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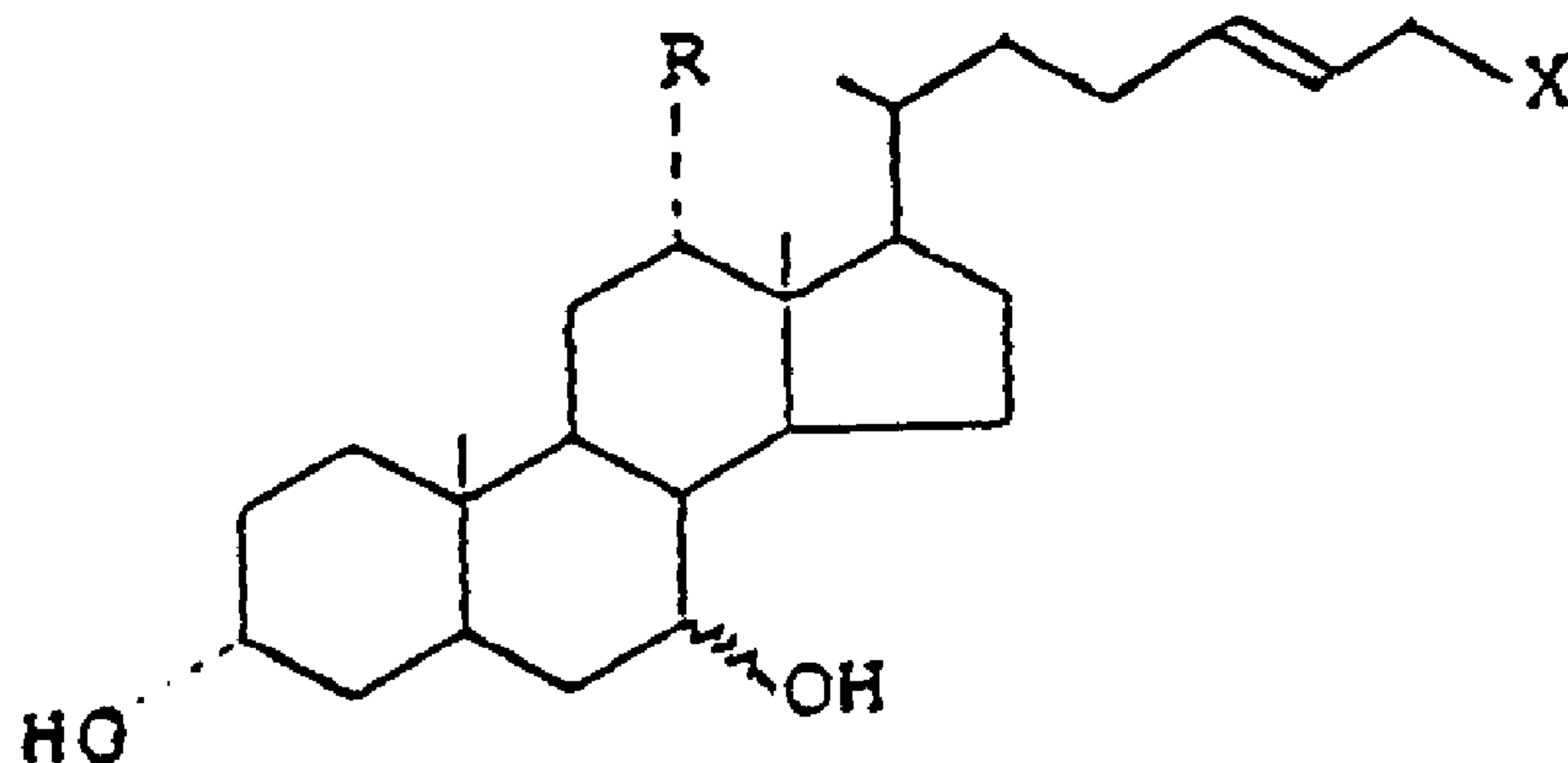
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(54) Titre : DERIVES INSATURES D'ACIDES BILIAIRES, PROCEDES DE PREPARATION ET COMPOSITIONS PHARMACEUTIQUES EN CONTENANT

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



( I )

