



(22) Date de dépôt/Filing Date: 1990/04/12

(41) Mise à la disp. pub./Open to Public Insp.: 1990/10/17

(45) Date de délivrance/Issue Date: 2001/08/14

(30) Priorité/Priority: 1989/04/17 (20170 A/89) IT

(51) Cl.Int.<sup>5</sup>/Int.Cl.<sup>5</sup> C07J 9/00, A61K 31/575

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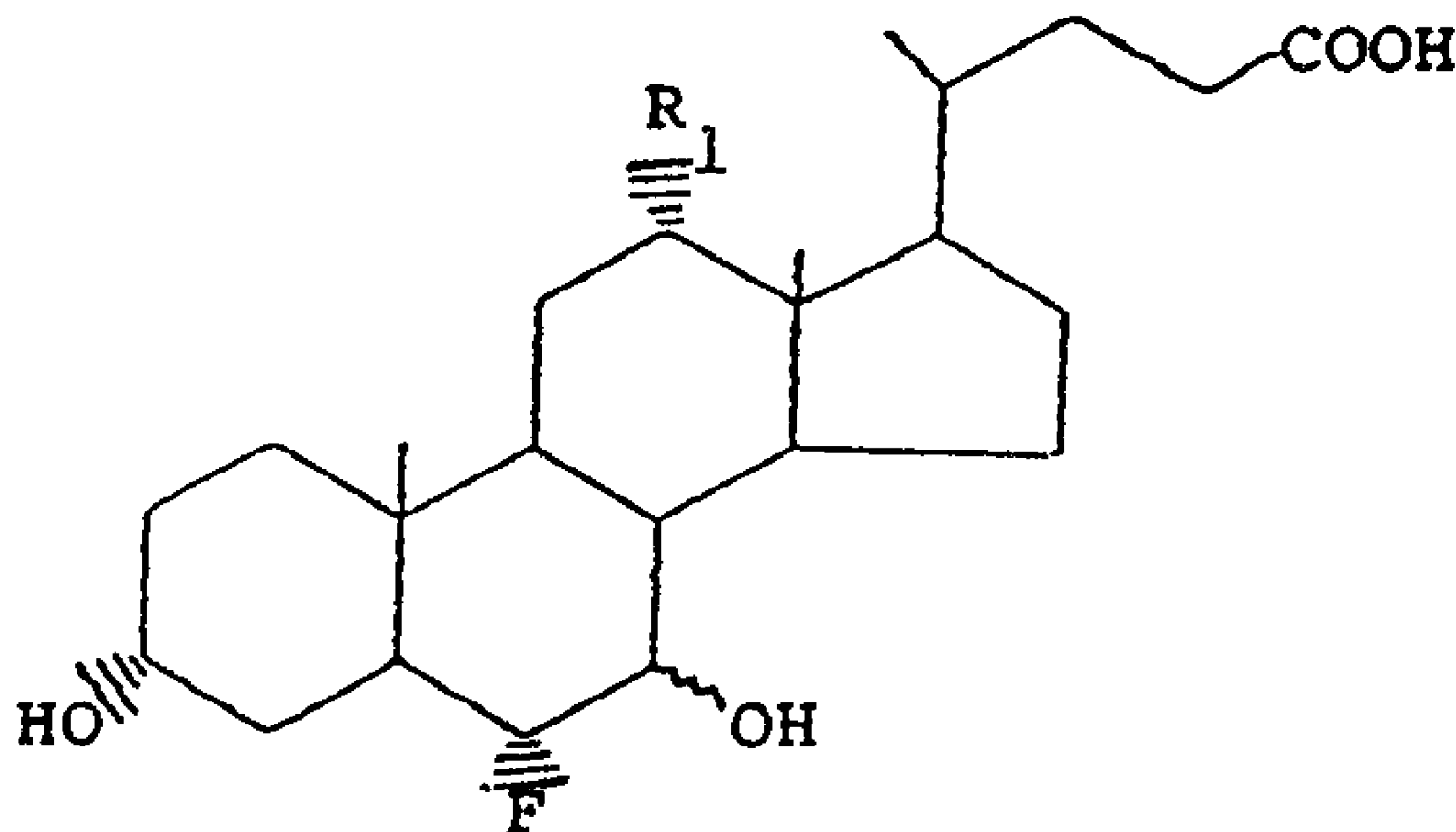
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(54) Titre : DERIVES D'ACIDE BILIAIRE; METHODE DE PREPARATION DESDITS DERIVES ET COMPOSITIONS PHARMACEUTIQUES UTILISANT CES DERIVES

(54) Title: FLUORINATED BILE ACID DERIVATIVES, PROCESSES FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM



( I )

(57) Abrégé/Abstract:

