Preparation and Physicochemical Properties of Natural (23*R*)-3α,7α,23- and (23*R*)-3α,12α,23-Trihydroxylated Bile Acids and Their (23*S*)-Epimers

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By submitting the ketene silyl acetal (**5a**) generated from 3α,7α-dihydroxy-5β-cholan-24-oic acid [chenodeoxycholic acid (**1a**)], to a variety of oxidants, a new efficient route to (23*R*)-3α,7α,23-trihydroxy-5β-cholan-24-oic acid [phocaecholic acid, (**3a**)] and its (23*S*)-epimer (**4a**) has been developed. By this route, the first synthesis of 3α,12α,23-trihydroxy-5β-cholan-24-oic acid (bitocholic acid) has been achieved and the stereochemistry at C-23 conclusively assigned as *R*. Aqueous physical chemical properties of (**3a**) have been studied and compared with those of (**1a**) and 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid [cholic acid, (**1b**)], in order to gain insight into the influence of the hydroxy group at C-23 on the physical properties of bile acids.

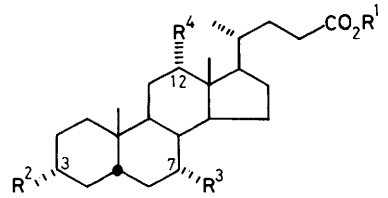
23-Hydroxylated bile acids,[1] interesting from the standpoint of both comparative biochemistry and structure/activity studies, have been isolated from the bile of the snakes of the family *Colubridae* and sub-family *Viperinae*, from that of seals and other marine mammals of the order *Pinnipedia* and, recently, reported to be also present in the bile of ducks.[2]

Three 23-hydroxy bile acids have so far been isolated. Two of them, (23*R*)-3α,7α,12α,23-tetrahydroxy-5β-cholan-24-oic acid (**3b**) and (23*R*)-3α,7α,23-trihydroxy-5β-cholan-24-oic acid [phocaecholic acid, (**3a**)], occurring both in marine mammals and in snakes, were isolated for the first time in 1909 by Hammarsten[3] from seal bile and their structures assigned by Bergstrom in 1959.[4] The third one, bitocholic acid, reported to be present only in the bile of snakes, was isolated for the first time in 1961 by Haslewood[5] from puff-adder (*Bitis arietans*) bile and identified as 3α,12α,23ξ-trihydroxy-5β-cholan-24-oic acid (**2**) by its sequential lead tetra-acetate/chromic acid oxidative conversion into nordeoxycholic acid.

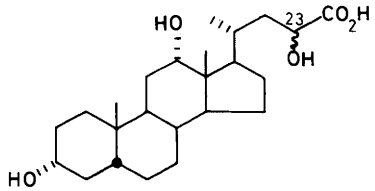
Subsequently, Ikawa and Tammar[6] reported that the bile acid fraction of the gall-bladder bile of snakes of the sub-family *Viperinae* consists mainly (41—57%) of bitocholic acid (**2**) and hypothesized as its main biosynthetic pathway the C-23 hydroxylation of cholic acid (**1b**), mainly by the microsomal fraction of snake liver, followed by 7α-dehydroxylation of the resulting 3α,7α,12α,23-tetrahydroxy-5β-cholan-24-oic acid by intestinal bacterial enzymes.

In 1982 Kutner and Jaworska[7] established as (*R*) the absolute configuration at C-23 of natural 23-hydroxy acids (**3b**) and (**3a**), by comparative molecular rotation and c.d. measurements of the (23*R*)-hydroxy methyl esters (**3f**) and (**3m**) and their (23*S*)-epimers, isolated from the C-23 epimeric mixture obtained by oxidizing the lithium enolates generated from cholic acid and chenodeoxycholic acid derivatives (**3d**) and (**3c**), respectively, with MoO_5–py·HMPA. In this paper, the preparation of the natural (23*R*)-hydroxy acids (**3a**) and (**3b**) as well as that of their (23*S*)-epimers from the corresponding esters was not reported.

Neither bitocholic acid nor corresponding derivatives have so far been synthesized and, therefore, the configuration at C-23 of this natural bile acid is still obscure.



(1)



(2)

(1)

	R^1	R^2	R^3	R^4
a	H	OH	OH	H
b	H	OH	OH	OH
c	Me	OCO_2Et	OCO_2Et	H
d	H	OH	H	OH
e	Me	OCO_2Et	H	OCO_2Et
f	Me	OH	OH	H
e	Me	OH	H	OH

As a part of a broad program devoted to the study of physicochemical and biological properties of bile acids,[8] we became interested in the study of 23-hydroxylated bile acids. In this connection, and in order to assign conclusively the configuration at C-23 of bitocholic acid (**2**), large quantities of (23*R*)-3α,7α,23- and (23*R*)-3α,12α,23-trihydroxy bile acids as well as their corresponding (23*S*)-epimers, were needed. With this aim, a new efficient route to 23-hydroxy bile acids involving the α-oxygenation of silylalkenes[9] was developed and applied to the preparation of the above compounds.

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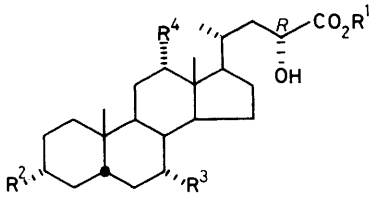
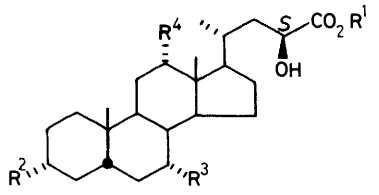


(3)

	R^1	R^2	R^3	R^4
a	H	OH	OH	H
b	H	OH	OH	OH
c	Et	OTHP	OTHP	H
d	Et	OTHP	OTHP	OTHP
e	Me	OH	OCO_2Et	H
f	Me	OH	OH	H
g	Me	OH	H	OCO_2Et
h	H	OH	H	OH
i	Me	OH	H	OH
m	Me	OH	OH	OH



(4)

	R^1	R^2	R^3	R^4
a	H	OH	OH	H
b	Me	OH	OCO_2Et	H
c	Me	OH	OH	H
d	Me	OH	H	OCO_2Et
e	H	OH	H	OH
f	Me	OH	H	OH

Results and Discussion

Initially, the behaviour of the ketene silyl acetal (**5a**) derived from chenodeoxycholic acid (**1a**) towards a variety of oxidizing agents was explored. Methyl 3α,7α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**1c**)[10] was subjected to kinetically controlled *O*-silylation[11] to give quantitatively the corresponding ketene silyl acetal (**5a**).

Ozonolysis[12] of (**5a**) at −78 °C for 1 h afforded an equimolecular mixture of the methyl α-trimethylsilyloxy ester (**7a**) and the aldehyde (**8**). A likely mechanism accounting for the above results is shown in Scheme 1. According to this mechanism, loss of oxygen from the zwitterion (**9**) followed by silatropic rearrangement *via* (**10**), would lead to (**7a**) (route **a**), while cleavage of molozonide (**11**) would account for the formation of the aldehyde (**8**) (route **b**). While attempts to favour route **b** by changing a variety of reaction parameters failed, we were able to direct the ozonolysis process towards the selective formation of the 23-hydroxy derivative (**7b**) (49% yield) by adding a catalytic amount of BF_3-Et_2O to the reaction mixture before introducing ozone.