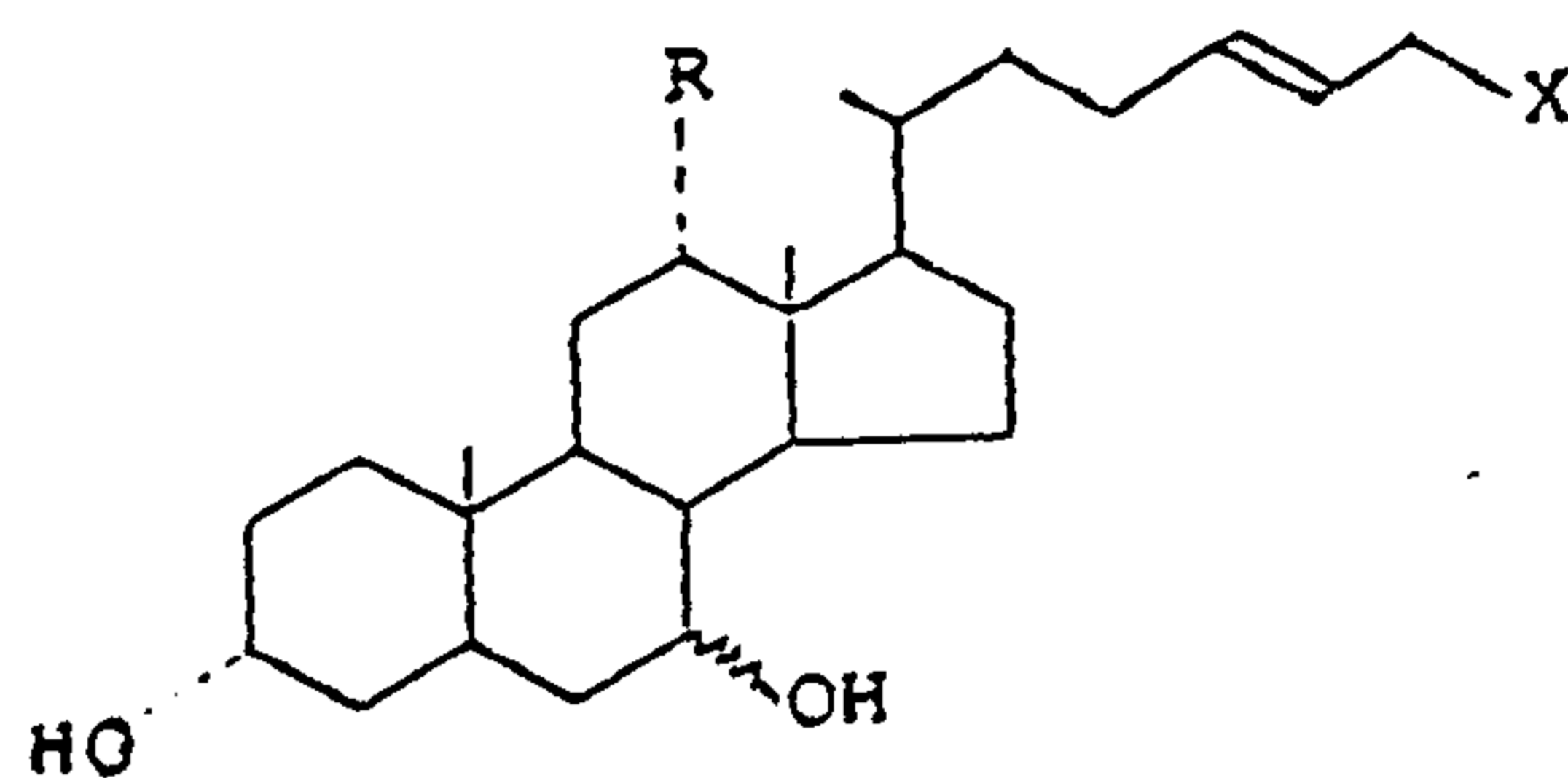
(57) Abrégé/Abstract:

Bile acid derivatives of formula I (see formula I) wherein : R is hydrogen or hydroxy; X is a carboxy or a SO<sub>3</sub>H group, have valuable pharmacologic properties.

ABSTRACTBILE ACID UNSATURATED DERIVATIVES, PROCESSES FOR THE  
PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITIONS  
CONTAINING THEM

Bile acid derivatives of formula I

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(I)

wherein :

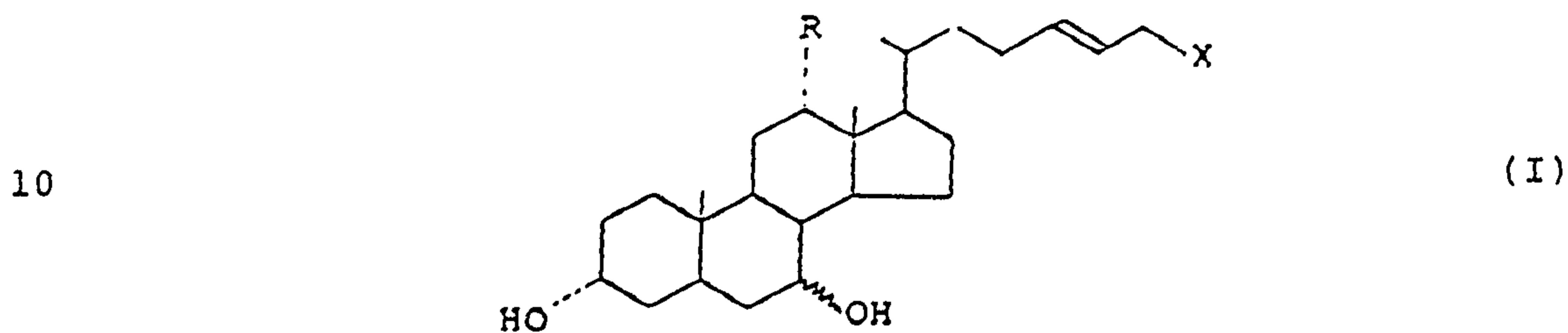
R is hydrogen or hydroxy;

15 X is a carboxy or a  $\text{SO}_3\text{H}$  group, have valuable  
pharmacologic properties.

BILE ACID UNSATURATED 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 as the active ingredients.