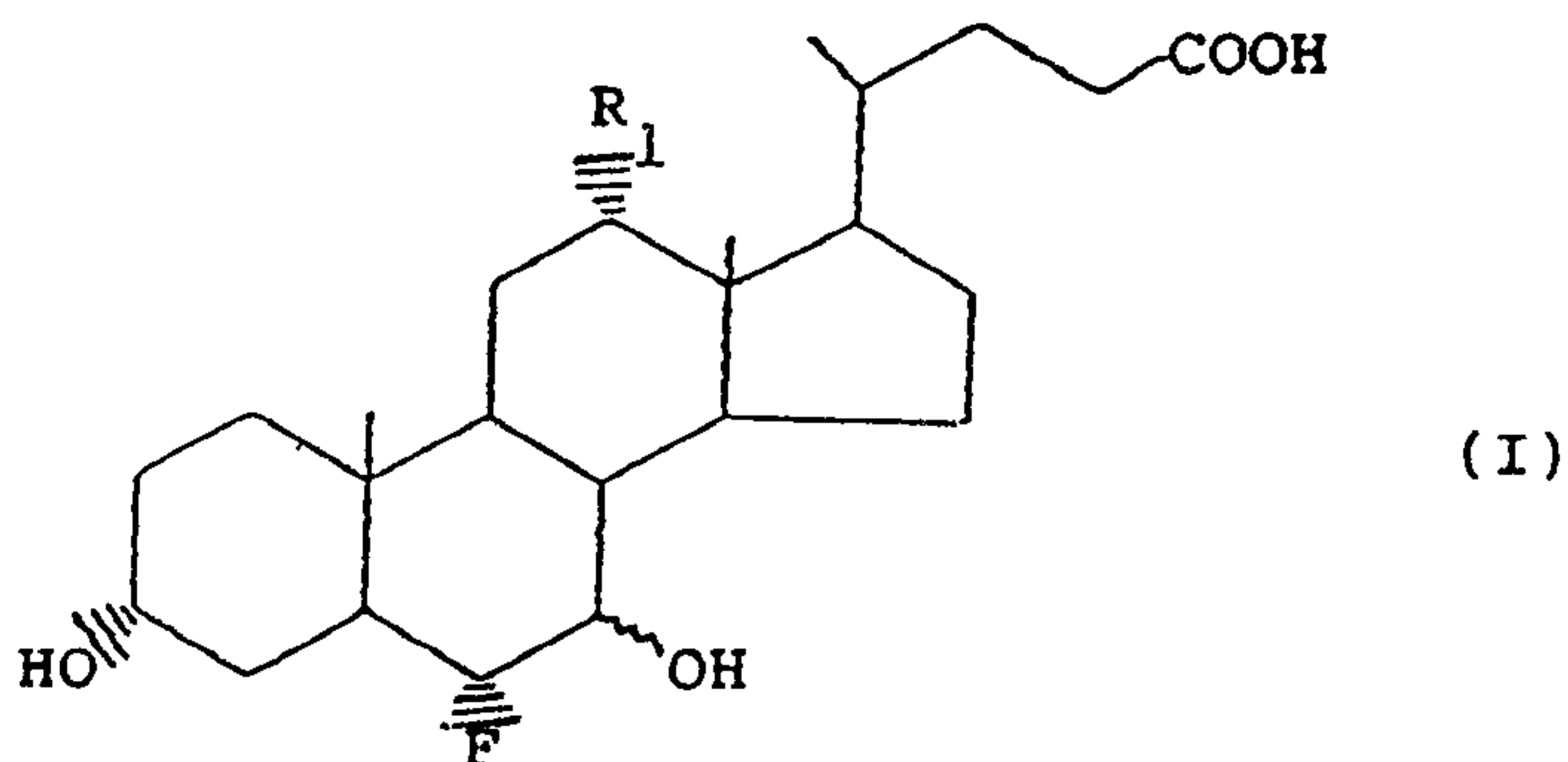
A compounds of general formula I (see formula I) wherein R<sub>1</sub> is hydrogen or hydroxy, and the hydroxy group at the 7-position can be either in or B configuration, are valuable in human therapy. Compounds I can be prepared by fluorination of the suitably protected 6-hydroxy-7-keto-derivatives.



Abstract

FLUORINATED BILE ACID DERIVATIVES, PROCESSES FOR THE  
PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITIONS  
CONTAINING THEM

A compounds of general formula I

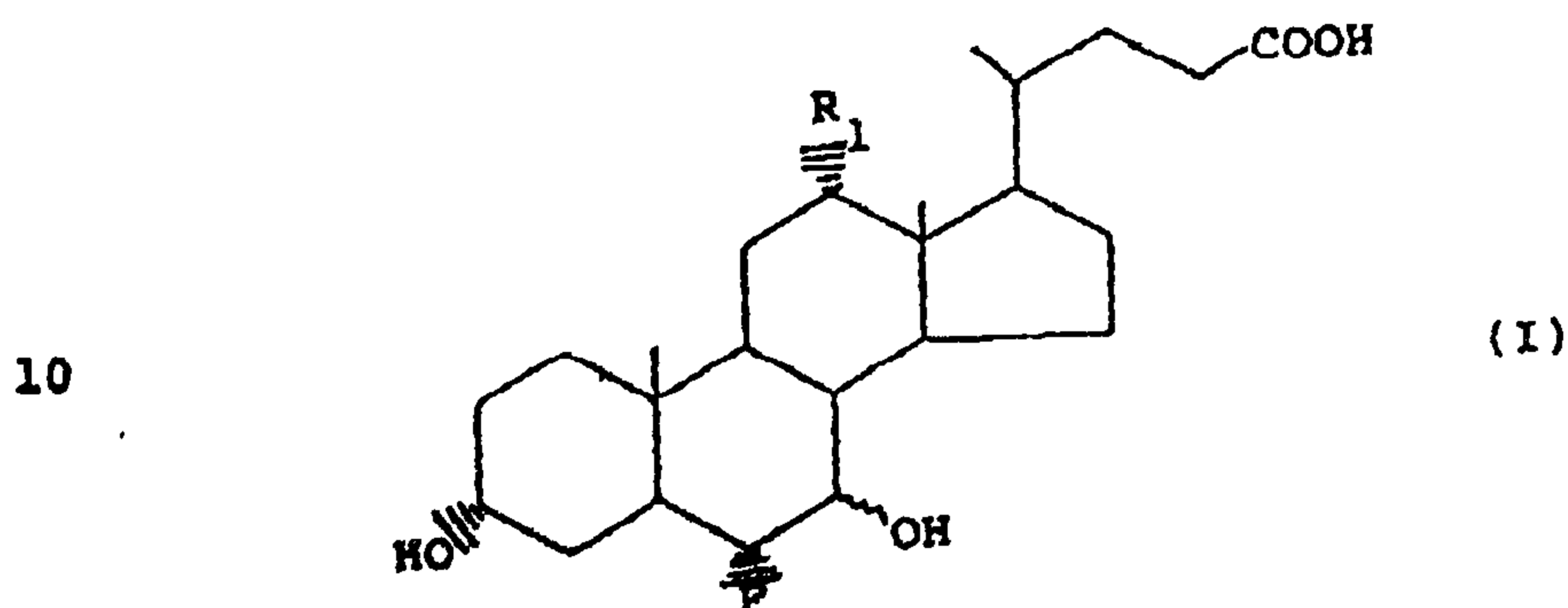


wherein  $R_1$  is hydrogen or hydroxy, and the hydroxy group at the 7-position can be either in  $\alpha$  or  $\beta$  configuration, are valuable in human therapy. Compounds I can be prepared by fluorination of the suitably protected 6 -hydroxy-7-keto-derivatives.

FLUORINATED BILE ACID DERIVATIVES, PROCESSES FOR THE  
PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITIONS  
CONTAINING THEM

The present invention relates to bile acid derivatives, to a process for the preparation thereof and to pharmaceutical compositions containing them.

The derivatives of the present invention have the  
 5 following general formula I



wherein  $R_1$  is hydrogen or hydroxy, and the hydroxy group  
 15 at 7-position can be either in  $\alpha$  or  $\beta$  configuration.