Further attempts to optimize route **a** as well as to exploit route **b** (representing a one carbon degradation of an ester) synthetically are currently under investigation. As an alternative oxidative route, the peracid oxidation, a procedure first applied to ketene silyl acetals by Rubottom,[13] was then investigated. When (**5a**) was exposed to 3-chloroperoxybenzoic acid (MCPBA) in hexane and the crude reaction mixture treated with triethylammonium fluoride, the hydroxy ester (**7b**) was obtained in 55% yield with 45% of the ester (**1c**) recovered.

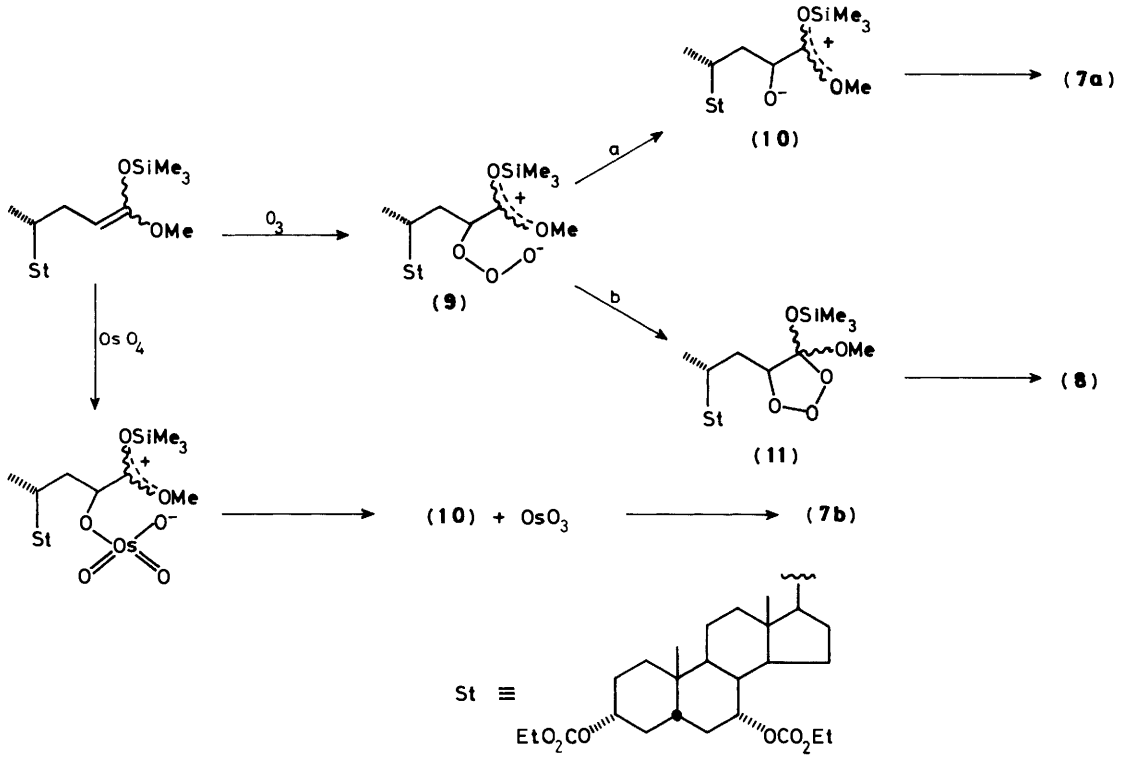
The behaviour of ketene silyl acetals toward osmium tetraoxide has not been reported yet. Stirring of (**5a**) with this oxidant in dioxane at room temperature for 16 h, followed by treatment with hydrogen sulphide led to the formation of α-hydroxy ester (**7b**) (59% yield) besides the ester (**1c**). A plausible mechanism for the formation of (**7b**) by this route, shown in Scheme 1, involves the initial formation of the zwitterion favoured, also in this case, by the strong stabilization of the positive charge by two oxygens, followed sequentially by loss of osmium(VI) oxide, silatropic rearrangement, and deprotection of the 23-silyloxy group in the acidic reaction medium.

Finally, the lead(IV) acetate (LTA) oxidation of ketene silyl acetal (**5a**), a reaction first reported by Rubottom[14] in 1982, was investigated and shown to be, in terms of yield, the method of choice for the preparation of the title compounds. Thus, sequential treatment of (**5a**) with LTA in dichloromethane at −15 °C for 15 min and triethylammonium fluoride, afforded α-acetoxy ester (**6a**) in 91% yield as a nearly equal mixture of C-23 epimers.

Having devised an efficient route to the C-23 hydroxylation of (**1a**), our attention was turned towards the separation of the C-23 epimers. Ultrasonic irradiation of a solution of (**6a**) in methanol in the presence of potassium carbonate afforded a corresponding mixture of (23*R*)- and (23*S*)-hydroxy compounds (**3e**) and (**4b**) which could be separated by medium pressure chromatography. Alkaline hydrolysis of the less polar ethoxycarbonyloxy derivative (**3e**) afforded the desired (23*R*)-3α,7α,23-trihydroxy-5β-cholan-24-oic acid [phocaecholic, (**3a**)] and, analogously, the more polar compound (**4b**) gave (23*S*)-3α,7α,23-trihydroxy-5β-cholan-24-oic acid (**4a**). Both (**3a**) and (**4a**) were then converted into the corresponding methyl esters (**3f**) and (**4c**) with physical constants identical with those reported in the literature.[7]

The application of the above sequence to the preparation of bitocholic acid (**2**) was investigated next. Methyl 3α,12α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**1e**) was prepared from deoxycholic acid (**1d**) and converted quantitatively into the corresponding silyl ketene acetal (**5b**). Sequential LTA oxidation and triethylammonium fluoride treatment of (**5b**) afforded α-acetoxy ester (**6b**) (85% yield) which was submitted to mild alkaline treatment as described above to give a nearly equal mixture of C-23 epimeric hydroxy compounds (**3g**) and (**4d**). Separation of these two isomers by medium pressure chromatography followed by alkaline hydrolysis of the less polar isomer afforded (23*R*)-3α,12α,23-trihydroxy-5β-cholan-24-oic acid (**3h**) which was identified with bitocholic acid by comparing the physical properties of (**3h**) with those reported in the literature for the natural acid.[5] Analogously, the more polar isomer was hydrolysed to give the corresponding (23*S*)-3α,12α,23-trihydroxy-5β-cholan-24-oic acid (**4e**).

The stereochemistry at C-23 of (**3h**) and (**4e**) was assigned by c.d. measurements and ^{13}C n.m.r. spectroscopy. The c.d. spectra (Figure 1) showed that the isomer (**3i**) had a large negative Cotton effect at 210 nm, indicating a 23*R*(α_F) configuration for bitocholic acid while the 23*S*(β_F)-epimer (**4f**) exhibited a curve opposite in sign, by analogy with previously reported data for 23-hydroxy bile acids,[7,15] and in general agreement with the known behaviour of (*R*)- and (*S*)-α-hydroxy acids.[16] ^{13}C N.m.r. data for the side chain carbons of compounds (**1f**), (**3f**), (**4c**),



Scheme 1.

