acide biliaire ou d'acide gras de sel biliaire selon l'une des revendications 1 à 15 ou d'une composition pharmaceutique selon l'une des revendications 16 à 19 pour la fabrication d'un médicament pour la prévention et/ou la réduction de l'artériosclérose. 35

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Fig. 1

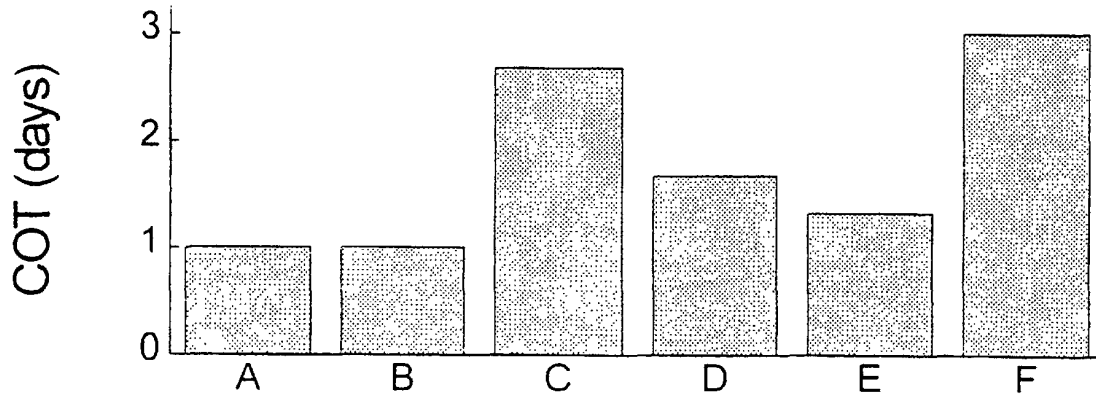


Fig. 2

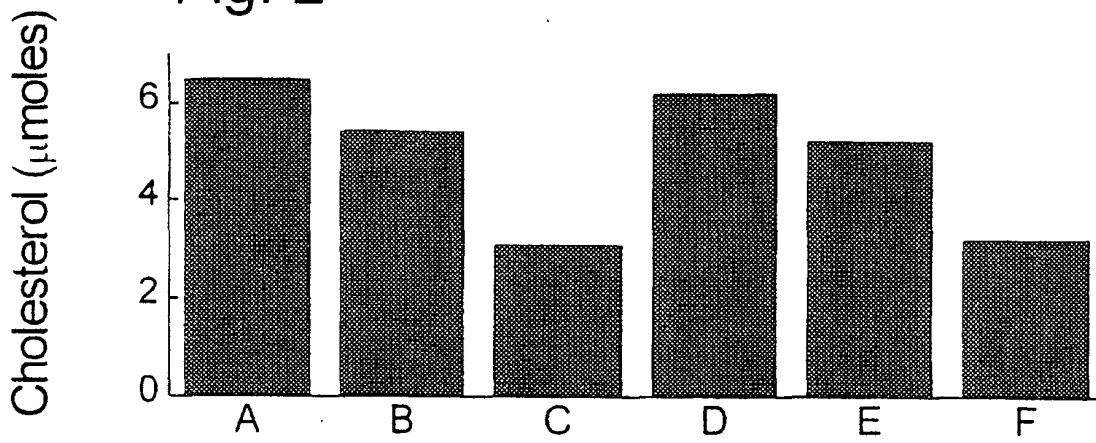
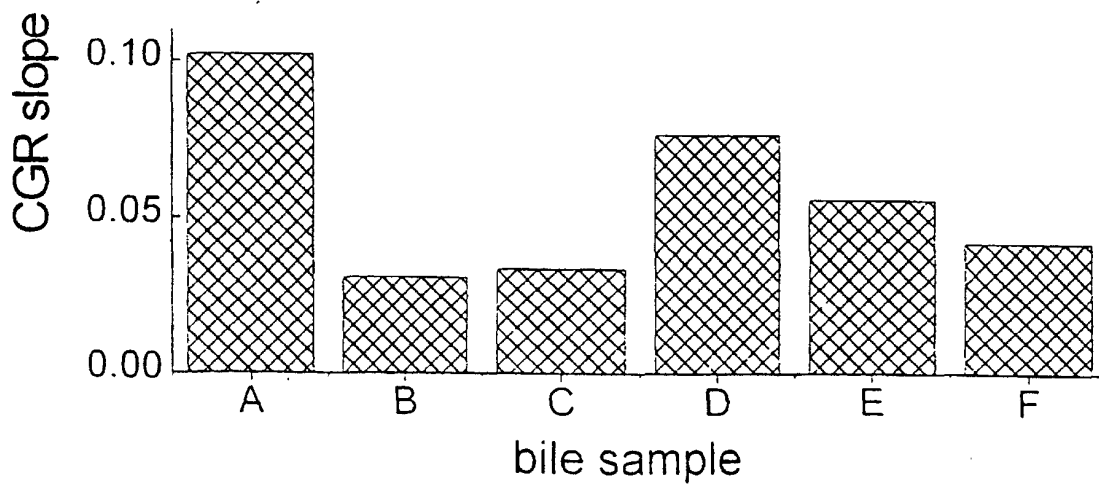


