NOVEL DERIVATIVES OF 3α,7α-DIHYDROXY-5β-CHOLAN-24-OIC ACID (CHENODEOXYCHOLIC ACID) AND 3α,7β-DIHYDROXY-5β-CHOLAN-24-OIC ACID (URSODEOXYCHOLIC ACID)

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ABSTRACT

Several 7-acyl cheno- and ursodeoxycholic acids were obtained in good yields starting from the corresponding cheno- and ursodeoxycholic acids, by a diacylation-selective hydrolysis procedure. A superior method for the synthesis of the 7-oleyl derivatives, by a selective acylation procedure, is also presented.

INTRODUCTION

Therapy with cheno- and ursodeoxycholic acids in the field of biliary gallstone disease is well established [1, 2]. However, the activity of these acids against the formation of biliary stones is limited, owing to metabolic inactivation and side effects [3-6]. It has been found that the 7-acyl derivatives [3] may resist inactivation, thus permitting a significant reduction in the daily doses and/or an increase in the intervals between each administration. It is therefore very important to obtain derivatives, which permit a continuous recycling of 3,7-dihydroxy-5β-cholan-24-oic acids.

EXPERIMENTAL

^{1}H NMR spectra were recorded with a Varian EM-360 or a Bruker WP-80 using tetramethylsilane as internal standard. IR spectra were recorded with a Perkin Elmer 457 spectrophotometer. Elemental analyses were performed with a Perkin Elmer 240 instrument. Silica gel 60 F_{254} plates

(Merck) were used for analytical TLC and 270-400 mesh silica gel (Merck) was used for chromatography. Organic extracts were dried over Na_2SO_4 and filtered before removal of the solvent under reduced pressure. Pyridine was distilled from BaO just before use. Freshly distilled acyl chlorides or anhydrides were always used.

Diacylation of methyl 3α,7α-dihydroxy-5β-cholan-24-oate (I) and of methyl 3α,7β-dihydroxy-5β-cholan-24-oate (II)

Freshly distilled acyl chloride or anhydride (0.27 mol) was added to a solution of (I) or (II) (0.108 mol) in dry pyridine (220 mL). The mixture was refluxed for 2 h. After cooling, the mixture was poured over ice and acidified with 4 M HCl to pH 2. The suspension was extracted 3 times with ethyl ether, the organic extracts were collected and worked up as described by Hauser et al [7] (85-95% yield). The diacylated products were used as such for the subsequent hydrolysis. Except for acetyl and butyroyl derivatives, the more reactive acyl chlorides were always used.

Selective hydrolysis of methyl 3α,7α-diacyloxy-5β-cholan-24-oate (III) and of methyl 3α,7β-diacyloxy-5β-cholan-24-oate (IV) to give (V) and (VI) respectively

A solution of the diacyl derivative (1 mmol) in methanol (4.4 mL) was added to water (2.2 mL) and 50% aqueous KOH (0.23 mL). The mixture was heated to reflux in an inert atmosphere up to the point when TLC (benzene/ethyl acetate 8/2-6/4) indicated that hydrolysis had occurred (1-3 h). Analytical data are summarized in Tables 1 and 2.

In the case of (IVa) and (IVb) the reaction was carried out at 60°C. In the case of (IVg) a catalytic amount of 2,3-di-tert-butyl-4-methylphenol was added. A further addition of 50% aqueous KOH (0.23 mL was sometimes necessary to speed the reaction. The methanol was evaporated under vacuum, the residue was acidified to pH 2 with 4 M HCl and extracted with CH_2Cl_2. The extracts were worked up as before [7].

Methyl 3α-methoxycarbonyloxy-7α-hydroxy-5β-cholan-24-oate (VII)

Methyl chloroformate (0.895 mL, 11.6 mmol) was slowly added to a stirred suspension of (I) (1g, 2.46 mmol) in dry pyridine (25 mL) at 0°C. After 3 h at 0°C the mixture was poured onto ice and acidified with 4 M HCl to pH 2.