Therefore, compounds I are the 6- $\alpha$ -fluoro derivatives of the following natural bile acids: ursodeoxycholic (UDCA) ( $3\alpha$ ,  $7\beta$  OH), ursocholic ( $3\alpha$ ,  $7\beta$  OH;  $R_1$  = OH), chenodeoxycholic ( $3\alpha$ ,  $7\alpha$  OH) and cholic ( $3\alpha$ ,  $7\alpha$   
 20 OH;  $R_1$  = OH) acids.

The present invention also relates to the physiologically acceptable salts of compounds I, as well as to possible glycine or taurine conjugated forms. Moreover, the invention also relates to the single isomers or diastereoisomers of compounds I and to the mixtures thereof.  
 25

The above cited bile acids have been used for a long

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time in human therapy for the treatment of biliary  
calculosis, as antidyspeptic, eupeptic, antidyslipidemic and  
choleretic agents, and generally in all those pathological  
conditions in which a stimulation of bile flow and a  
5 qualitative and/or quantitative change thereof are required.

Therapeutic characteristics of natural molecules  
promoted the development of a number of synthetic or semi-  
synthetic derivatives in the attempt to obtain improved  
drugs as regard pharmacokinetic, metabolic or chemico-  
10 physical aspects (lipophilia/hydrophilia ratio, stability,  
critical micellar concentration). See, for instance, EP  
101554 published February 29, 1984, EP 124068 published  
November 7, 1984, EP 135782 published April 3, 1985 and EP  
186023 published July 2, 1986, and USA-P 4648995 issued March  
15 10, 1987, 4460509 issued July 17, 1984, 4545938 issued  
October 8, 1985.

The above cited US patents particularly disclose 7-  
methyl, 7-hydroxy derivatives which, in comparison with the  
natural molecules, should provide the advantage of a higher  
20 resistance to 7-dehydroxylation by intestinal bacterial  
flora, and accordingly a prolonged half-life as well as an  
increase in stability.

These and other advantages are provided by the  
compounds of the present invention, which compounds are  
25 characterized by the presence of a fluorine atom at the 6-  
position, the 7-position being substantially unchanged in  
comparison with the natural molecule, which is per se  
advantageous since 7-position has been found to be  
critical as regard pharmacological activity.

30 Fluorine at the 6- $\alpha$ -position makes the molecule less  
liable to 7-dehydroxylation.

Moreover, fluorinated derivatives show an increased

pharmacological activity and favourable pharmaco-kinetic characteristics, in comparison with natural bile acids.