Fig. 3



PaIC

Hamster bile

pure in bile

control PaIC fed

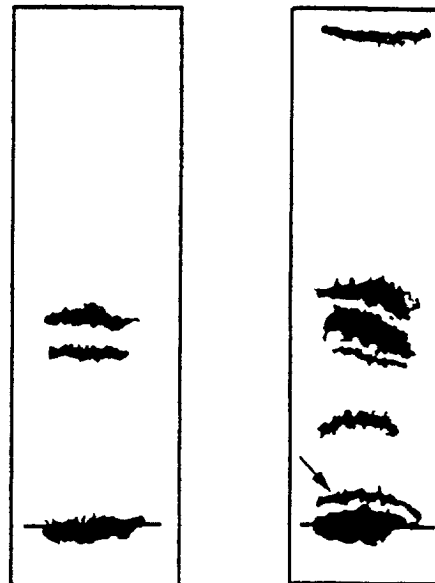
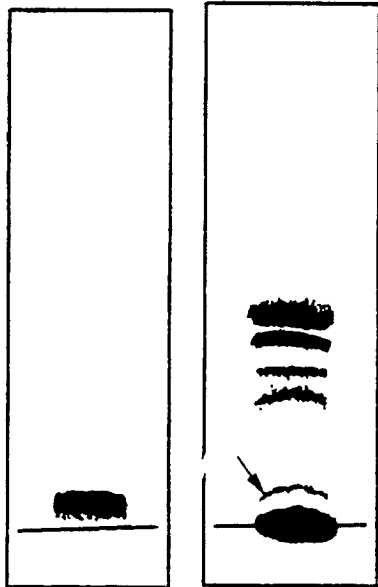