5 The compounds of the invention have the following general formula I



wherein :

R is hydrogen or hydroxy;

15 X is a carboxy or a  $\text{SO}_3\text{H}$  group.

The present invention also relates to the non toxic salts as well as to the glycine and taurine conjugated forms of the compounds of the invention.

In compounds I, X is preferably a carboxy group.

20 Bile acids, particularly cholic acid, ursodeoxycholic acid, chenodeoxycholic acid,ursocholic acid, have been used for a long time in human therapy as choleric, anticholestatic and antilithiasis agents.

25 Now it has been found that a change in the side chain at the 17-position of the natural molecules by introducing a trans double bond at the 24-position, involves surprising effects on the physico-chemical, biological and biopharmacological characteristics of

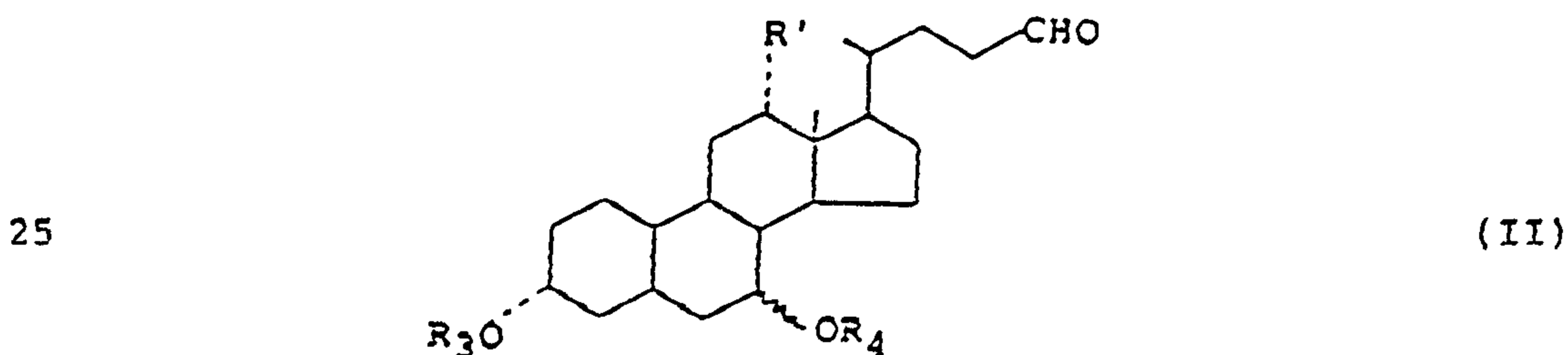
the compounds of the invention compared with the natural ones.

Particularly, a better metabolic stability, an increase in detergency and lipophilicity, a higher capability to form micells, an increased choleric effect were evidenced, the secretion of cholesterol and phospholipids being unchanged.

Therefore, compounds I can be used in human therapy for the treatment of all those pathologies on which natural bile acids are known to exert a favourable pharmacological effect. Compounds I can be administered in form of suitable pharmaceutical formulations, which can be prepared according to conventional techniques and excipients, such as those described in "Remington's Pharmaceutical Sciences Handbook", Mack Pub. Co., N.Y., USA.

The envisaged daily dosages will range from 100 mg to 2.5 g depending on the disease to be treated as well as the patient's conditions.

Compounds of formula I are prepared, according to the invention, by reacting compounds of formula II



wherein R' is hydrogen or a protected hydroxy group, and R<sub>3</sub> and R<sub>4</sub> are hydroxy-protecting groups, with a phosphorus ylide of formula III

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76.72 mmoles) and p-toluenesulphonic acid (1.55 g; 8.15 mmoles) in dioxane (160 ml), kept under magnetic stirring at room temperature. At the end of the addition the reaction mixture was concentrated under vacuum, taken up into ethyl ether (100 ml) then washed with water (3 x 50 ml) and saturated water (1 x 50 ml). After drying over sodium sulfate and evaporation of the solvents, the residue (45.00 g) was subjected to flash chromatography on SiO<sub>2</sub> (Ø = 5 cm, h = 20 cm): eluting with 95/5 chloroform/methanol, 34.4 g of compound (2) were obtained, (80%);  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 1710 cm<sup>-1</sup> (C = O); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 0,70 (s, 3H, 18-CH<sub>3</sub>); 0,90-1,00 (m, 6H, 19-CH<sub>3</sub>); 3,3-4,05 (m, 6H, 3-CH-, 7-CH-, 2x-OCH<sub>2</sub>); 4,40-4,70 (m, 2H, 2x-O-CH-O-).

15 3α,7β-Ditetrahydropyranyloxy-5β-cholan-24-ol (3).