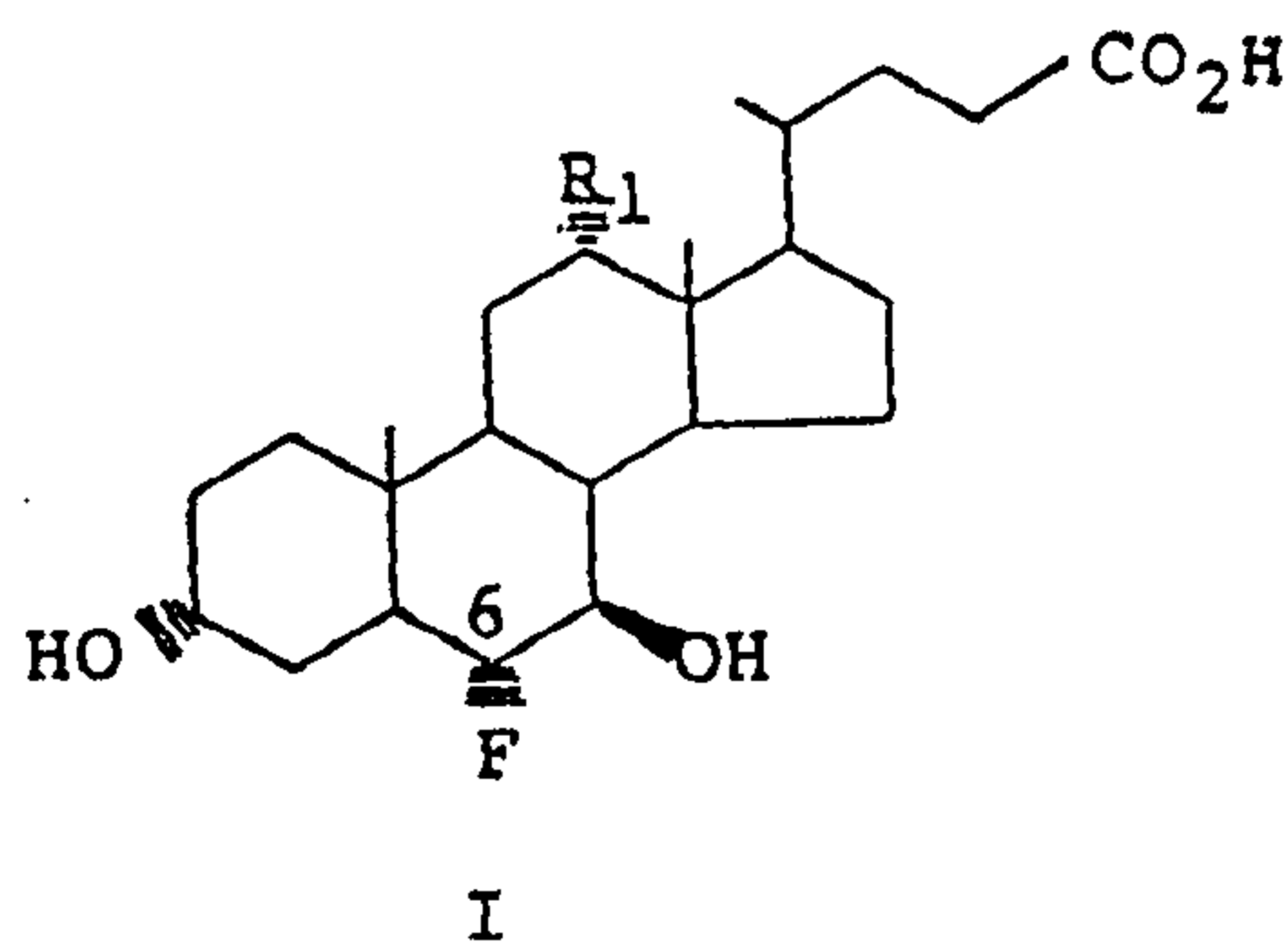
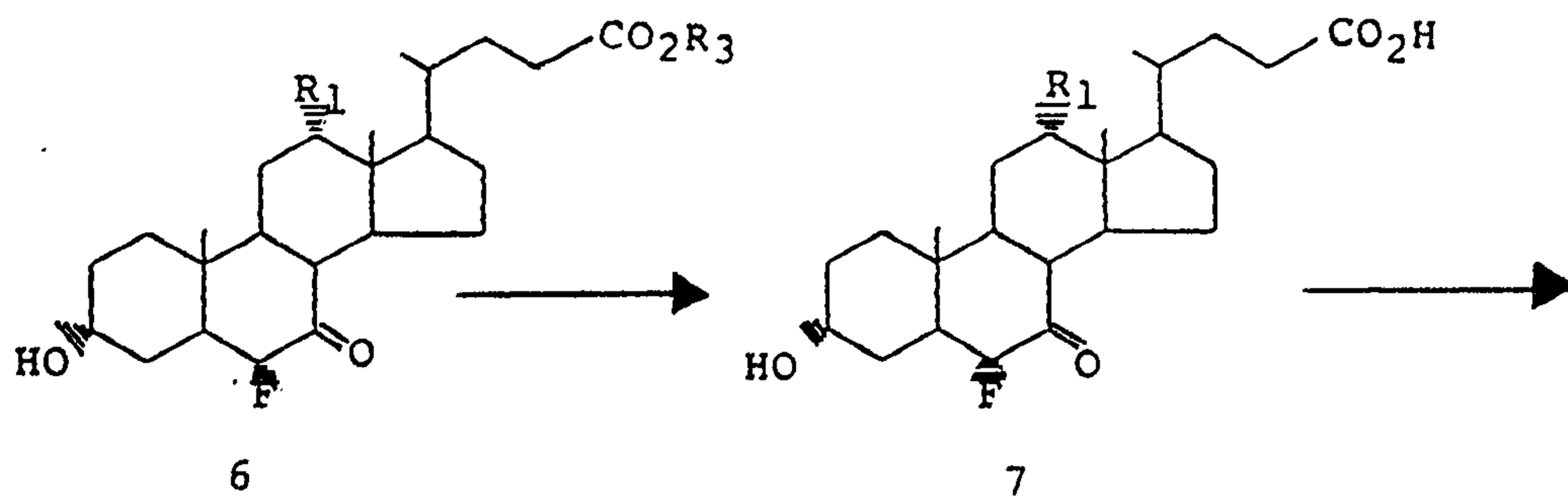
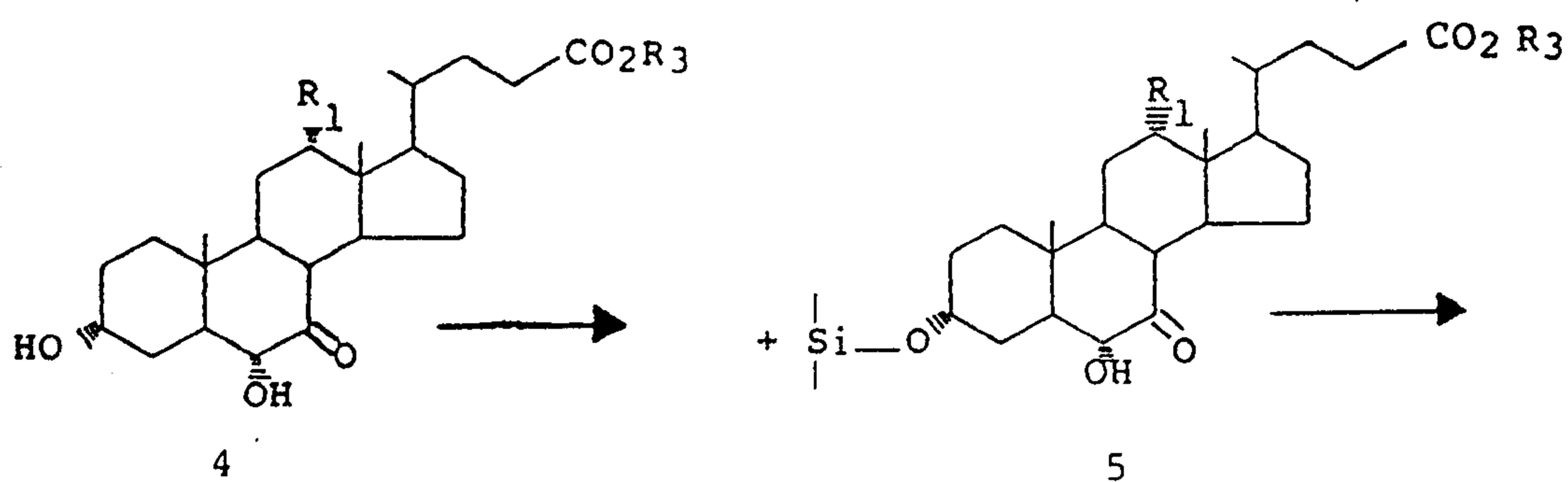
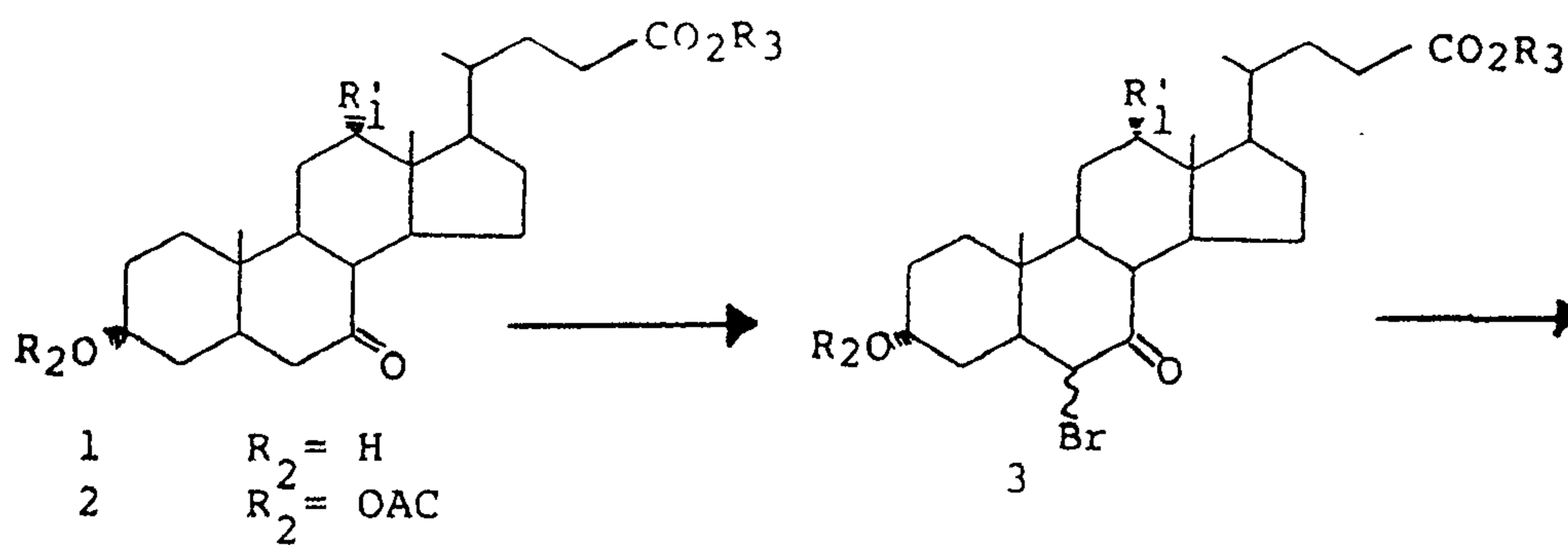
5 The compounds of the invention have a very low acute toxicity ( $LD_{50}$  in the mouse per os  $> 5$  g/kg), and are very useful particularly for the treatment of cholelithiasis, biliary dyskinesias, hypercholesterolemia, atherosclerosis, dispepsias and generally in all those pathological conditions for which the therapeutical usefulness of the known bile acids has been recognized.