Table 1. ^{13}C N.m.r. chemical shifts of the side chain carbons

No. of carbon	(1f)	(3f) *R*	(4c) *S*	(1g)	(3i) *R*	(4f) *S*
20	35.20	32.30	33.60	35.10	32.37	34.39
21	18.20	17.97	19.50	17.10	17.08	18.26
22	30.90	41.50	41.40	31.00	40.88	41.29
23	30.90	68.40	69.90	30.80	68.43	70.14
24	174.50	176.50	176.20	174.50	176.69	172.20
Me ester	51.30	52.30	52.30	51.20	52.36	52.15

(**1g**), (**3i**), and (**4f**) are reported in Table 1. It is known from *X*-ray analysis[17] that bile acids exist in two main rotameric populations, the first fully extended, and the other gauche about the C(20)–C(22) bond, both of them characterized by an anti-parallel relationship between 17-H and 20-H.[18] Accordingly, the side chains of 23-hydroxy bile acids such as (**4a**) and (**3a**) can be represented in two sets (Figure 2), I and II for the most favoured and III and IV for the next favourable 23*S*- and 23*R*-conformers, respectively. In I and II, there is only one gauche interaction around C-23, whereas in III and IV there is an additional gauche interaction between C-23 and C-17. The diagnostic difference in the chemical shift of C-23 which is more upfield in the 23*R*-hydroxy esters (**3f**) and (**3i**) than the corresponding 23*S*-isomers can be attributed to a parallel 1,3 interaction of the 23*S*-hydroxy group with C-17, with a consequent diminution of the gauche rotameric population III in the *S*-series.

The availability of the 23*R*-epimeric pairs (**3a**) and (**3h**) and their corresponding 23*S*-epimers (**4a**) and (**4e**) gave us the opportunity of comparative physicochemical studies. Selected physical properties of the naturally occurring (23*R*)-hydroxy bile acids (**3a**) and (**3h**), their corresponding (23*S*)-epimers and some ester derivatives are reported in Table 2. The occurrence in the bile of some animal species of mixtures of OH-epimers at C-23 has previously been suggested by Haslewood in order to explain conflicting literature data.[5] Now that the above data are known, the search and the identification of naturally occurring (23*S*)-hydroxy bile acids such as (**4a**) and (**4e**) become realistic.

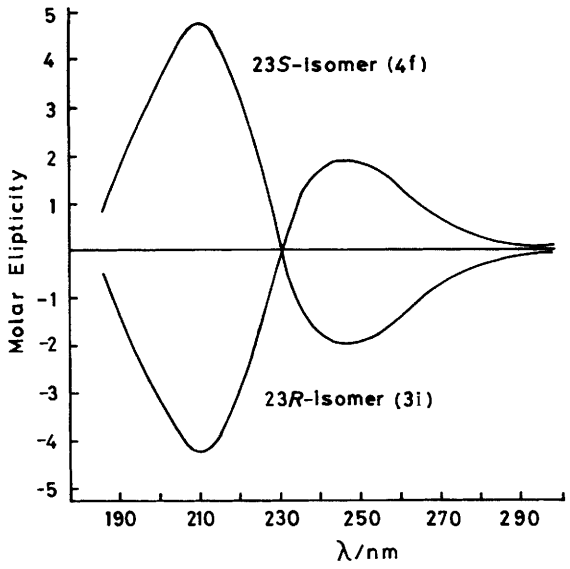
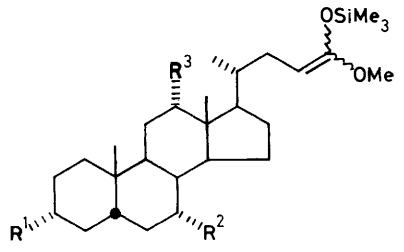


Figure 1. C.d. spectra of (**3i**) and (**4f**)

Finally, bulk aqueous physicochemical properties of phocaecholic acid (**3a**), chosen as representative of 23-OH bile acids, and its 23*S*-epimer were studied and compared with those of chenodeoxycholic acid (**1a**) and cholic acid (**1b**).[19] The results, reported in Table 3, permit preliminary considerations to be made on the influence of an OH group at C-23 on the physical properties of bile acids.

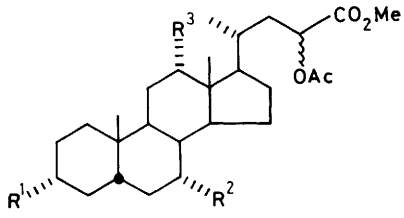
Although amphiphatic molecules, bile acids differ from common anionic surfactants in that they possess a rigid nucleus and micellar aggregation is considered to require an hydro-



(5)

a, $R^1 = OCO_2Et$, $R^2 = OCO_2Et$, $R^3 = H$

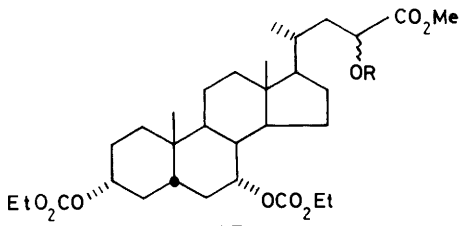
b, $R^1 = OCO_2Et$, $R^2 = H$, $R^3 = OCO_2Et$



(6)

a, $R^1 = OCO_2Et$, $R^2 = OCO_2Et$, $R^3 = H$

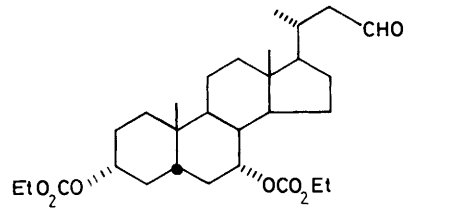
b, $R^1 = OCO_2Et$, $R^2 = H$, $R^3 = OCO_2Et$



(7)

a, R = $SiMe_3$

b, R = H



(8)

phobic β-side to permit molecular association, an α-face made hydrophilic by the presence in the nucleus of 1—3 OH groups, and a short side chain ending with a polar terminus. Our results show that the introduction of a 23-OH group (either *R* or *S*) in the side chain of (**1a**) leads to an increase of the critical micellar concentration (c.m.c.) values with an effect fully comparable with that induced by the introduction of an α-OH group at C-12. Since phocaecholic acid (**3a**), its 23*S*-epimer (**4a**), and cholic acid (**1b**) exhibit not only close c.m.c. values, but also similar aggregation number and micellar size, it can be concluded that the three trihydroxylated bile acids share very similar detergent properties.

While the hydrophilicity of the two 23-OH isomers (**3a**) and (**4a**), indirectly evaluated by reverse phase C-18 h.p.l.c. and expressed as K′ 'retention factor',[20,21] and the water solubility values of the corresponding protonated acids (evaluated at pH = 2) were also shown to be fully comparable with those exhibited by cholic acid (**1b**), significant differences were found in the thermodynamic pK_a values obtained by potentiometric titration methods as previously reported.[22] Indeed, the two 23-OH isomers (**3a**) and (**4a**) show pK_a values of ≃3.8, lower than that exhibited by cholic acid (≃5.02), a difference which can be attributed to the presence in the former compounds of an α-hydroxy carboxylic entity and which is in agreement with reported data for α-hydroxy carboxylic acids.[23]

The phylogenetic implications of the above data as well as the possibility to exploit in medicine the peculiar properties of these 'unique' bile acids are currently under study.