To a solution of compound (2) (9.80 g; 17.48 mmoles) in anhydrous tetrahydrofuran (250 ml), at -70°C under magnetic stirring and nitrogen atmosphere, N-methylmorpholine (1.76 g; 17.48 mmoles) and isobutyl chloroformate (2.87 g; 21.00 mmoles) were added. The reaction mixture was left to react for 30 min., the separated solid was then removed by filtration and the filtrate was added to a sodium borohydride solution (1.65 g; 43.60 mmoles) in water (10 ml) kept at -10°C under magnetic stirring at room temperature, in about 30 min., and the resulting mixture was left to react overnight at r.t. Thereafter the reaction mixture was diluted with ethyl acetate (20 ml) and water (80 ml), acidified with 5% hydrochloric acid and extracted with ethyl acetate (3 x 50 ml). The combined organic phases were washed with saturated water (1 x 50 ml), dried

over sodium sulfate and concentrated under vacuum. A residue (9.40 g) was obtained which was subjected to flash chromatography on  $\text{SiO}_2$  ( $\phi = 5.5$  cm,  $h = 20$  cm): eluting with 99/1 chloroform/methanol, 6.80 g of compound (3) were obtained, (71%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  0,70 (s, 3H, 18- $\text{CH}_3$ ); 0,90-1,00 (m, 6H, 19- $\text{CH}_3$ , 21- $\text{CH}_3$ ); 2,15-2,30 (s, 1H, -OH); 3,30-4,10 (m, 8H, 3-CH-, 7-CH, 24- $\text{CH}_2$ -, 2x-O- $\text{CH}_2$ -); 4,40-4,75 (m, 2H, 2x-O-CH-O-).

3 $\alpha$ ,7 $\beta$ -Ditetrahydropyranyloxy-5 $\beta$ -cholan-24-al (4).

10 To a solution of oxalyl chloride (8.17 g; 64.35 mmoles) in methylene chloride (140 ml) kept at  $-70^\circ\text{C}$  under magnetic stirring and nitrogen atmosphere, dimethylsulfoxide (11.00 g; 141.15 mmoles) diluted in methylene chloride (10 ml) was added in about 10 min.

15 The reaction mixture was left to stand for 10 min., then a solution of compound (3) (29.60 g; 54.15 mmoles) in methylene chloride (20 ml) and dimethylsulfoxide (20 ml) was dropped therein in about 20 min. 15 Min. after, triethylamine (29.73 g; 294.40 mmoles) was added and

20 the resulting mixture was kept under stirring for 10 min. The reaction mixture was warmed to r.t., diluted with water (200 ml), the organic phase was separated and the aqueous one was extracted with methylene chloride (3 x 50 ml). The combined organic phases were

25 washed with saturated water (1 x 50 ml), dried over sodium sulfate and concentrated under vacuum. The residue (31.50 g) was subjected to flash chromatography on  $\text{SiO}_2$  ( $\phi = 5.5$  cm,  $h = 25$  cm): eluting with 4/6 ethyl ether/petroleum ether, 25.60 g of compound (4) were

30 obtained, (87%);  $\text{max}$  ( $\text{CHCl}_3$ ):  $1725\text{ cm}^{-1}$  (C = O);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  0,70 (s, 3H, 18- $\text{CH}_3$ ); 0,90-0,95 (m, 6H, 19- $\text{CH}_3$ , 21- $\text{CH}_3$ ); 3,10-4,10 (m,

6H, 3-CH-, 7-CH- e 2x-OCH<sub>2</sub>-); 4,35-4,70 (m, 2H, 2x-O-CH-O-); 9,60 (s, 1H, -CHO).

Trans-3 $\alpha$ ,7 $\beta$ -ditetrahydropyranyloxy-5 $\beta$ -24-cholesterol-21-oic acid.

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To a suspension of 2-carboxyethyl(triphenyl)phosphonium chloride (12.30 g; 33.13 mmoles) /previously prepared by reacting 3-chloropropionic acid (7.42 g; 68.38 mmoles) with triphenylphosphine (18.00 g; 68.38 mmoles) in xylene (100 ml) under reflux/ in anhydrous tetrahydrofuran (80 ml) and anhydrous dimethylformamide (20 ml), kept at 0°C under magnetic stirring and nitrogen atmosphere, a solution of hexamethyldisilazane (12.65 ml; 60.00 mmoles) and n-butyl lithium (31.40 ml; 56.30 mmoles) in anhydrous tetrahydrofuran (40 ml) was added in about 10 min. The reaction mixture was left to react for 15 min. at 0°C, then it was cooled to -70°C and a solution of compound (4) (9.00 g; 16.52 mmoles) in anhydrous tetrahydrofuran (80 ml) was dropped therein in 40 min. The resulting mixture was left to react at -70°C for 30 min., at r.t. for 60 min. and under reflux for 4 hrs. The reaction mixture was concentrated under vacuum, the crude product was taken up into ethyl acetate (100 ml), acidified with 2% hydrochloric acid (100 ml), the organic phase was separated and the aqueous one was extracted with ethyl acetate (3 x 100 ml). The combined organic phases were washed with saturated water (1 x 100 ml), dried over sodium sulfate and concentrated under vacuum. The residue (20.00 g) was subjected to flash chromatography on SiO<sub>2</sub> ( $\phi$  = 4 cm, h = 20 cm): eluting first with 6/4



petroleum ether/ethyl ether, then with 98/2 chloroform/methanol, 9.00 g of a solid impure product were obtained. The subsequent purification was carried out suspending the solid in ethyl ether and, after filtration and evaporation of the solvent, 3.00 g of pure compound (5) were obtained (30%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  0,70 (s, 3H, 18- $\text{CH}_3$ ); 0,90-1,00 (m, 6H, 19- $\text{CH}_3$ , 21- $\text{CH}_3$ ); 2,95-3,10 (m, 2H, 26- $\text{CH}_2$ -); 3,20-4,00 (m, 6H, 3-CH-, 6-CH-, 2x-O- $\text{CH}_2$ -); 4,30-4,75 (m, 2H, 2x-O-CH-O-); 5,35-6,55 (m, 2H, 24-CH-, 25-CH-); 5,65-6,05 (br, 1H, -COOH).