10 The compounds of the invention, for the envisaged therapeutical uses, are administered in form of pharmaceutical compositions prepared according to known techniques and excipients, as described e.g. in "Remington's Pharmaceutical Sciences Handbook", Hack Pub. Co., N.Y. USA.

15 The preferred administration route is the oral one, and the daily doses, which will vary depending on the pathology to be treated and the patient's conditions, will in principle be comprised from 50 to 500 mg, one or more times a day.

20 Examples of suitable pharmaceutical compositions comprise capsules, tablets, sugar-coated pills, syrups, granulates, solutions, vials. The compounds of the invention can also be administered by local perfusion, before or after surgical operations, in form of  
25 dispersible solutions or powders.

The process for the preparation of compounds I is carried out according to the scheme reported hereinbelow, in which  $R_2$  is a hydroxy protecting group,  $R_1$  is hydrogen or a protected hydroxy group,  $R_3$  is a carboxy protecting  
30 group and  $R_1$  is as defined in formula I.



The bromination reaction can be carried out using bromine in acetic acid, N-bromosuccinimide or other conventional bromination agents.

5 Stereospecific substitution of the bromine atom at the 6-position is usually effected by reaction with an alkali hydroxide, preferably potassium hydroxide, in an alcoholic or water-alcoholic solution. The introduced 6-hydroxy group is sterically hindered and therefore a selective protection of the 3-hydroxy group and possibly  
10 of the 11-hydroxy group can be effected. Preferred protecting groups are, for example, silanes having bulky alkyl groups, such as tert-butyldimethyl-silyl. The hydroxy group can be replaced by the fluorine atom using an appropriate fluoride ion source, for example  
15 diethylamino-sulfur-trifluoride, (DAST) in anhydrous solvents or with quaternary ammonium fluorides, such as tetra-t-butylammonium fluoride.

Elimination of the hydroxy and carboxy protecting groups and reduction of the 7-keto group give compounds  
20 of formula I.

Suitable hydroxy protecting groups are esters, such as acetates, trichloroacetates, formates, benzoates, benzyloxy carbonyl derivatives, carbonates; ethers such as tetrahydropyranyl ethers, silyl derivatives and the  
25 like. Suitable carboxy protecting groups are esters such as methyl, t-butyl, benzyl, benzhydryl, trityl, p-nitrobenzyl, trimethylsilyl and tetrahydropyranyl esters, amides, hydrazides and the like.

Although all the known protecting groups can be used  
30 as far as they are inert under the selected reaction

conditions, the acetic ester is preferred as the hydroxy protecting group and the methyl ester is preferred as the carboxy protecting group.

5 The following examples further illustrate the invention without limiting it.

EXAMPLE 1

Preparation of 3 $\alpha$ , 7 $\beta$ -dihydroxy-6 $\alpha$ -fluoro-5 $\beta$ -cholanoic acid.

a) Methyl 3 $\alpha$ -acetoxy-7-keto-5 $\beta$ -cholanoate.

10 A solution of methyl 3 $\alpha$ -hydroxy-7-keto-5 $\beta$ -cholanoate (10 g, 24.72 mmol) in methylene chloride (40 ml) was added with acetic anhydride (3.79 g, 37.1 mmol), triethylamine (3.63 g, 36 mmol) and 4-pyrrolidino-  
15 pyridine (0.11 g, 0.74 mmol) and the reaction mixture was left to react for 3 hours under strong magnetic stirring, then it was diluted with methylene chloride (50 ml) and water (50 ml) and acidified with 10% hydrochloric acid. The organic phase was separated and the aqueous phase was extracted with methylene chloride (3 x 20 ml).  
20 The combined organic phases were washed with brine (1 x 30 ml), dried over sodium sulfate and concentrated under vacuum. The residue (11.0 g) was subjected to flash chromatography on silica, eluting with methylene chloride/methanol 99:1, to obtain 10.5 g of a pure  
25 product (95%).

b) Methyl 3 $\alpha$ -acetoxy-6-bromo-7-keto-5 $\beta$ -cholanoate.

A solution of methyl 3 $\alpha$ -acetoxy-7-keto-5 $\beta$ -cholanoate (5.6 g, 12.54 mmol) in acetic acid (100 ml) was added with a bromine solution (2.8 g) in acetic acid (10 ml)  
30 and immediately after with 48% hydrobromic acid (0.74

ml). The resulting reaction mixture was left to react overnight under strong magnetic stirring, then it was poured into water (300 ml) and filtered under vacuum to obtain a solid (8,03 g) which was subjected to flash chromatography on silica, eluting with methylene chloride/methanol 99.5:0.5, to yield 6.0 g of a pure product (91%).

c) Methyl 3 $\alpha$ , 6 $\alpha$ -dihydroxy-7-keto-5 $\beta$ -cholanoate.

