Structure of the Steroidal Lactone isolated from Turtle Bile: (22*S*,25*R*)-3α,7α,12α-Trihydroxy-5β-cholestano-26,22-lactone

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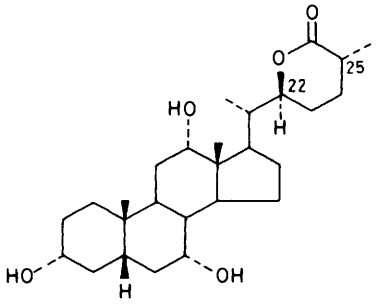
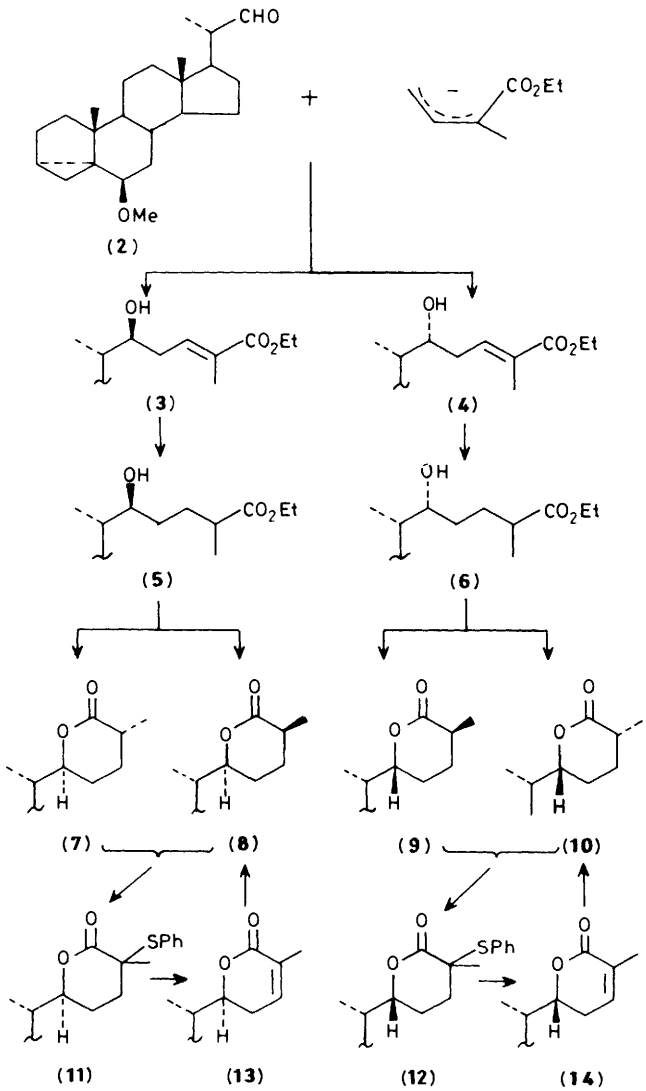
The structure of the steroidal lactone (previously designated as tetrahydroxysterocholanic acid lactone) isolated from the bile of *Amyda japonica* (turtle) has been characterized as (22*S*,25*R*)-3α,7α,12α-trihydroxy-5β-cholestano-26,22-lactone (**1**). The 22*S*,25*R* configuration was determined by ^{1}H n.m.r. comparison with the reference compounds, four possible stereoisomers (with respect to the C-22 and C-25 positions) of 6β-methoxy-3α,5-cyclo-5α-cholestano-26,22-lactones (**7**)—(**10**).

More than 30 years ago the isolation of two steroidal lactonic substances designated as tetrahydroxysterocholanic acid lactone (TSL) and tetrahydroxyisosterocholanic acid lactone (TISL) from the bile of *Amyda japonica* (turtle) and *Emys orbicularis* (tortoise) was reported.[1] Although 3α,7α,12α,22ξ-tetrahydroxycoprostanoic lactone was proposed as a putative structure of TSL in subsequent studies,[2] conclusive evidence on the structure has not been published until now. We envisaged that the TSL side-chain stereostructure as well as its gross structure could be established by ^{1}H n.m.r. comparison with model steroidal compounds having a 26,22-lactone moiety with known C-22 and C-25 stereochemistry. This paper describes the preparation of the model compounds and their ^{1}H n.m.r. comparison, which allowed us to characterize the structure of TSL as (22*S*,25*R*)-3α,7α,12α-trihydroxy-5β-cholestano-26,22-lactone (**1**).