Fig. 4A

Fig. 4B

Fig. 5A

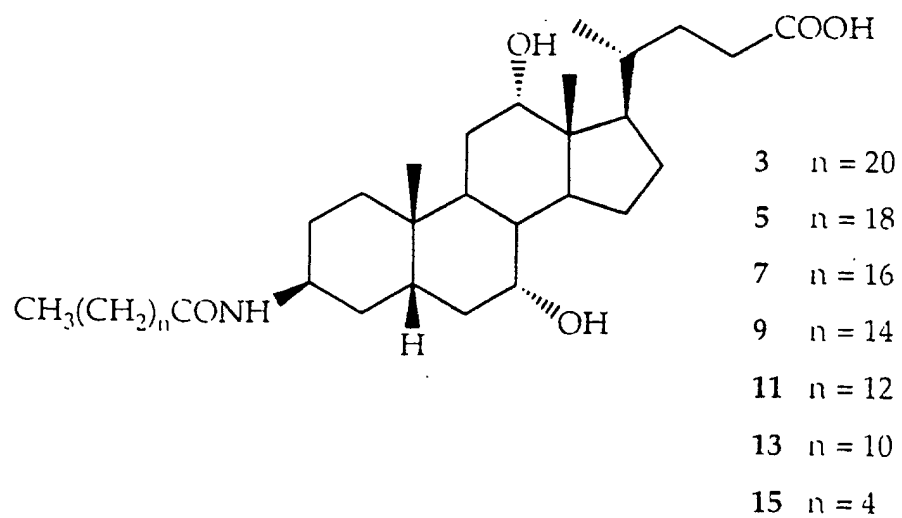
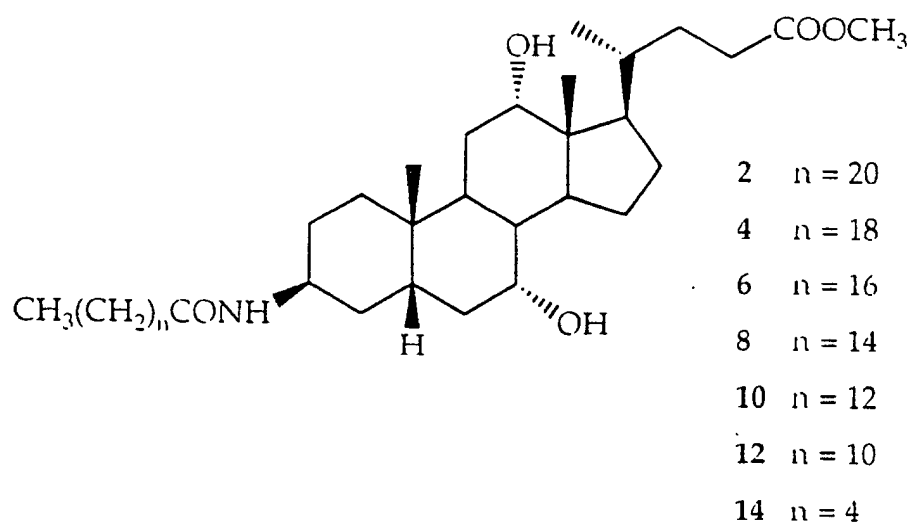
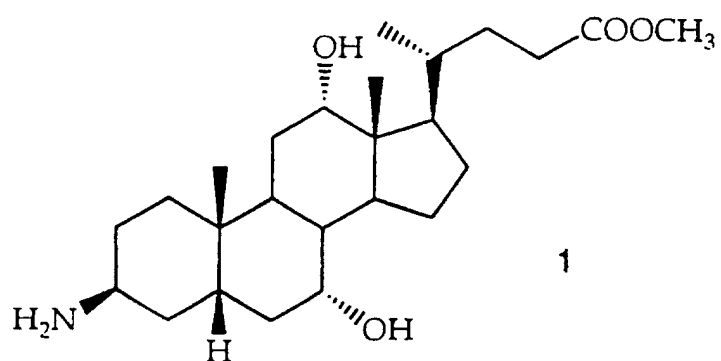