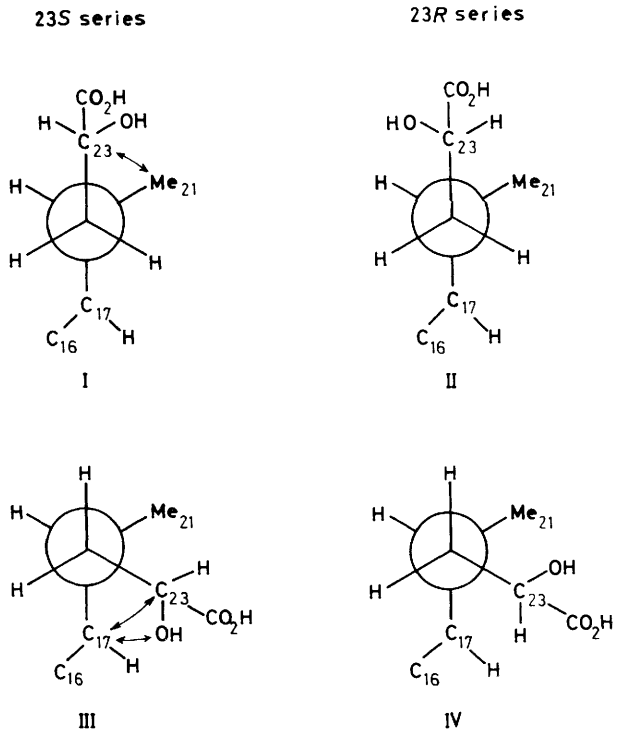


Figure 2.

Table 2. Physical properties of 23-hydroxy bile acids

Bile acid	M.p. (°C)	$[\alpha]_D$	Relative polarity on SiO_2	Sign of c.d.
(**3a**)	225 [lit.,[3] 222—224]	+21 [lit.,[3] +11]	Less polar	−
(**4a**)	215	+15	More polar	+
(**3h**)	230	+48	Less polar	−
(**4e**)	225	+38	More polar	+
(**3i**)	114—123	+44.2		

Table 3. Physicochemical properties

OH Position	Solubility (μM)	C.m.c. (mM)	Hydrophilicity (K′)	pK_a
3α 7α	27 ± 6	9 ± 0.5	2.05 ± 0.08	5.07 ± 0.02
3α 7α 12α	235 ± 25	13 ± 1	1.08 ± 0.02	5.08 ± 0.04
3α 7α 23*R*	250 ± 30	12 ± 1	0.95 ± 0.02	3.80 ± 0.02
3α 7α 23*S*	180 ± 12	13 ± 2	0.91 ± 0.02	3.80 ± 0.04

Experimental

General Methods.—M.p.s were determined on a Kofler micro hot-stage apparatus and are uncorrected. Specific rotations

were recorded on a Roussel Jouan digital 71 polarimeter. I.r. spectra were determined with a Perkin-Elmer 1320 spectrometer. ^{1}H N.m.r. spectra were taken on a Varian EM 390 spectrometer. ^{13}C N.m.r. spectra were taken on a Bruker WP80SY spectrometer, the carbon shifts are in p.p.m. downfield from tetramethylsilane. C.d. spectra were taken on a Jasco J-500A spectropolarimeter. Ultrasonic irradiations were performed on a Bransonic 220 apparatus. All reactions involving organometallic reagents were performed in dry apparatus under argon. Dioxane, dichloromethane and tetrahydrofuran (THF) were distilled from $LiAlH_4$ immediately prior to use. Di-isopropylamine was distilled from calcium hydride and stored over 4 Å molecular sieves. Hexane was distilled from sodium and chlorotrimethylsilane was distilled from anhydrous barium oxide. Column chromatography was performed on Merck silica gel (0.063—0.200 mm). Flash chromatography was performed on Merck silica gel (0.040—0.063 mm). Medium pressure chromatography was performed on Merck LiChroprep Si 60 (0.040—0.063 mm, lobar columns). Light petroleum refers to the fraction boiling in the range 40—70 °C.

Methyl 3α,7α-*Bis*(*ethoxycarbonyloxy*)-5β-*cholan*-24-*oate* (**1c**).—Ethyl chloroformate (19.4 g, 179 mmol) was added dropwise in 40 min to a solution of methyl 3α,7α-dihydroxy-5β-cholan-24-oate (**1f**) (12.0 g, 29.5 mmol) in anhydrous dioxane (300 ml) containing pyridine (18 ml) with vigorous mechanical stirring at 0 °C. When the addition was completed, stirring was continued for 5 days at room temperature. The reaction mixture was then diluted with water (100 ml) and extracted with ether (3 × 100 ml). The combined organic phases were washed with 3M hydrochloric acid (2 × 70 ml) and dried ($MgSO_4$). Evaporation of the solvent gave a residue (17.9 g) which was subjected to flash chromatography: elution with light petroleum–ether (4:1) yielded *pure* (**1c**) (9.39 g, 58%), m.p. 117—118 °C (light petroleum); $\nu_{max.}$($CHCl_3$) 1 730 and 1 710 cm^{-1} (CO); δ_H($CDCl_3$) 0.67 (3 H, s, 18-Me), 0.98 (3 H, s, 19-Me), 1.33 (6 H, t, *J* 7 Hz, 2 × OCH_2*Me*), 3.70 (3 H, s, CO_2Me), 4.18 (4 H, q, *J* 7 Hz, 2 × OCH_2Me), 4.40 (1 H, br m, 3-C*H*OCO_2Et), and 4.70 (1 H, m, 7-C*H*OCO_2Et) (Found: C, 67.55; H, 9.15. $C_{31}H_{50}O_8$ requires C, 67.61; H, 9.15%).

Methyl Trimethylsilyl Ketene Acetal of (**1c**) [(**5a**)].—A cooled (−78 °C) solution of ester (**1c**) (2.0 g, 3.63 mmol) in THF (15 ml) was added dropwise during 15 min to a stirred solution of lithium di-isopropylamide [from addition of butyl-lithium in hexane (2.6 ml of a 1.60M solution) to a solution of di-isopropylamine (0.43 g) in THF (15 ml)] kept under argon at −78 °C. After the mixture had been stirred for 20 min, chlorotrimethylsilane (1.0 ml) was rapidly added and the resulting solution stirred at −78 °C for 2 h; it was then allowed to warm to room temperature when the solvent was evaporated off. Hexane (80 ml) was added to the residue and the resulting suspension filtered. Evaporation of the solvent gave *pure* (**5a**) (2.26 g), 100%), $\nu_{max.}$($CHCl_3$) 1 740 cm^{-1} (CO); δ_H($CDCl_3$) 0.24 (9 H, s, $OSiMe_3$), 0.67 (3 H, s, 18-Me), 0.95 (3 H, s, 19-Me), 1.33 (6 H, t, *J* 7 Hz, 2 × OCH_2*Me*), 3.48 (3 H, s, OMe), 3.61 (1 H, m, 23-CH), 4.18 (4 H, q, *J* 7 Hz, 2 × OCH_2Me), 4.40 (1 H, br m, 3-C*H*OCO_2Et), and 4.60 (1 H, m, 7-C*H*OCO_2Et) (Found: C, 65.55; H, 9.35; O, 20.55. $C_{34}H_{58}O_8Si$ requires C, 65.56; H, 9.38; O, 20.55%).