The white suspension was filtered by suction, washed three times with water and dried. The crude product was recrystallized from isopropyl ether (93% yield); mp 113°C, TLC R_f 0.6 (benzene/ethyl acetate 7/3); IR ($CHCl_3$) 2920, 2840, 1740 cm^{-1}; ^1H nmr ($CDCl_3$) 0.6-2.6(m,steroid nucleus), 3.66(3H,s,MeOCOOC-3), 3.74(3H,s,MeOCOC-24), 3.82 (1H,m,HC-7), 4.4(1H,m,HC-3). Anal. Calcd. for $C_{27}H_{44}O_6$: C 69.79, H 9.54. Found: C 69.85, H 9.50%.

Methyl 3α-methoxycarbonyloxy-7α-[(Z) octadec-9-enoyloxy]-5β-cholan-24-oate (IX)

The same general procedure described for the diacylation of (I) and (II) was followed but 1.5 mol equiv of oleyl chloride was used.

After 1 h refluxing the mixture was treated with methanol (20 mol

TABLE 1. Analytical Data (V)

(V)	MP °C	Recryst. Solv.	Formula	Analysis Calcd. %	Found %
a	192-4	Methanol	$C_{28}H_{46}O_5$	C 72.73 H 9.96	73.01 10.22
b	135-5	Methanol-water	$C_{31}H_{44}O_5$	C 74.13 H 10.42	73.81 10.61
c	173-5	Methanol-water	$C_{28}H_{54}O_5$	C 73.04 H 9.57	72.87 9.48
d	97-9	Et_2O-hexane	$C_{36}H_{62}O_5$	C 75.26 H 10.80	75.09 10.96
e	oil		$C_{40}H_{70}O_5$	C 76.19 H 11.11	76.37 10.97
f	oil		$C_{42}H_{72}O_5$	C 76.83 H 10.98	77.00 11.05
g	oil		$C_{42}H_{70}O_5$	C 77.06 H 10.70	77.02 10.90

^{1}H nmr ($CDCl_3$) (selected values) 3.15/3.27-3.80/4.00 (1H,m,CHOH), 4.70/4.85-5.00/5.90 (1H,m,CHOCO), 5.90/6.65-6.30/7.10 (2H,m,CO_2H, OH). In addition (Vf) and (Vg) gave signals at 5.27-5.60 (2H,m,CH=CH), and at 5.05-5.75 (4H,m,2 CH=CH) respectively. IR ($CHCl_3$) 3540, 3420, 1720 cm^{-1}.

equiv), cooled to room temperature and quenched as described earlier. Methyl oleate was removed by decantation at 0°C from methanol. Crude (IX), obtained as an oil, was used as such for the subsequent hydrolysis. A pure sample of (IX) was obtained by chromatography (hexane/ethyl acetate). Oil, TLC, R_f=0.6 (benzene/ethyl acetate 95/5); IR ($CHCl_3$), 2910, 2830, 1740, 1435, 1270 cm^{-1}; ^{1}H nmr ($CDCl_3$) 0.5-2.45(m,steroid nucleus + aliphatic chain), 3.66(3H,s,MeOC-24), 3.75(3H,s,MeOCOOC-3), 4.46(1H,m,HC-3), 4.82(1H,m,HC-7), 5.33(2H,bt,CH-CH). Anal. Calcd. for $C_{45}H_{76}O_7$ C 74.29, H 10.44. Found C 74.15, H 10.51%.

3α-Hydroxy-7α-[(Z) octadec-9-enoyloxy]-5β-cholan-24-oic acid (Vf) from IX

A 25% aqueous solution of KOH (4.2 mL) was added to a methanolic solution (17 mL) of (IX) (1.2 g, 1.73 mmol) and the mixture was refluxed for 2 h. The work-up procedure is identical to the previously reported selective hydrolysis; 85% yield starting from (VII).

TABLE 2. Analytical Data (VI)