Fig. 5B

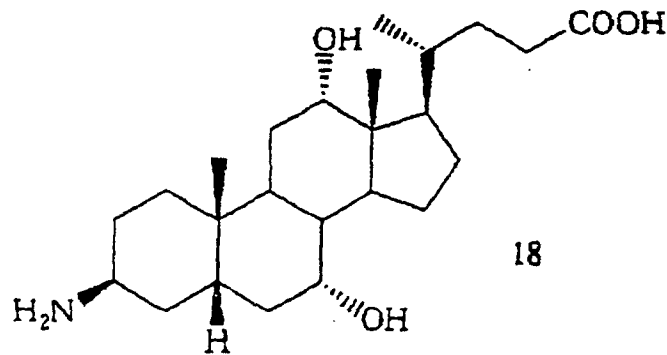
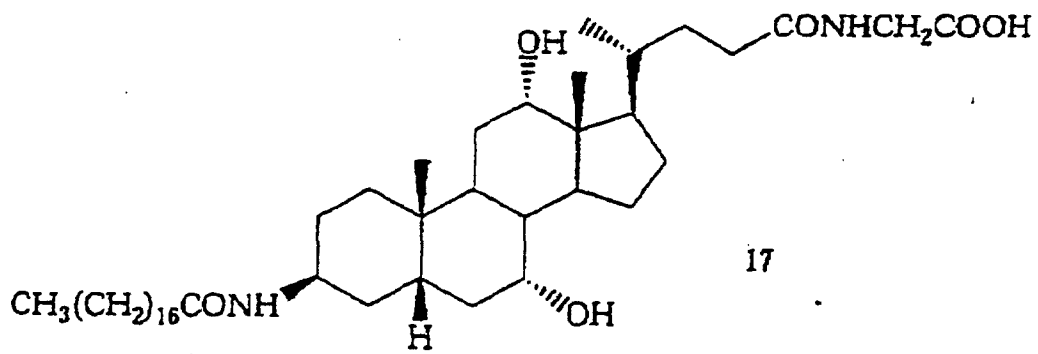
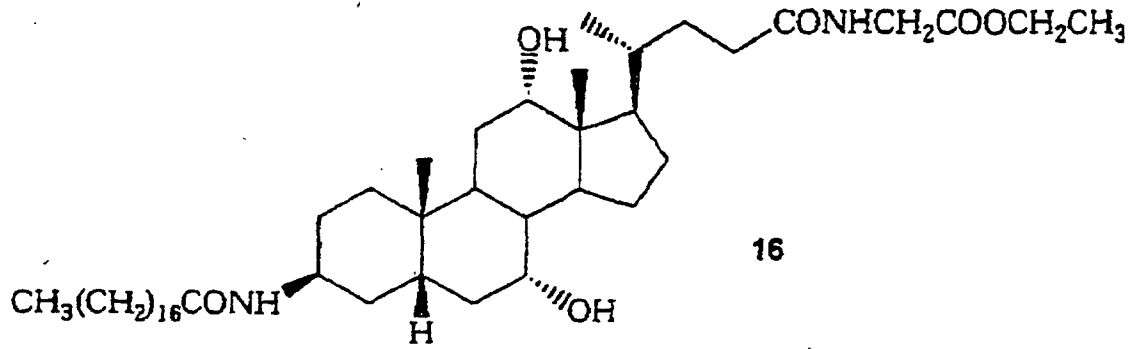


Fig. 5C

(reference example)