Methyl 23-*Acetoxy*-3α,7α-*bis*(*ethoxycarbonyloxy*)-5β-*cholan*-24-*oate* (**6a**).—A solution of methyl trimethylsilyl ketene acetal (**5a**) (0.70 g, 1.12 mmol) in dichloromethane (5 ml) was added dropwise during 15 min to an acetic acid-free solution of lead tetra-acetate (0.55 g, 1.24 mmol) in dichloromethane (15 ml) stirred at −15 °C under an argon atmosphere. When the addition was complete, the resulting mixture was stirred for 30 min at room temperature, filtered through Celite and the filtrate added to triethylammonium fluoride (0.408 g, 3.40 mmol). The resulting solution was stirred at room temperature for 12 h after which dichloromethane was added and the reaction mixture washed with saturated aqueous sodium hydrogen carbonate (3 × 10 ml), 1.5M hydrochloric acid (2 × 10 ml), water (2 × 10 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Flash chromatography of the residue (0.75 g) and elution with light petroleum and then light petroleum–ether (3:2) gave the pure 23-*acetoxy derivative* (**6a**) (0.620 g, 91%), $\nu_{max.}$($CHCl_3$) 1 735 cm^{-1} (CO); δ_H[mixture of isomers: ($CDCl_3$)] 0.67 (3 H, s, 18-Me), 0.95 (3 H, s, 19-Me), 1.29 (6 H, t, *J* 7 Hz, 2 × OCH_2*Me*), 2.09 and 2.12 (3 H, 2s, OCOMe), 3.70 (3 H, s, CO_2Me), 4.16 (4 H, q, *J* 7 Hz, 2 × OCH_2Me), 4.42 (1 H, br m, 3-C*H*OCO_2Et), 4.76 (1 H, m, 7-C*H*OCO_2Et), and 5.05 (1 H, m, 23-C*H*OAc) (Found: C, 65.05; H, 8.65. $C_{33}H_{52}O_{10}$ requires C, 65.10; H, 8.61%).

Selective Hydrolysis of Methyl 23-*Acetoxy*-3α,7α-*bis*(*ethoxycarbonyloxy*)-5β-*cholan*-24-*oate* (**6a**).—Methyl 23-acetoxy-3α,7α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**6a**) (0.20 g, 0.33 mmol) was added to a saturated methanolic solution of potassium carbonate (9 ml) and the resulting mixture was submitted to ultrasonic irradiation for 5 min at room temperature. This mixture was then magnetically stirred for 2.5 h. After evaporation of the solvent, the residue (0.175 g) was subjected to medium pressure chromatography: elution with chloroform afforded methyl 3α,7α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (**7b**) (0.030 g, 16%), as an inseparable mixture of the 23-OH epimers. Further elution with the same solvent afforded (23*R*)-methyl 7α-ethoxycarbonyloxy-3α,23-dihydroxy-5β-cholan-24-oate (**3e**) (0.064 g, 39%), m.p. 80—82 °C; $\nu_{max.}$($CHCl_3$) 3 500 (OH) and 1 730 cm^{-1} (CO); δ_H($CDCl_3$) 0.70 (3 H, s, 18-Me), 0.93 (3 H, s, 19-Me), 1.01 (3 H, d, 21-Me), 1.30 (3 H, t, *J* 7 Hz, OCH_2*Me*), 3.43 (1 H, br m, 3-C*H*OH), 3.77 (3 H, s, CO_2Me), 4.20 (3 H, br q, *J* 7 Hz, OCH_2Me and 23-C*H*OH), and 4.73 (1 H, m, C*H*OCO_2Et) (Found: C, 68.0; H, 9.4. $C_{28}H_{46}O_7$ requires C, 67.98; H, 9.37%).

Further elution with the same solvent afforded (23*S*)-methyl 7α-(ethoxycarbonyloxy)-3α,23-dihydroxy-5β-cholan-24-oate (**4b**) (0.060 g, 37%), m.p. 65—67 °C (Found: C, 68.05; H, 9.4. $C_{28}H_{46}O_7$ requires C, 67.98; H, 9.37%).

Finally, further elution with chloroform–methanol (9:1) afforded 3α,7α,23-trihydroxy-5β-cholan-24-oic acid (0.009 g, 7%) as an inseparable mixture of the two 23-OH epimers.

(23R)-3α,7α,23-*Trihydroxy*-5β-*cholan*-24-*oic Acid* (**3a**) (*Phocaecholic Acid*).—(23*R*)-Methyl 7α-ethoxycarbonyloxy-3α,23-dihydroxy-5β-cholan-24-oate (**3e**) (0.300 g, 0.6 mmol) was added to a warm methanolic solution of 2M KOH (18 ml). The mixture was heated at reflux for 5 h, poured into iced water (20 ml), acidified with 3M hydrochloric acid, and extracted with ethyl acetate (4 × 10 ml). The combined organic phases were washed with water (2 × 10 ml), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue (0.250 g) and elution with chloroform–methanol (85:15) gave the pure acid (**3a**) (0.230 g, 93%), m.p. 225 °C; $[\alpha]_D^{20}$ +21 (*c* 1.06 in EtOH) (lit.,[3] 222—224 °C; lit.[3] $[\alpha]_D$ +11, EtOH; lit.,[24] $[\alpha]_D$ +18, EtOH) (Found: C, 70.55; H, 9.8. Calc. for $C_{24}H_{40}O_5$: C, 70.50; H, 9.79%).

(23S)-3α,7α,23-*Trihydroxy*-5β-*cholan*-24-*oic acid* (**4a**).—(23*S*)-Methyl 7α-(ethoxycarbonyloxy)-3α,23-dihydroxy-5β-cholan-24-oate (**4b**) (0.200 g, 0.4 mmol) was added to a warm methanolic solution of 2M KOH (15 ml). The mixture was heated under reflux for 5 h, poured into iced water (20 ml), acidified with 3M hydrochloric acid, and extracted with ethyl

acetate (4 × 10 ml). The combined organic phases were washed with water (2 × 10 ml), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue (0.180 g) and elution with chloroform–methanol (85:15) gave the *pure acid* (**4a**) (0.150 g, 92%), m.p. 215 °C; $[\alpha]_D^{20}$ +15 (*c* 1 in EtOH) (Found: C, 70.6; H, 9.75. $C_{24}H_{40}O_5$ requires C, 70.50; H, 9.79%).