A solution of methyl 3 $\alpha$ -acetoxy-6-bromo-7-keto-5 $\beta$ -cholanoate (10.0 g, 19.025 mmoles) in methanol (600 ml) was added to a solution of potassium hydroxide (24 g) in water (100 ml), dropwise, in 1 hour. The resulting mixture was left to react for 48 hours under argon atmosphere, with magnetic stirring. The reaction mixture was diluted with water (100 ml), acidified with 10% hydrochloric acid and extracted with chloroform (3 x 200 ml). The combined organic phases were washed with brine (1 x 50 ml) and dried over sodium sulfate. After evaporation of the solvent, the residue (7.7 g) was reacted with p-toluenesulfonic acid (0.5 g) in methanol (250 ml) under magnetic stirring for one night. The reaction mixture was concentrated under vacuum, taken up into chloroform (150 ml) and washed first with water (3 x 30 ml), then with brine (1 x 50 ml). The organic phase was dried over sodium sulfate and concentrated under vacuum. The residue (8.0 g) was subjected to flash chromatography on silica, eluting with chloroform/methanol 98:2, to obtain 4.5 g of a pure product (56%).

d) Methyl 3 $\alpha$ -tert-butyltrimethylsilyloxy-6 $\alpha$ -hydroxy-7-keto-5 $\beta$ -cholanoate.

A solution of methyl 3 $\alpha$ , 6 $\alpha$ -dihydroxy-7-keto-5 $\beta$ -cholanoate (4.4 g, 10.45 mmoles) in anhydrous dimethylformamide (120 ml) were added with imidazole (7.4 g, 108.8 mmoles), anhydrous pyridine (1.07 ml) and tert-butyltrimethylchlorosilane (3.3 g, 21.9 mmoles); the mixture was left to react for 4 hours under strong magnetic stirring, under argon atmosphere, then it was diluted with methylene chloride (100 ml), washed with water (3 x 50 ml), then with brine (1 x 50 ml). The organic phase was dried over sodium sulfate and concentrated under vacuum. The residue (6.20 g) was subjected to flash chromatography on silica, eluting with chloroform, to obtain 3.36 g of a pure product (60%).

e) Methyl 3 $\alpha$ -hydroxy-6 $\alpha$ -fluoro-7-keto-5 $\beta$ -cholanoate.

A solution of methyl 3 $\alpha$ -tert-butyltrimethylsilyloxy-6 $\alpha$ -hydroxy-7-keto-5 $\beta$ -cholanoate (2.4 g, 4.49 mmoles) in methylene chloride (50 ml) was added dropwise in one hour to a solution of diethylamino-sulfur-trifluoride (2.72 ml, 20.0 mmoles) in methylene chloride (50 ml) and the mixture was left to react overnight under argon atmosphere, with magnetic stirring. The reaction mixture was washed with water (1 x 30 ml), subsequently with a sodium bicarbonate saturated solution (2 x 20 ml), finally with brine (1 x 30 ml). The organic phase was dried over sodium sulfate and concentrated under vacuum. The residue (2.0 g) was subjected to flash chromatography on silica, eluting with methylene chloride, to obtain 0.6 g of a pure product (32%).

f) 3 $\alpha$ -hydroxy-6 $\alpha$ -fluoro-7-keto-5 $\beta$ -cholanoic acid.

A solution of methyl 3 $\alpha$ -hydroxy-6 $\alpha$ -fluoro-7-keto-5 $\beta$ -

-cholanoate (0.6 g, 1.42 mmoles) in methanol (46 ml) was added to a methanol solution of 10% potassium hydroxide (82 ml) and the resulting solution was kept under magnetic stirring overnight. Then methanol was removed under vacuum, the residue was taken up into water (50 ml), acidified with 10% hydrochloric acid and extracted with methylene chloride (3 x 30 ml). The combined organic phases were washed with brine (1 x 20 ml) and dried over sodium sulfate. After evaporation of the solvent, the residue (0.62 g) was subjected to flash chromatography on silica, eluting with methylene chloride/methanol 95:5, to obtain 0.55 g of a pure product (95%).

g) 3 $\alpha$ , 7 $\beta$ -dihydroxy-6 $\alpha$ -fluoro-5 $\beta$ -cholanoic acid.

A solution of 3 $\alpha$ -hydroxy-6 $\alpha$ -fluoro-7-keto-5 $\beta$ -cholanoic acid (0.42 g, 1.02 mmoles) in tetrahydrofuran (44 ml) was added with sodium borohydride (0.165 g, 4.36 mmoles) in methanol (11 ml) and the resulting mixture was left to react for one hour under magnetic stirring. Then the reaction mixture was diluted with ethyl acetate (20 ml), added with water (20 ml) and acidified with 10% hydrochloric acid (10 ml). The organic phase was separated, the aqueous phase was extracted with methylene chloride (3 x 20 ml); the combined organic phases were washed with brine (1 x 30 ml) and dried over sodium sulfate. After evaporating the solvent, the residue (0.42 g) was subjected to flash chromatography on silica, eluting with methylene chloride/methanol 98:2, to obtain 0.03 g of the starting product and 0.16 g of the title product (41%), the structure of which was confirmed by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, <sup>19</sup>F-NMR and mass spectroscopies.