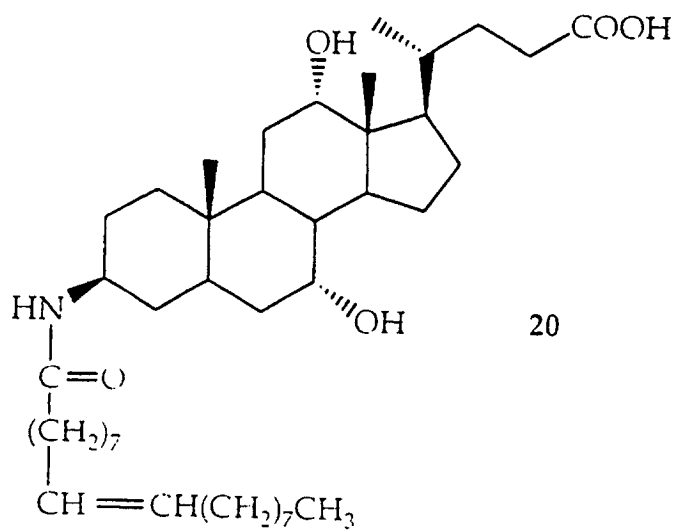
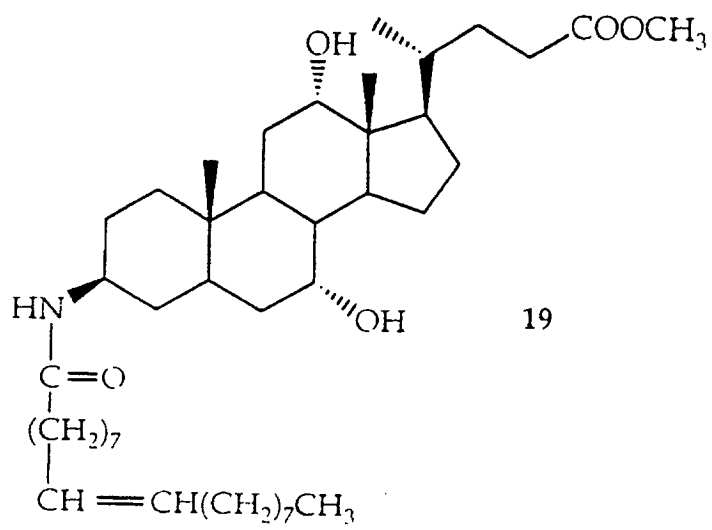
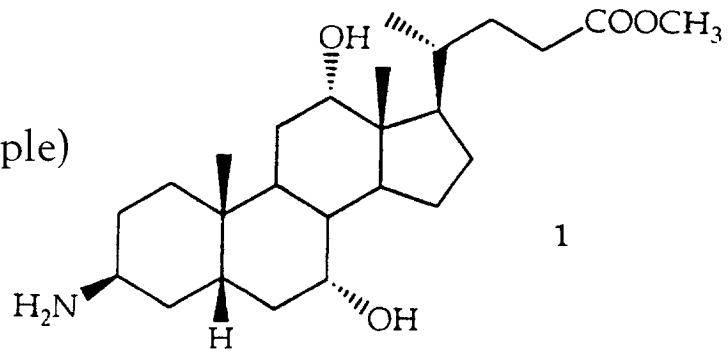