Ozonolysis of Compound (**5a**).—Ozone was bubbled at −78 °C through a stirred solution of the methyl trimethylsilyl ketene acetal (**5a**) (2.0 g, 3.2 mmol) in dichloromethane (100 ml) until a light blue colour appeared (1 h). The reaction mixture was then added dropwise to a suspension of zinc dust (9 g) in 5M acetic acid (50 ml) and stirred for 1 h at room temperature. The zinc dust was filtered off and washed with dichloromethane (3 × 10 ml) and the aqueous layer separated and extracted with dichloromethane (3 × 30 ml). The combined organic phases were washed with saturated aqueous hydrogen carbonate and brine, dried (Na_2SO_4), and evaporated under reduced pressure. Flash chromatography of the residue (2.0 g) and elution with light petroleum–ether (95:5) afforded *methyl* 3α,7α-*bis*(*ethoxycarbonyloxy*)-23-*trimethylsilyloxy*-5β-*cholan*-24-*oate* (**7a**) (1.0 g, 49%), as an inseparable mixture of 23-$OSiMe_3$ epimers: $\nu_{max.}(CHCl_3)$ 1 730 cm^{-1} (CO); δ_H[mixture of isomers: ($CDCl_3$)] 0.21 (9 H, s, $OSiMe_3$), 0.72 (3 H, s, 18-Me), 1.00 (3 H, s, 19-Me), 1.38 (6 H, t, *J* 7 Hz, 2 × OCH_2*Me*), 3.78 (3 H, s, CO_2Me), 4.23 (5 H, q, *J* 7 Hz, 2 × O*CH*$_2$Me and 23-C*H*O$SiMe_3$), 4.50 (1 H, br m, 3-C*H*OCO_2Et), and 4.76 (1 H, m, 7-C*H*OCO_2Et) (Found: C, 63.9; H, 9.15; O, 22.5. $C_{34}H_{58}O_9Si$ requires C, 63.92; H, 9.15; O, 22.54%). Further elution with light petroleum–ether (9:1) afforded the *nor-aldehyde* (**8**) (0.230 g, 14%), m.p. 122—125 °C, $\nu_{max.}$ 1 735 cm^{-1} (CO); $\delta_H(CDCl_3)$ 0.70 (3 H, s, 18-Me), 0.93 (3 H, s, 19-Me), 1.00 (3 H, d, 21-Me), 1.29 (6 H, t, *J* 7 Hz, 2 × OCH_2*Me*), 4.20 (4 H, q, *J* 7 Hz, 2 × O*CH*$_2$Me), 4.43 (1 H, br m, 3-C*H*OCO_2Et), 4.69 (1 H, m, 7-C*H*OCO_2Et), and 9.71 (1 H, m, CHO) (Found: C, 76.1; H, 10.5. $C_{23}H_{38}O_3$ requires C, 76.14; H, 10.48%).

Finally, elution with light petroleum–ether (85:15) afforded methyl 3α,7α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (**7b**) (0.330 g, 18%) as an inseparable mixture of the two 23-OH epimers; $\nu_{max.}(CHCl_3)$ 3 500 (OH) and 1 730 cm^{-1} (CO); δ_H[mixture of isomers: ($CDCl_3$)] 0.68 (3 H, 2s, 18-Me), 0.92 (3 H, s, 19-Me), 1.00 (3 H, d, 21-Me), 1.30 (6 H, t, *J* 7 Hz, 2 × OCH_2*Me*), 2.83 (1 H, m, OH), 3.78 (3 H, s, CO_2Me), 4.20 (5 H, q, *J* 7 Hz, 2 × O*CH*$_2$Me and 23 C*H*OH), 4.48 (1 H, br m, 3-C*H*OCO_2Et), and 4.71 (1 H, m, 7-C*H*OCO_2Et) (Found: C, 65.6; H, 8.85. $C_{31}H_{50}O_9$ requires C, 65.70; H, 8.89%).

Ozonolysis of (**5a**) *in the Presence of* BF_3·Et_2O.—A cooled (−78 °C) solution of the methyl trimethylsilyl ketene acetal (**5a**) (1.0 g, 1.6 mmol) in dichloromethane (20 ml) was added dropwise to a solution of freshly distilled BF_3·Et_2O (0.023 g, 0.16 mmol) in dichloromethane (50 ml) with vigorous stirring at −18 °C. Ozone was then bubbled through this solution for 1.5 h, after which a stream of argon was used to remove the excess of ozone. The reaction mixture was evaporated under reduced pressure and the residue (0.90 g) was submitted to flash chromatography: elution with light petroleum–ether (85:15) yielded methyl 3α,7α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**1c**) (0.44 g). Further elution with the same solvents yielded methyl 3α,7α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (**7b**) (0.45 g, 49%) as an inseparable mixture of the two 23-OH epimers.

Selective Hydrolysis of Methyl 3α,7α-*Bis*(*ethoxycarbonyloxy*)-23-*hydroxy*-5β-*cholan*-24-*oate* (**7b**).—Methyl 3α,7α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (**7b**) (0.500 g, 0.88 mmol) was added to a saturated methanolic solution of potassium carbonate (20 ml) and the resulting mixture stirred for 5 h at room temperature. After evaporation of the solvent, the residue (0.43 g) was subjected to medium pressure chromatography: elution with chloroform afforded in the following order: starting material (0.035 g); (23*R*)-methyl 3α,23-dihydroxy-7α-(ethoxycarbonyloxy)-5β-cholan-24-oate (**3e**) (0.146 g, 33%); a mixture of the (23*R*)- and (23*S*)-derivatives (**3e**) and (**4b**) (0.010 g); (23*S*)-methyl 7α-bis(ethoxycarbonyloxy)-3α,23-dihydroxy-5β-cholan-24-oate (**4b**) (0.147 g, 34%). Finally, elution with the same solvent afforded 3α,7α,23-trihydroxy-5β-cholan-24-oic acid (0.075 g) as an inseparable mixture of the two 23-OH epimers.

Oxidation of Methyl Trimethylsilyl Ketene Acetal (**5a**) *with* OsO_4.—Osmium tetraoxide (0.203 g, 0.8 mmol) was added to a solution of (**5a**) (0.5 g, 0.8 mmol) in anhydrous dioxane (30 ml) and the resulting mixture was stirred under argon for 16 h at room temperature. Hydrogen sulphide was then bubbled through this mixture for 3 h; the reaction mixture was then filtered through Celite and the filtrate extracted with ether (3 × 15 ml) and dried ($MgSO_4$). After evaporation of the solvent, the residue (0.5 g) was chromatographed on silica gel: elution with light petroleum–ether (4:1) afforded methyl 3α,7α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**1c**) (0.16 g). Further elution with the same solvents afforded methyl 3α,7α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (**7b**) (0.270 g, 59%) as an inseparable mixture of the two 23-OH epimers.

Oxidation of the Methyl Trimethylsilyl Ketene Acetal (**5a**) *with MCPBA*.—A solution of the methyl trimethylsilyl ketene acetal (**5a**) (1.0 g, 1.61 mmol) in hexane (10 ml) was added dropwise in 5 min to a stirred solution of MCPBA (0.285 g, 1.65 mmol) in hexane (10 ml) kept under argon at −15 °C. When the addition was complete, stirring was continued at room temperature for 1 h, after which the reaction mixture was filtered, and the solvent evaporated under reduced pressure. The residue (1.1 g) was dissolved in dichloromethane (20 ml), added to triethylammonium fluoride (0.54 g, 4.46 mmol), and the resulting mixture stirred for 10 h at room temperature. The reaction mixture was then washed with aqueous saturated sodium hydrogen carbonate (2 × 20 ml), 3M hydrochloric acid (2 × 20 ml), and saturated aqueous sodium hydrogen carbonate (2 × 20 ml), dried ($MgSO_4$), and evaporated. The residue (1.0 g) was submitted to flash chromatography: elution with light petroleum–ether (9:1) afforded methyl 3α,7α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**1c**) (0.44 g). Further elution with the same solvents afforded methyl 3α,7α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (**7b**) (0.50 g, 55%) as an inseparable mixture of the two 23-OH epimers.