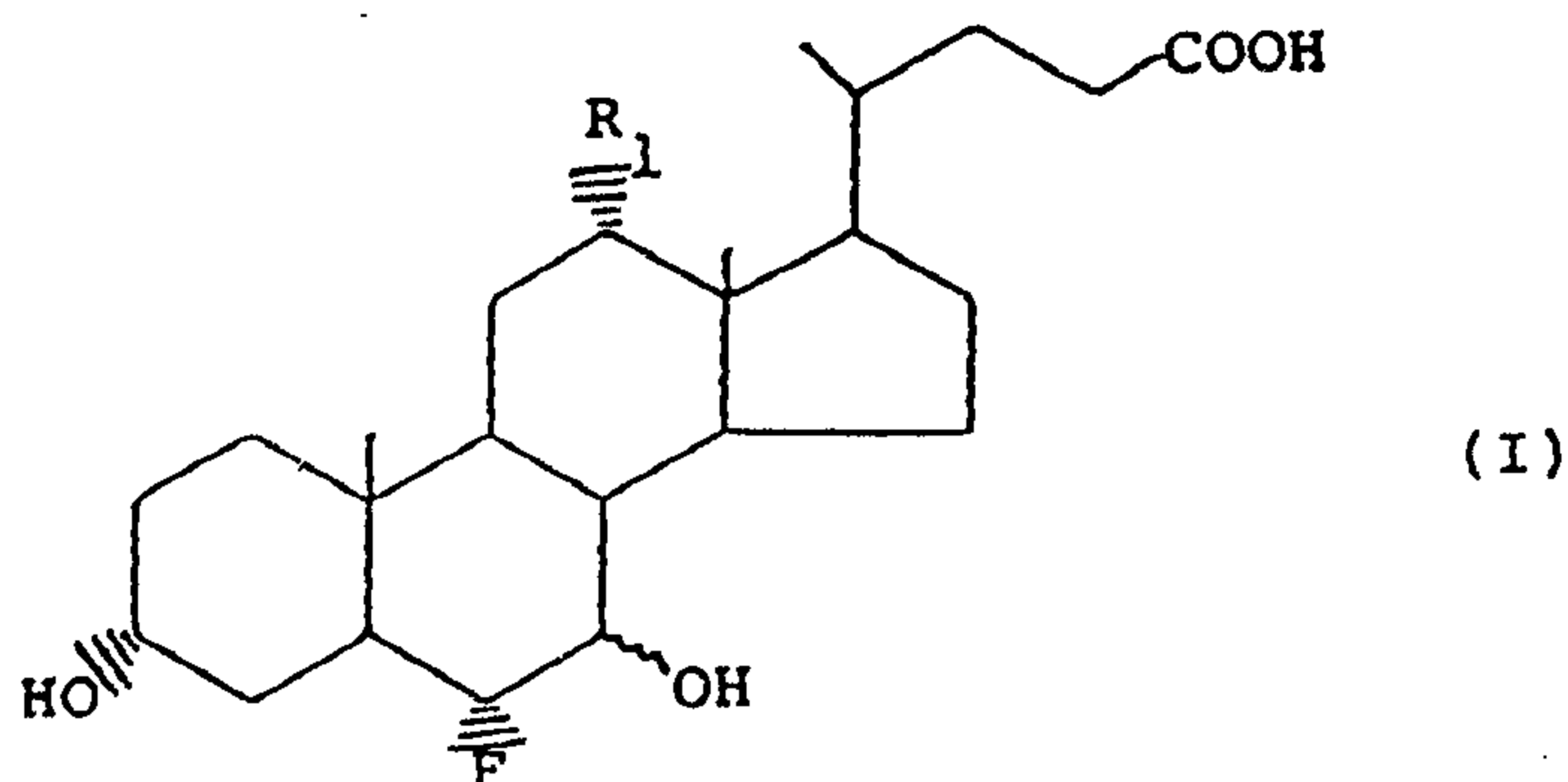
mp 198-201°C; IR (CHCl<sub>3</sub>) 1725 (C=O); <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub> + CD<sub>3</sub>OD) 0.7 (s, 3H, 18-CH<sub>3</sub>), 0.9-0.95 (m, 6H, 19-CH<sub>3</sub>, 21-CH<sub>3</sub>), 3.38 (m, 1H, 6-H), 3.7 (m, 2H, 3H, 7-H); <sup>13</sup>C-NMR (200 MHz, CDCl<sub>3</sub> + CD<sub>3</sub>OD) 75.43 C<sub>3</sub>, 79.199 C<sub>7</sub>, 95.45 C<sub>6</sub>. 176.24 (-COOH); <sup>19</sup>F-NMR(CDCl<sub>3</sub> + CD<sub>3</sub>OD) inner standard C<sub>6</sub>F<sub>6</sub>, δ -5.4; m/z 391 (9.18%), 390 (34.28%), 354.4 (2.64%). C<sub>24</sub>H<sub>39</sub>FO<sub>4</sub>.

CLAIMS

1. Compounds of general formula I

5

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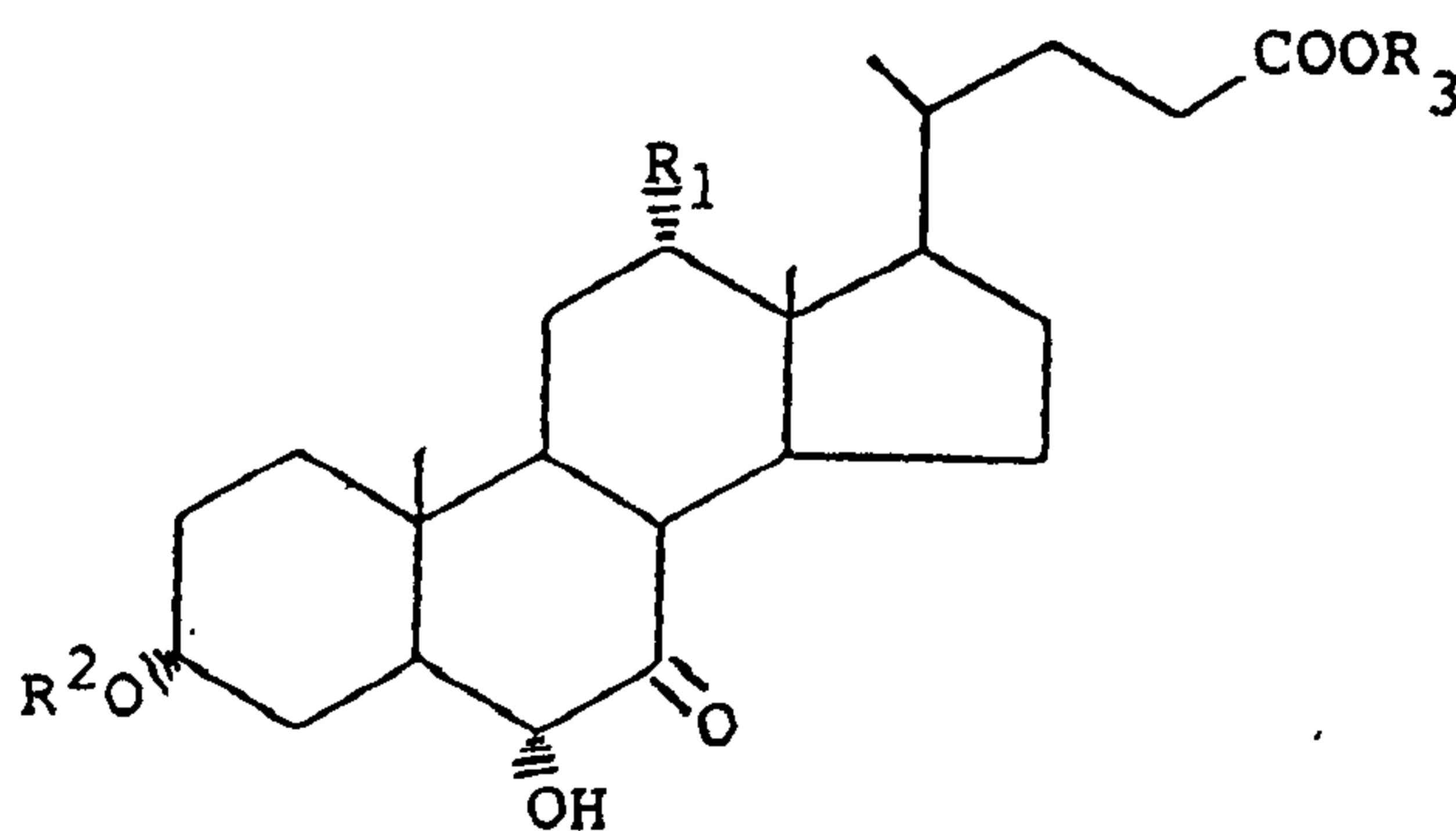
wherein  $R_1$  is hydrogen or hydroxy and the hydroxy group at the 7-position can be either in  $\alpha$  or  $\beta$  configuration, the pharmaceutically acceptable salts thereof and the glycine and taurine conjugated forms thereof.