Fig. 5D

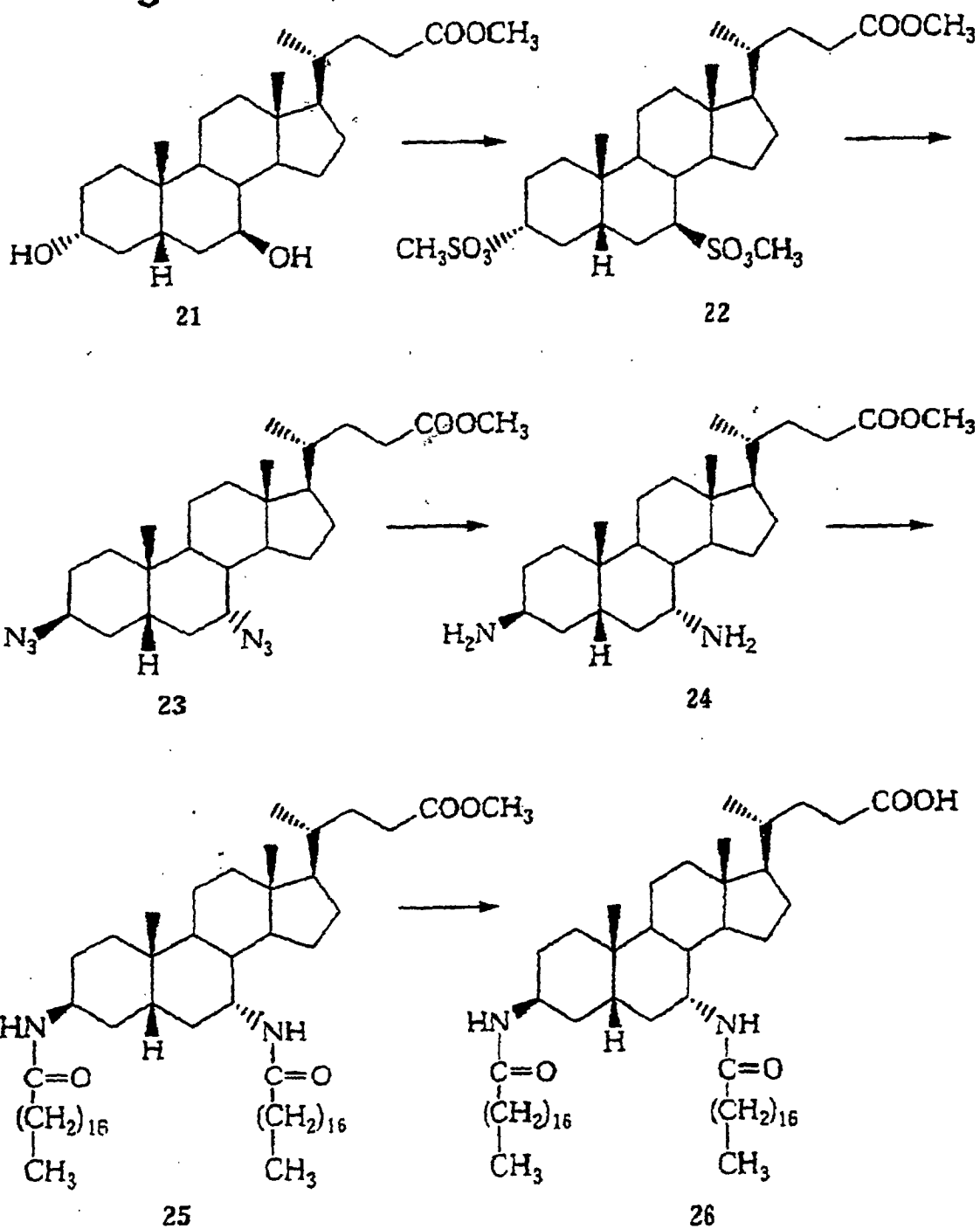


Fig. 6