Methyl 3α,12α-*Bis*(*ethoxycarbonyloxy*)-5β-*cholan*-24-*oate* (**1e**).—Ethyl chloroformate (21.1 g, 194 mmol) was added dropwise during 40 min to a solution of methyl 3α,12α-dihydroxy-5β-cholan-24-oate (**1g**) (4.3 g, 11 mmol) in anhydrous dioxane (120 ml) containing pyridine (19.5 ml) with vigorous mechanical stirring at 0 °C. When the addition was complete, stirring was continued for 7 days at room temperature. The reaction mixture was then diluted with water (40 ml) and extracted with ether (4 × 30 ml). The combined organic phases were washed with 3M hydrochloric acid (2 × 20 ml) and brine (2 × 20 ml) and dried ($MgSO_4$). Evaporation of the solvent gave a residue (5.0 g) which was subjected to flash chromatography: elution with light petroleum–ether (4:1) yielded *pure* (**1e**) (3.62 g, 62%), m.p. 92 °C (light petroleum); $\nu_{max.}(CHCl_3)$ 1 735 cm^{-1} (CO); $\delta_H(CDCl_3)$ 0.71 (3 H, s, 18-Me), 0.94 (3 H, s, 19-Me), 1.32 and 1.33 (6 H, 2t, *J* 7 Hz, 2 × OCH_2*Me*), 3.67 (3 H, s, CO_2Me), 4.11 and 4.12 (4 H, 2q, *J* 7 Hz, 2 × O*CH*$_2$Me), 4.57 (1 H, br m, 3-C*H*OCO_2Et), and 4.93 (1 H, m,

12-C*H*OCO$_2$Et) (Found: C, 67.6; H, 9.1. $C_{31}H_{50}O_8$ requires C, 67.61; H, 9.15%).

Methyl Trimethylsilyl Ketene Acetal of (**1e**) [(**5b**)].—A cooled (−78 °C) solution of the ester (**1e**) (2.0 g, 3.63 mmol) in THF (15 ml) was added dropwise during 15 min to a stirred solution of lithium di-isopropylamide [from addition of butyl-lithium in hexane (2.6 ml of a 1.6M solution) to a solution of di-isopropylamine (0.43 g) in THF (15 ml)] kept under argon at − 78 °C. The mixture was stirred for 20 min after which chlorotrimethylsilane (1.0 ml) was rapidly added and the resulting solution stirred at −78 °C for 2 h; the reaction mixture was allowed to warm to room temperature and then evaporated. Hexane (80 ml) was added to the residue and the resulting suspension filtered. Evaporation of the solvent gave *pure* (**5b**) (2.26 g, 100%), $\nu_{max.}$($CHCl_3$) 1 740 cm^{-1} (CO) δ_H($CDCl_3$) 0.20 (9 H, s, $OSiMe_3$), 0.70 (3 H, s, 18-Me), 0.90 (3 H, s, 19-Me), 1.30 (6 H, 2t, *J* 7 Hz, 2 × OCH_2*Me*), 3.41 (3 H, s, OMe), 3.55 (1 H, m, 23-CH), 4.10 (4 H, 2q, *J* 7 Hz, 2 × OCH_2Me), 4.50 (1 H, br m, 3-C*H*OCO$_2$Et), and 4.85 (1 H, m, 12-C*H*OCO$_2$Et) (Found: C, 65.5; H, 9.4; O, 20.5. $C_{34}H_{58}O_8Si$ requires C, 65.56; H, 9.38; O, 20.55%).

Methyl 23-*Acetoxy*-3α,12α-*bis*(*ethoxycarbonyloxy*)-5β-*cholan*-24-*oate* (**6b**).—A solution of methyl trimethylsilyl ketene acetal (**5b**) (2.0 g, 3.2 mmol) in dichloromethane (10 ml) was added dropwise during 25 min to an acetic acid-free solution of lead tetra-acetate (1.57 g, 3.54 mmol) in dichloromethane (50 ml) stirred at −15 °C under an argon atmosphere. Once the addition was complete, the resulting mixture was stirred for 30 min at room temperature, filtered through Celite and the filtrate added to triethylammonium fluoride (1.17 g, 9.75 mmol). The resulting solution was stirred at room temperature for 12 h, after which dichloromethane (40 ml) was added and the reaction mixture washed with saturated aqueous sodium hydrogen carbonate (3 × 10 ml), 1.5M hydrochloric acid (2 × 10 ml), and water (2 × 10 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Flash chromatography of the residue (2.18 g) and elution with light petroleum–ether (7:3) gave the pure 23-*acetoxy derivative* (**6b**) (1.48 g, 76%), $\nu_{max.}$($CHCl_3$) 1 730 cm^{-1} (CO); δ_H[mixture of isomers: ($CDCl_3$)] 0.72 (3 H, s, 18-Me), 0.91 (3 H, s, 19-Me), 1.32 and 1.34 (6 H, 2t, *J* 7 Hz, 2 × OCH_2*Me*), 2.10 (3 H, s, OCOMe), 3.70 (3 H, s, CO_2Me), 4.25 and 4.28 (4 H, 2q, *J* 7 Hz, 2 × OCH_2Me), 4.59 (1 H, br m, 3-C*H*OCO$_2$Et), and 4.97 (2 H, m, 7-C*H*OCO$_2$Et and 23-C*H*OAc) (Found: C, 65.15; H, 8.6. $C_{33}H_{52}O_{10}$ requires C, 65.10; H, 8.61%).

Selective Hydrolysis of Methyl 23-*Acetoxy*-3α,12α-*bis*(*ethoxycarbonyloxy*)-5β-*cholan*-24-*oate* (**6b**).—Methyl 23-acetoxy-3α,12α-bis(ethoxycarbonyloxy)-5β-cholan-24-oate (**6b**) (0.400 g, 0.65 mmol) was added to a saturated methanolic solution of potassium carbonate (40 ml) and the resulting mixture was submitted to ultrasonic irradiation for 5 min at room temperature. This mixture was then magnetically stirred for 5 h. After evaporation of the solvent, the residue (0.440 g) was subjected to medium pressure chromatography: elution with chloroform afforded methyl 3α,12α-bis(ethoxycarbonyloxy)-23-hydroxy-5β-cholan-24-oate (0.029 g) as an inseparable mixture of the two 23-OH epimers. Elution with the same solvent afforded (23R)-*methyl* 3α,23-*dihydroxy*-12α-*ethoxycarbonyloxy*-5β-*cholan*-24-*oate* (**3g**) (0.113 g, 35%); $\nu_{max.}$($CHCl_3$) 1 735 cm^{-1} (CO); δ_H($CDCl_3$) 0.77 (3 H, s, 18-Me), 0.93 (3 H, s, 19-Me), 1.33 (3 H, t, *J* 7 Hz, OCH_2*Me*), 3.60 (1 H, br m, 3-C*H*OH), 3.80 (3 H, s, CO_2Me), 4.21 (3 H, br q, *J* 7 Hz, OCH_2Me and 23-C*H*OH), and 5.00 (1 H, m, C*H*OCO$_2$Et) (Found: C, 67.9; H, 9.35. $C_{28}H_{46}O_7$ requires C, 67.98; H, 9.37%).

Further elution with the same solvent afforded a mixture of the (23*R*)- and (23*S*)-derivatives (**3g**) and (**4d**) (0.007 g). Further elution with the same solvent afforded (23S)-*methyl* 3α,23-*dihydroxy*-12α-*ethoxycarbonyloxy*-5β-*cholan*-24-*oate* (**4d**) (0.117 g, 36%), m.p. 95—96 °C (Found: C, 67.95; H, 9.4. $C_{28}H_{46}O_7$ requires C, 67.98; H, 9.37%). Finally, further elution with the same solvent afforded 3α,12α,23-trihydroxy-5β-cholan-24-oic acid (0.045 g) as mixture of the two 23-OH epimers.