Trans-3 $\alpha$ ,7 $\beta$ -dihydroxy-5 $\beta$ -24-cholen-27-oic acid (6).

Concentrated hydrochloric acid (10 ml) was added to a solution of compound (5) (3.00 g; 5.00 mmoles) in tetrahydrofuran (50 ml), kept under magnetic stirring at r.t. The reaction mixture was left to react for 2 hrs., then it was concentrated under vacuum, the residue was taken up into ethyl acetate (100 ml), diluted with water (100 ml), the organic phase was separated and the aqueous one was extracted with ethyl acetate (3 x 30 ml). The combined organic phases were washed with saturated water (1 x 50 ml), dried over sodium sulfate and concentrated under vacuum. The residue was flash chromatographed on  $\text{SiO}_2$  ( $\phi$  = 3.5 cm, h = 15 cm): eluting with chloroform containing 2.15% methanol, 1.30 g of compound were obtained (60%); m.p.

156-161°C;  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 1710  $\text{cm}^{-1}$  (C = O);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  0,70 (s, 3H, 18- $\text{CH}_3$ ); 0,90-1,00 (m, 6H, 19- $\text{CH}_3$ , 21- $\text{CH}_3$ ); 2,96-3,10 (m, 2H, 26- $\text{CH}_2$ -); 3,50-3,70 (m, 2H, 3-CH-, 7-CH-); 5,40-5,60 (m, 2H, 24-CH-, 25-CH-); 5,80-6,10 (m, 3H, 2x-CH e -COOH);  $^{13}\text{C-NMR}$  [ $(\text{CD}_3)_2\text{SO}$ ]  $\delta$  69,38 ( $\text{C}_3$ ); 69,64

(C<sub>7</sub>); 132,36 (C<sub>24</sub>); 133,57 (C<sub>25</sub>); 172,4 (C<sub>27</sub>).

Following the same procedure, but starting from the appropriate bile acids, the following derivatives were prepared :

- 5 - trans 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5 $\beta$ -24-cholen-27-oic acid
- trans 3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -24-cholen-27-oic acid
- trans 3 $\alpha$ ,7 $\beta$ ,12 $\alpha$ -trihydroxy-5 $\beta$ -24-cholen-27-oic acid

The physico-chemical characteristics of 3,7 $\beta$ -dihydroxy-5 $\beta$ -24-cholen-27-oic acid, hereinafter also defined with the abbreviation TOL-UDCA for sake of shortness, are reported in the following Table 1, in comparison with those of ursodeoxycholic acid (UDCA) and of the glycine and taurine conjugated thereof.

Table 1 Physico-chemical characteristics

15	Critical micellar concentration (CMC)	Retention times with C <sub>18</sub> -HPLC (K')	Solubility ( $\mu$ M)	pKa	
	UDCA	19	3,66	9	5,06
20	T-UDCA	8	0,98	-	2
	G-UDCA	12	1,06	3	3,90
	TOL-UDCA	5	9,12	10	5,02

25 Trans 3 $\alpha$ ,7 $\beta$ -dihydroxy-5 $\beta$ -24-cholen-27-oic acid (TOL-UDCA) was studied compared with ursodeoxycholic acid and the glycine and taurine conjugated thereof also in the following pharmacological tests :

- hepatic and intestinal metabolism (in vivo and in vitro)
- 30 - hepatic and intestinal absorption
- bile secretion

- effects on the secretion of bile, bile acids, cholesterol and phospholipids.

TOL-UDCA was administered to rat by intravenous and intraduodenal routes at a dose of 6  $\mu$ moles/min/kg during 1 hour.

Hepatic absorption and intestinal metabolism.

When administered intravenously, TOL-UDCA is absorbed by liver and only partially secreted in bile. The compound is recovered in the bile with a slow kinetic and it is poorly conjugated with glycine and taurine. On the contrary, UDCA is completely transformed into the conjugated forms thereof, i.e. tauro-UDCA and, to a lesser extent, glyco-UDCA.

At the end of the infusion, TOL-UDCA disappears from the bile with a slow kinetic and a noticeable amount of this bile acid is still present 2 hours after.

When administered intraduodenally, TOL-UDCA is efficiently absorbed by intestine with a higher recovery than that of glyco-UDCA and tauro-UDCA.

When incubated with human faeces under aerobic and anaerobic conditions, the compound is poorly metabolized (7-dehydroxylate) with a significantly lower kinetic than that of UDCA and of the conjugated forms thereof.

Effect on bile flow and bile lipid secretion

Intravenous administration of TOL-UDCA increases bile flow and this effect is stronger than the one of UDCA natural analogs and of the conjugated forms thereof (see Table 2).