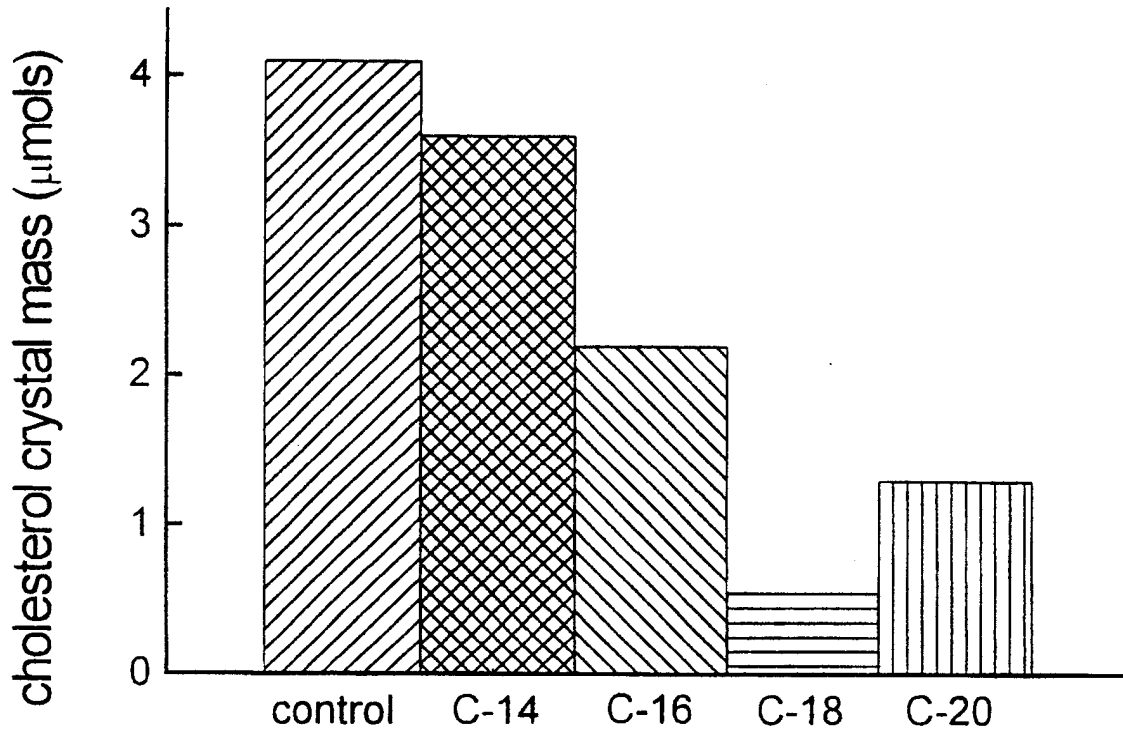


Fig. 7

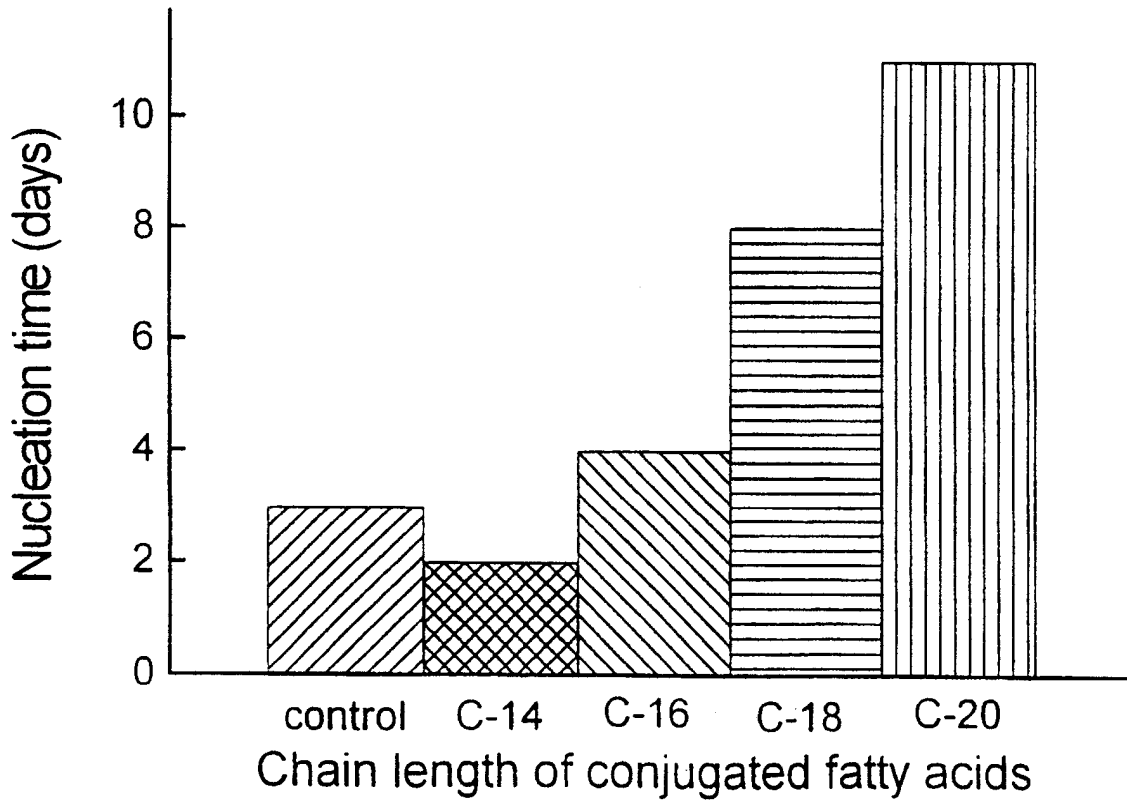