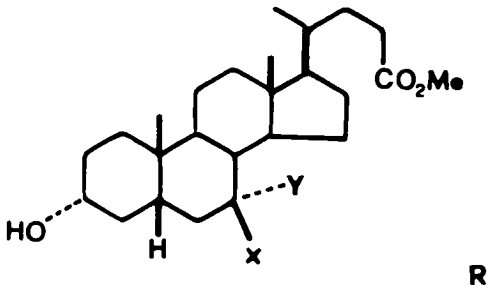
(VI)	MP °C	Recryst. Solv.	Formula	Analysis Calcd. %	Found %
a	184-5	Ethyl acetate	$C_{28}H_{46}O_5$	C 72.73 H 9.96	72.52 10.19
b	200-2	Ethyl acetate	$C_{32}H_{54}O_5$	C 74.13 H 10.42	74.00 10.79
c	214-6	Methanol	$C_{28}H_{44}O_5$	C 73.04 H 9.57	72.74 9.87
d	oil		$C_{36}H_{62}O_5$	C 75.26 H 10.80	74.70 11.12
f	oil		$C_{42}H_{72}O_5$	C 76.83 H 10.98	77.08 11.01
g	oil		$C_{42}H_{70}O_5$	C 77.06 H 10.70	77.12 10.83

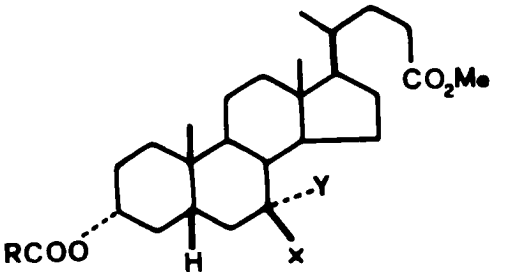
^{1}H nmr ($CDCl_3$) (selected values) 3.55/3.60-4.05-4.15 (1H,m,CHOH), 4.75/4.80-5.25/5.30 (1H,m,CHOCO). IR ($CHCl_3$) 3540, 3420, 1720 cm^{-1}. In addition (VIf) and (VIg) gave signals at 5.27-5.60 (2H,m,CH=CH), and at 5.05-5.75 (4H,m,2 CH=CH) respectively.

Methyl 3α-t-butylcarbonyloxy-7β-hydroxy-5β-cholan-24-oate (VIII) and methyl 3α-t-butylcarbonyloxy-7β-[(Z) octadec-9-en-oyloxy]-5β-cholan-24-oate (X) from (II)

Pivaloyl chloride (1 mL, 8.12 mmol) was slowly added to a solution of (II) (3 g, 7.39 mmol) in dry pyridine (10 mL) at 0°C. After 3 h stirring, oleyl chloride was added and the mixture was refluxed for 1 h. Methanol (2 mL) was then added, and after cooling, the solution was poured into ice and acidified with 4 M HCl to pH 3. Usual work-up afforded crude (X). Methyl oleate was removed by the same procedure described in the preparation of (IX).

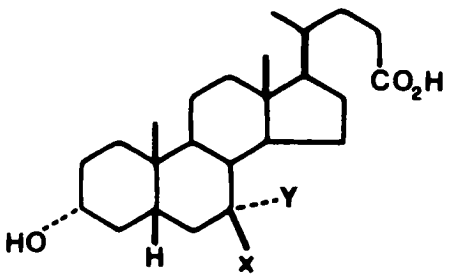
A pure sample of (VIII) was obtained by quenching a small amount of the reaction mixture prior to the addition of the chloride. mp 106°C, TLC R_f 0.35 (hexane/ethyl acetate 75/25), IR ($CHCl_3$) 2910, 2860, 1610, 1160 cm^{-1}; ^{1}H nmr ($CDCl_3$) 0.6-2.4(m,steroid nucleus), 1.18(9H,s,Me_3C), 3.6(1H,m,HC-7), 3.66(3H,s,MeO), 4.65 (1H,m,HC-3). Anal. Calcd. for $C_{30}H_{50}O_5$: C 73.43, H 10.27. Found: C 73.75, H 10.10%.





(I) X = H, Y = OH
(II) X = OS, Y = H

(III) X = H, Y = OCOR
(IV) X = OCOR, Y = H
(VII) X = H, Y = OH, R = OMe
(VIII) X = OH, Y = H, R = Me_3C
(IX) X = H, Y = $OCO(CH_2)_7CH = CH(CH_2)_7Me$, R = OMe
(X) $OCO(CH_2)_7CH = CH(CH_2)_7Me$, Y = H, R = Me_3C