Table 2 Effect of TOL-UDCA on bile flow and secretion

	$SM_{Vo}$	$SM_{AB}$	$SM_{XOL}$	$SM_{PL}$
5	$\mu\text{l}/\text{min}/\text{kg}$		$\mu\text{mol}/\text{min}/\text{kg}$	
UDCA	60	3,02	0,022	0,22
T-UDCA	35	2,40	0,014	0,18
G-UDCA	40	2,10	0,023	0,39
10 TOL-UDCA	90	1,02	0,020	0,12

$SM_{Vo}$  = maximum bile flow

$SM$  = maximum bile lipid secretion

15 No significant differences are evidenced on cholesterol and phospholipid transport and secretion compared with UDCA and its conjugated forms.

The pharmacokinetic characteristics of compound TOL-UDCA can be summarized as follows :

20 a) efficient absorption by liver and poor secretion in bile, as a consequence of a poor conjugation;

b) very good absorption by intestine after intraduodenal administration, to a higher degree than natural analogs such as glyco-UDCA and tauro-UDCA, as a  
25 consequence of both the active and passive transports;

c) very slow intestinal metabolization, particularly 7-dehydroxylation, compared with UDCA, likely as a consequence of a good resistance to intestinal bacterial flora;

30 d) strong choleric effect after intravenous or intraduodenal administrations, which effect is higher

than that of UDCA.

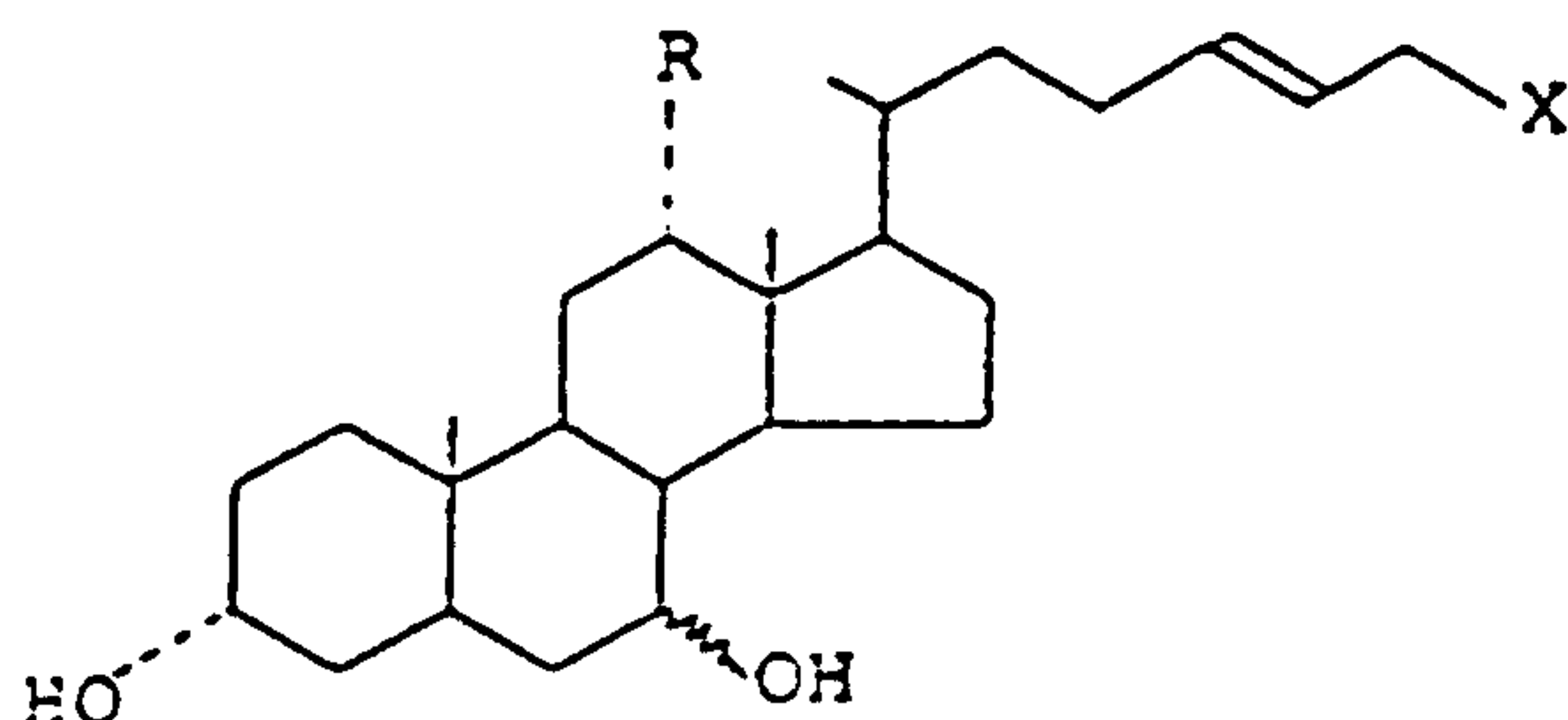
In conclusion, the compounds of the invention provide an improvement in the structure/activity studies, since the presence of a double bond in the side chain give the compounds an optimum conformation for intestinal absorption. Hepatic absorption is also efficient.

Therefore a compound such as TOL-UDCA, which accumulates in the hepatic cells, can advantageously be used in the treatment of cholestatic syndromes.