Fig. 8

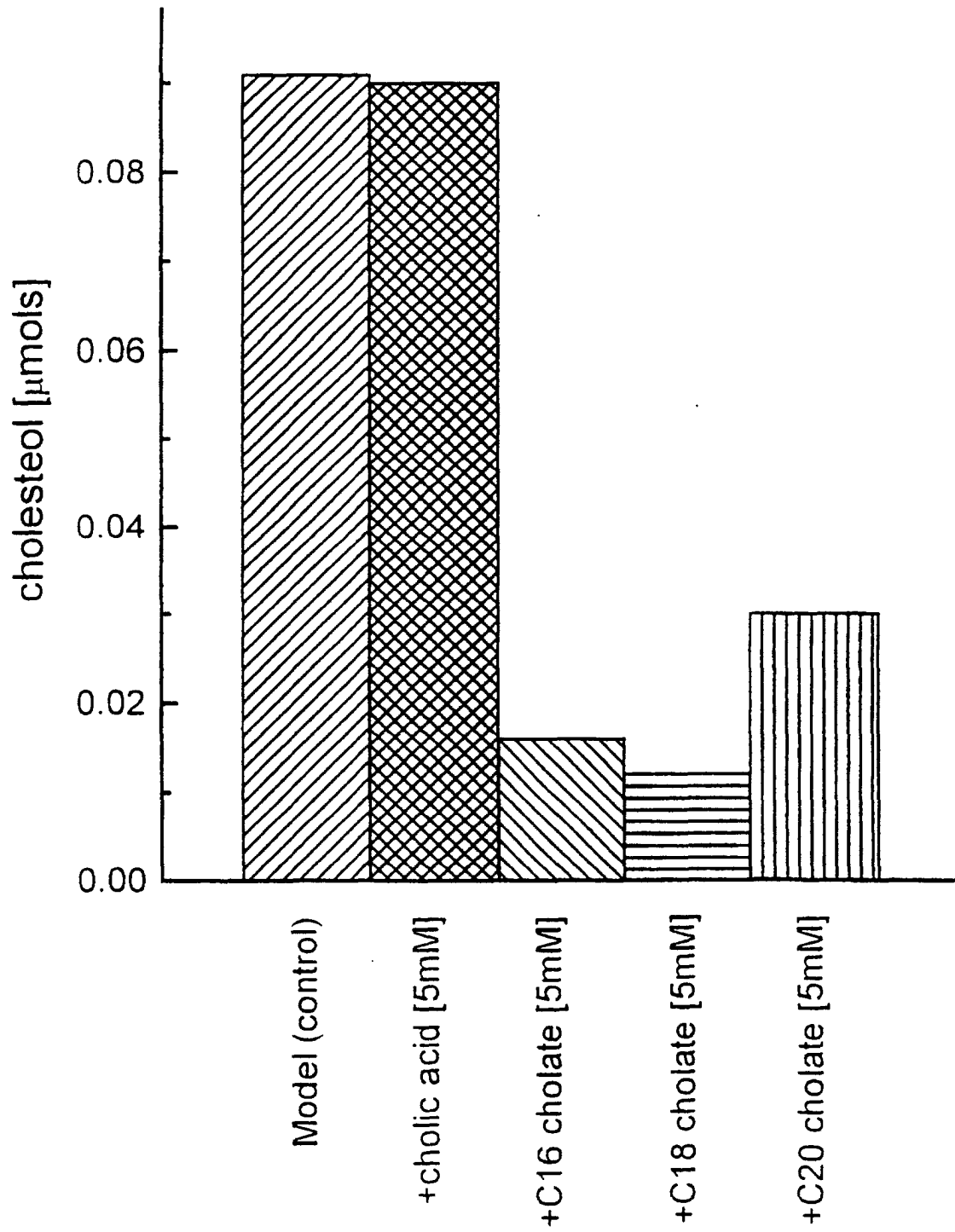


Fig. 9