(23R)-3α,12α,23-*Trihydroxy*-5β-*cholan*-24-*oic acid* (**3h**) (*Bitocholic Acid*).—(23*R*)-Methyl 3α,23-dihydroxy-12α-ethoxycarbonyloxy-5β-cholan-24-oate (**3g**) (0.100 g, 0.2 mmol) was added to a warm methanolic solution of 2M KOH (10 ml). The mixture was heated under reflux for 5 h, poured into iced water (20 ml), acidified with 3M hydrochloric acid, and extracted with ethyl acetate (4 × 10 ml). The combined organic phases were washed with water (2 × 10 ml), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue (0.080 g) and elution with chloroform–methanol (85:15) gave the pure acid (**3h**) (0.076 g, 92%), m.p. 230—232 °C; $[\alpha]_D^{20}$ +48 (*c* 0.856 in EtOH) [lit.,[5] $[\alpha]_D$ +49 (*c* 1.3 in EtOH)] (Found: C, 70.45; H, 9.85. $C_{24}H_{40}O_5$ requires C, 70.50; H, 9.79%).

(23S)-3α,12α,23-*Trihydroxy*-5β-*cholan*-24-*oic Acid* (**4e**).—(23*S*)-Methyl 3α,23-dihydroxy-12α-ethoxycarbonyloxy-5β-cholan-24-oate (**4d**) (0.100 g, 0.2 mmol) was added to a warm methanolic solution of 2M KOH (10 ml). The mixture was heated under reflux for 5 h, poured into iced water (20 ml), acidified with 3M hydrochloric acid, and extracted with ethyl acetate (4 × 10 ml). The combined organic phases were washed with water (2 × 10 ml), dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the residue (0.080 g) and elution with chloroform–methanol (85:15) gave the pure acid (**4e**) (0.077 g, 94%), m.p. 225 °C; $[\alpha]_D^{20}$ +38 (*c* 1.8 in EtOH) (Found: C, 70.55; H, 9.75. $C_{24}H_{40}O_5$ requires C, 70.50; H, 9.79%).

(23R)-*Methyl* 3α,7α,23-*Trihydroxy*-5β-*cholan*-24-*oate* (**3b**).—Toluene-4-sulphonic acid (0.005 g, 0.026 mmol) was added to a solution of (**3a**) (0.350 g, 0.86 mmol) in methanol (40 ml) and the resulting mixture was stirred for 12 h at room temperature. It was then poured into iced water (30 ml) and extracted with chloroform (3 × 15 ml). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. Flash filtration of the residue (0.360 g) on silica gel and elution with chloroform–methanol (98:2) afforded (**3f**) (0.340 g, 94%), m.p. 110 °C; $\nu_{max.}$($CHCl_3$) 3 500 (OH) and 1 730 cm^{-1} (CO); δ_H($CDCl_3$ + CD_3OD) 0.69 (3 H, s, 18-Me), 0.90 (3 H, s, 19-Me), 1.01 (3 H, d, *J* 6 Hz, 21-Me), 3.38 (1 H, m, 3-C*H*OH), 3.78 (3 H, s, CO_2Me), 3.98 (3 H, s, 3 × OH), and 4.24 (1 H, m, 23-C*H*OH); $[\alpha]_D^{20}$ +8.5 (*c* 0.42 in $CHCl_3$) [lit.,[7] m.p. 110—112 °C, $[\alpha]_D$ +8 (*c* 1 in $CHCl_3$)].

(23S)-*Methyl* 3α,7α,23-*Trihydroxy*-5β-*cholan*-24-*oate* (**4c**).—This compound was prepared as above in 94% yield, m.p. 196—197 °C; $\nu_{max.}$($CHCl_3$) 3 500 (OH) and 1 730 cm^{-1} (CO); δ_H($CDCl_3$ + CD_3OD) 0.69 (3 H, s, 18-Me), 0.91 (3 H, s, 19-Me), 1.01 (3 H, d, *J* 6 Hz, 21-Me), 3.38 (1 H, m, 3-C*H*OH), 3.76 (3 H, s, CO_2Me), 4.00 (3 H, s, 3 × OH), and 4.24 (1 H, m, 23-C*H*OH); $[\alpha]_D^{20}$ +22 (*c* 0.58 in $CHCl_3$) [lit.,[7] m.p. 197—199 °C, $[\alpha]_D$ +20 (*c* 0.50 in $CHCl_3$)].

(23R)-*Methyl* 3α,12α,23-*Trihydroxy*-5β-*cholan*-24-*oate* (**3i**).—This compound was prepared as above in 91% yield, m.p. 119—120 °C; $\nu_{max.}$($CHCl_3$) 3 500 (OH) and 1 730 cm^{-1} (CO); δ_H($CDCl_3$ + CD_3OD) 0.71 (3 H, s, 18-Me), 0.91 (3 H, s, 19-Me), 1.10 (3 H, m, 21-Me), 3.45 (4 H, m, 3 × OH and 3-C*H*OH), 3.81 (3 H, s, CO_2Me), 4.05 (1 H, m, 12-C*H*OH), and

4.28 (1 H, m, 23-C*H*OH); $[\alpha]_D^{20}$ +44.1 (*c* 0.34 in abs. EtOH) [lit.,[5] m.p. 114—123 °C, $[\alpha]_D^{23}$ +44.2 (*c* 0.34 in EtOH)].

(23S)-*Methyl* 3α,12α,23-*Trihydroxy*-5β-*cholan*-24-*oate* (**4f**).—This compound was prepared as above in 91% yield, m.p. 143—144 °C; $\nu_{max.}(CHCl_3)$ 3 500 (OH) and 1 730 cm^{-1} (CO); $\delta_H(CDCl_3 + CD_3OD)$ 0.69 (3 H, s, 18-Me), 0.91 (3 H, s, 19-Me), 1.07 (3 H, d, *J* 6 Hz, 21-Me), 3.17 (4 H, m, 3 × OH and 3-C*H*OH), 3.97 (3 H, s, CO_2Me), 4.01 (1 H, m, 12-C*H*OH), and 4.26 (1 H, m, 23-C*H*OH); $[\alpha]_D^{20}$ +57.6 (*c* 0.34 in abs. EtOH).

Physicochemical Properties.—Critical micellar concentration (c.m.c.) values were measured in water by the dye solubilization method[25] and, in particular, with azulene (Aldrich Chemical Co., Milwaukee WIS) and Orange OT (gift from Dr. K. Mysels, Chemistry Department, University of California at San Diego, La Jolla, CA) as water-insoluble dyes. The water solubility of the protonated form was measured on saturated solutions at pH = 3 as previously reported[26] after filtration on a Millipore (0.22 μm).

Acidity constants were determined by potentiometric measurements in a solution of aqueous methanol at different mole fractions. The pK_a values, estimated in water by means of previously assessed correlations from the pK_a values in mixed solvents, are in close agreement with each other.[22] All measurements were carried out at 25 ± 0.01 °C.

Hydrophobic-hydrophilic properties of bile salts were determined by reverse-phase high performance liquid chromatography.[21] H.p.l.c. was performed using a Water Inc. (Milford, MA) liquid chromatograph. A C-18 reverse phase column 5 μ pore size and 10 cm length was used. The analysis was carried out under isocratic conditions. As a mobile phase a mixture of propan-2-ol–H_2O (8:17, v/v), pH = 7 was used. A retention factor (K′) was calculated from the relative mobilities of the separated bile acids.[20]

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