CLAIMS

1. Compounds of formula I

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(I)

10 wherein :

R is hydrogen or hydroxy;

X is a carboxy or a SO<sub>3</sub>H group.

2. Compounds of formula I wherein X is a carboxy group.

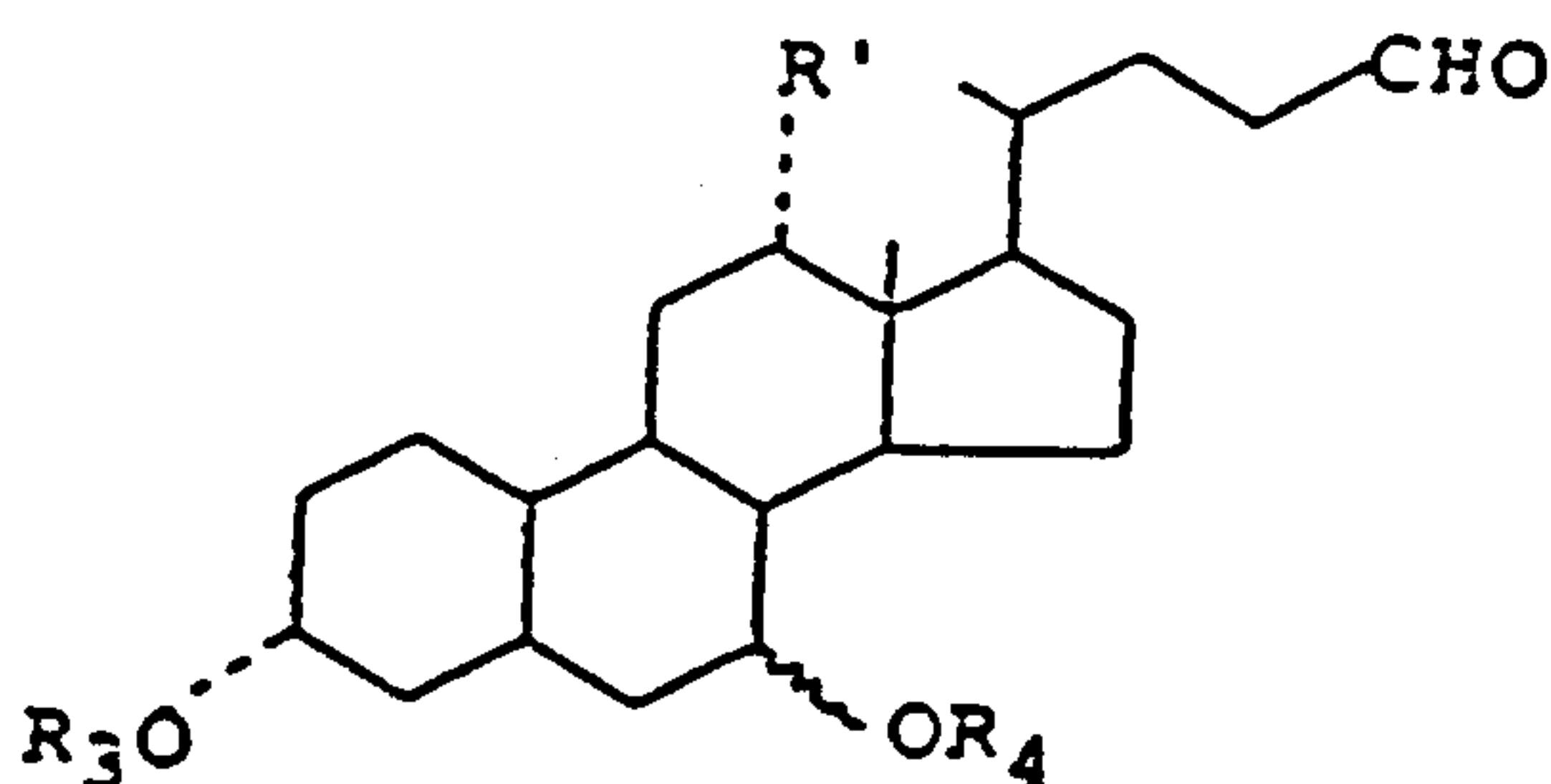
15 3. Compounds of claim 1 or 2, wherein R is hydroxy and the hydroxy group in 7 is in  $\alpha$  configuration.

4. Compounds of claim 1 or 2, wherein R is hydrogen and the hydroxy group in 7 is in  $\alpha$  configuration.

20 5. Compounds of claim 1 or 2, wherein R is hydrogen and the hydroxy group in 7 is in  $\beta$  configuration.