(V) X = H, Y = OCOR
(VI) X = OCOR, Y = H

a R = Me, b R = $Me(CH_2)_3$

c R = ◁ , d R = $Me(CH_2)_{10}$

e R = $Me(CH_2)_{14}$

f R = $Me(CH_2)_7CH = CH(CH_2)_7$

g R = $Me(CH_2)_3(CH_2CH=CH)_2(CH_2)_7$

A pure sample of (X) was obtained by column chromatography purification (hexane/ethyl acetate); oil, TLC R_f 0.6 (hexane/ethyl acetate 95/5); IR ($CHCl_3$) 2910, 2860, 1610, 1160 cm^{-1}; ^1H nmr ($CDCL_3$) 0.6-2.5 (m,steroid nucleus + aliphatic chain), 1.16 (9H,s,Me_3C), 3.66 (3H,s,MeO), 4.6 (2H,m,HC-3 and HC-7), 5.3 (2H,bt, CH=CH). Anal. Calcd. for $C_{48}H_{82}O_6$: C 76.34, H 10.94. Found: C 75.98, H 10.35%.

3α-hydroxy-7β-[(Z)-octadec-9-en-oyloxy]-5β-cholan-24-oic acid (VIf) from (X)

The same procedure described for the synthesis of (Vf) starting from (IX) was followed; 1 h refluxing was necessary. Usual work-up afforded crude (X) that was purified from pivalic acid by steam-distillation. Further purification by chromatography afforded pure (VIf) (75% yield from VIII).

RESULTS AND DISCUSSION

The synthesis of the 7-acyl derivatives of cheno- and ursodeoxycholic acids involves a differentiation in the reactivity of the two hydroxyl groups of the starting acids. In such steroids the hydroxyl group at C-3 is, in general, more reactive towards acylation or hydrolysis than the more shielded hydroxyl group at C-7. This behavior is even more pronounced in the case of the axial hydroxyl of the cheno series.

We therefore speculated that acylation at the desired position could be conveniently achieved by a diacylation-selective hydrolysis two-step sequence on the corresponding methyl esters [3]. Methyl cheno- and ursodeoxycholates (I) and (II) were acylated in pyridine under standard conditions to give the corresponding 3,7 diacetate, dibutyrate, dicaprylate, dicyclopropanecarboxylate, dilaurate, dipalmitate, dioleate and dilinoleate in excellent yields. Selective hydrolysis of the crude 3,7 diacylated products was obtained by treatment with aqueous methanolic KOH at different temperatures (see Experimental) depending on the substrate. Chromatographic purification afforded in each case the pure 7-acyl derivative free of the corresponding acid.

Although all the derivatives described here could be obtained by this method, a regioselective acylation procedure was still desirable, avoiding chromatographic purification of the final products from the acid produced during the hydrolysis.

Accordingly, a selective protection of the 3-hydroxy position was studied. It is well known that methyl cholate affords the 3-ethoxycarbonyl (cathyl) derivative in nearly quantative yield even in the presence of a great excess of ethoxycarbonylchloride [8]. This selective protection also worked very well with methyl chenodeoxycholate (I). Methyl 3-methoxycarbonyl-cheno deoxycholate (VII) could be oleylated exclusively at the 7-position under standard conditions affording methyl 3-methoxycarbonyl-7-oleyl-chenodeoxycholate (IX). Subsequent mild basic treatment gave, after acidification, the desired 7-oleyl derivative (Vg) in nearly quantitative yield. The more difficult hydroxyl differentiation in the urso series could be obtained efficiently only by the use of bulky acyl protecting groups. Indeed, methyl ursodeoxycholate (II) was found to react exclusively at the C-3 position with pivaloyl chloride affording methyl 3-pivaloylursodeoxycholate (VIII) in good yield. Subsequent in situ reaction with oleyl chloride gave methyl 3-pivaloyl-7-oleylursodeoxycholate (XI). Unreacted oleyl chloride still present could be transformed into the corresponding methyl ester by direct treatment of the reaction mixture with methanol prior to the work-up. The subsequent selective hydrolysis at the C-3 position by means of aqueous methanolic KOH afforded the desired 7-oleyl-ursodeoxycholate (VIf).

Thus, a suitable diacylation-selective hydrolysis two-step procedure is here presented for the general synthesis of 7-acyl cheno- and urso-

deoxycholic acids. Furthermore, in the case of long chain acyl groups, protection of the C-3 hydroxyl group greatly simplifies the procedure and avoids the waste of valuable fatty acids and time-consuming chromatographic purification.

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