2.  $3\alpha, 7\beta$ -dihydroxy- $6\alpha$ -fluoro- $5\beta$ -cholan-24-oic acid.

3.  $3\alpha, 7\beta, 12\alpha$ -trihydroxy- $6\alpha$ -fluoro- $5\beta$ -cholan-24-oic acid.

4. A process for the preparation of the compounds of claim 1, in which process a compound of formula

25



wherein  $R_2$  is an hydroxy protecting group,  $R_1$  is hydrogen

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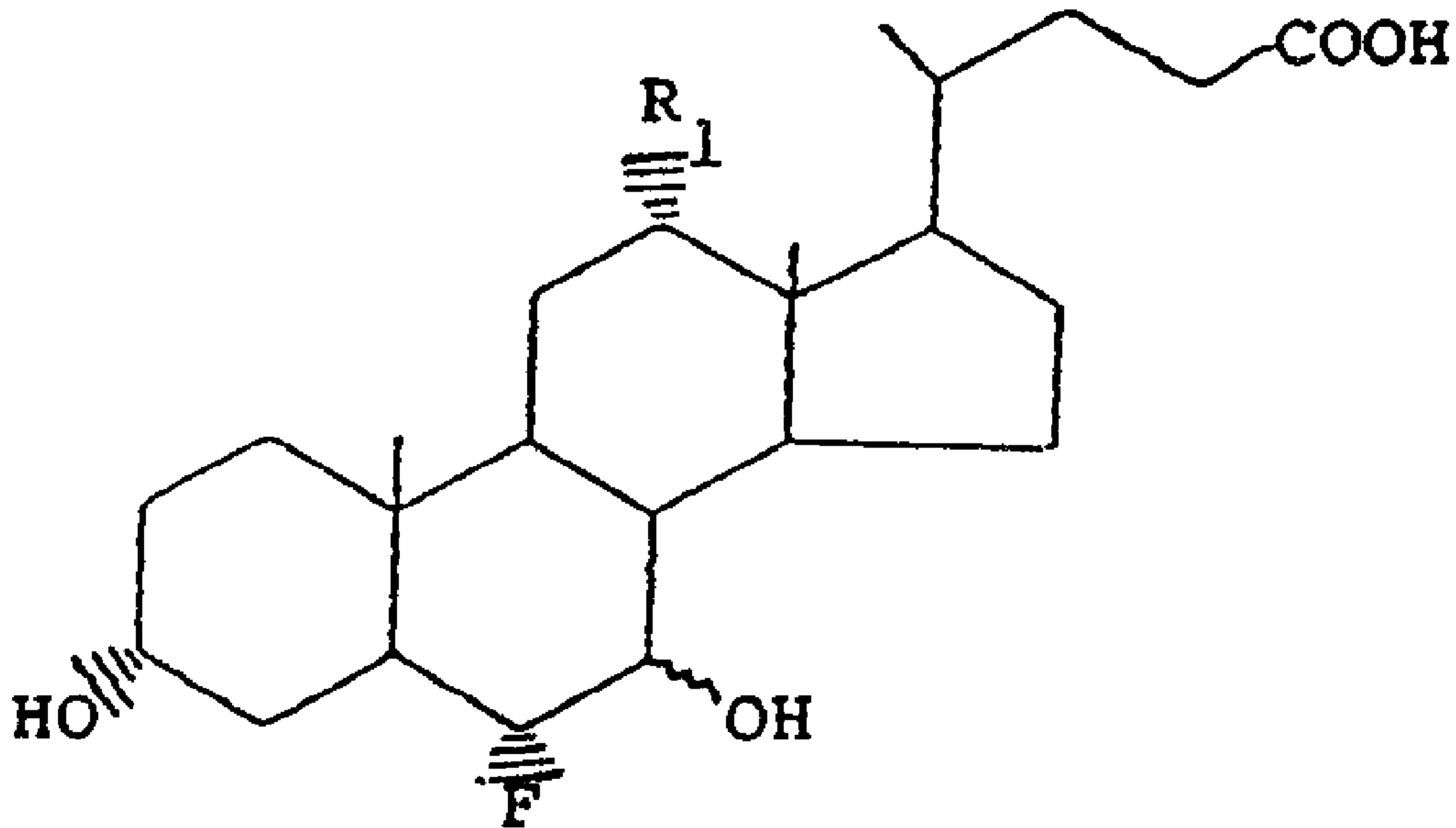
or a protected hydroxy group, and  $R_3$  is a carboxy protecting group, is subjected to a substitution reaction with the fluoride ion and the resulting compound is subsequently deprotected and reduced.

5 5. A process as claimed in claim 4, in which the substitution is carried out with diethylamino-sulfur-trifluoride.

6. The compounds as claimed in any one of claims 1, 2 or 3 as therapeutical agents.

10 7. Pharmaceutical compositions containing as the active ingredient a compound as claimed in any one of claims 1, 2 or 3, in admixture with a suited carrier or excipient.

8. The use of the compounds as claimed in any one of claims 1, 2 or 3 for the preparation of a medicament.



(I)