6. Compounds of claim 1 or 2, wherein R is hydroxy and the hydroxy group in 7 is in  $\beta$  configuration.

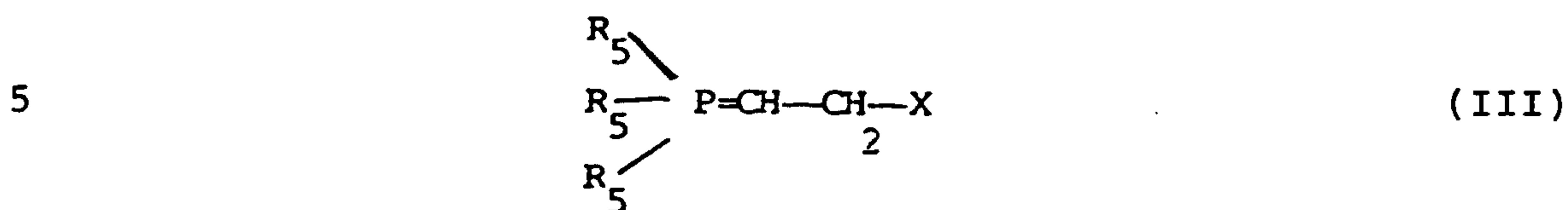
25 7. A process for the preparation of a compound according to any one of claims 1 to 6, which comprises reacting the compounds of formula II



(II)

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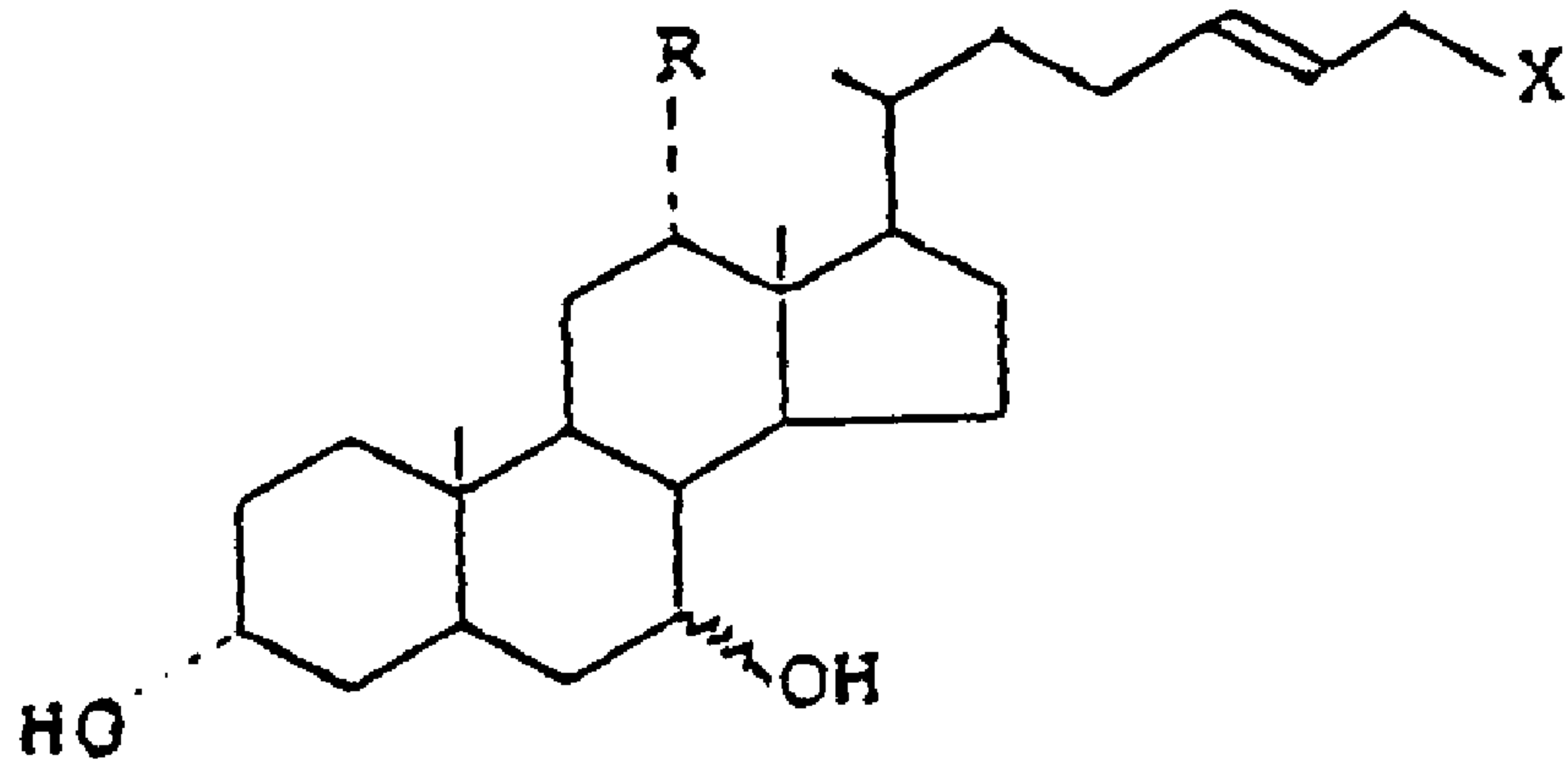
wherein R' is hydrogen or a hydroxy protected group and R<sub>3</sub> and R<sub>4</sub> are hydroxy-protecting groups, with a phosphorus ylide of formula III



wherein X is as defined in claim 1 and R<sub>5</sub> is an alkyl or phenyl group.

8. 10 Pharmaceutical compositions containing a compound according to any one of claims 1 to 6 as the active ingredient, in admixture with a pharmaceutically acceptable excipient.

9. The use of a compound according to any one of claims 1 to 6 for the preparation of a medicament having choleric, anticholestatic and antilithiasis actions.



(I)