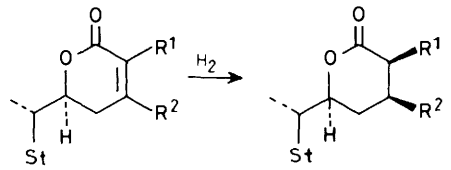
(**1**)

The four stereoisomers, (22*S*,25*R*)- (**7**), (22*S*, 25*S*)- (**8**), (22*R*,25*S*)- (**9**), and (22*R*,25*R*)- (**10**) -6β-methoxy-3α,5-cyclo-5α-cholestano-26,22-lactones, as model steroids, were prepared as outlined in the Scheme. The C-22 aldehyde (**2**),[3] readily available from stigmasterol in three steps, was treated with the enolate anion [generated by lithium di-isopropylamide (LDA)] of ethyl tiglate in tetrahydrofuran (THF)/hexamethylphosphoric triamide (HMPA). As expected from our previous paper,[4] the coupling occurred only at the γ-position to afford the less polar, major (22*S*)-alcohol (**3**) and the more polar, minor (22*R*)-epimer (**4**) in 45 and 17% yield, respectively. Since it is well known that nucleophilic carbanion attack toward C-22 aldehyde furnishes the 22α-F (22*S* in this case) isomer as the major product,[5] the less polar product (**3**) was deduced to be the (22*S*)-isomer. The stereoselectivity in this reaction [(3.2:1 for (**3**):(**4**), the ratio being determined by high-performance liquid chromatographic (h.p.l.c.) analysis of the crude product] was rather lower than that observed in the addition of 2,3-dimethylcrotonate esters in which the formation of the (22*R*)-isomer was not observed.[4]

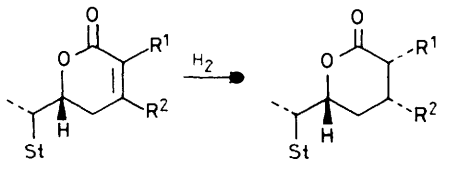


Catalytic hydrogenation of compound (**3**) over 10% Pd–C in ethyl acetate afforded the saturated ester (**5**) in 87% yield, presumably as a C-25 epimeric mixture. Hydrolysis (KOH–methanol–water) of compound (**5**) followed by lactonization (acidification with HCl and then evaporation in the presence of *p*-TsOH) afforded a *ca.* 1 : 1 mixture of the (22*S*)-lactones in 37% yield, which was separated by preparative t.l.c. (p.l.c.) into the less polar isomer (**7**) and the more polar one (**8**). The C-25 stereochemistry of the two lactones was determined from the following experimental results.

In the course of our synthetic studies toward withanolides,[6] we have recently found that hydrogenation of (22*S*)- or (22*R*)-unsaturated δ-lactones as shown below afforded essentially a single saturated lactone in each case. The stereochemical relationship in this reaction is represented in equations (1) and (2); the attack of hydrogen takes place from the side (plane) opposite that of the C_{20} steroid substituent.*†



(1)



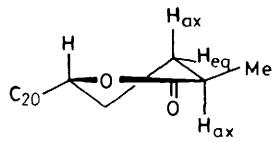
(2)

This empirical rule was applied to the assignment of the C-25 stereochemistry of compounds (**7**) and (**8**). The mixture of the (22*S*)-lactones (**7**) and (**8**) was treated with LDA and then with diphenyl disulphide to give the sulphide (**11**) in 75% yield. Oxidation of sulphide (**11**) with *m*-chloroperbenzoic acid (MCPBA), and subsequent elimination of the resulting sulphoxide upon heating, afforded the (22*S*)-unsaturated lactone (**13**) in 40% yield. The ^{1}H n.m.r. signal for the 22-H of lactone (**13**) showed characteristic coupling at δ_H 4.45 (dd, *J* 12 and 4 Hz), which is in accord with the data for (22*S*)-unsaturated lactones.[4,7] Hydrogenation (10% Pd–C in dioxane) of compound (**13**) proceeded cleanly to give a saturated lactone which was identified as the more polar lactone (**8**). Thus it is concluded, according to equation (1), that the polar lactone (**8**) has (22*S*,25*S*) configuration, while the less polar isomer (**7**) is (22*S*,25*R*).

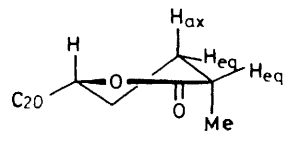
A parallel experiment was undertaken starting from the unsaturated ester (**4**). The saturated ester (**6**), obtained by hydrogenation of compound (**4**) in 86% yield, was converted in the same manner as described for ester (**5**) into the two isomeric (22*R*)-lactones (*ca.* 1 : 1 mixture) in 35% yield. Separation of the less polar isomer (**9**) and the more polar one (**10**) was effected by p.l.c. For the determination of the C-25 stereochemistry of lactones (**9**) and (**10**), the unsaturated (22*R*)-lactone (**14**) was prepared in 48% yield from the mixture of (**9**) and (**10**) *via* the sulphide intermediate (**12**) as described for compound (**11**). The ^{1}H n.m.r. signal for the 22-H of compound (**14**) was observed at δ_H 4.33 (dt, *J* 12 and 4 Hz), the coupling pattern of which is characteristic of naturally occurring (22*R*)-withanolides.[8] Hydrogenation of compound (**14**) afforded the more polar lactone (**10**). Thus it is concluded that the more polar lactone (**10**) has (22*R*,25*R*) stereochemistry, while the less polar one (**9**) has (22*R*,25*S*) according to equation (2).

Having the stereochemically defined model compounds (**7**)—(**10**) in hand, we then compared their ^{1}H n.m.r. (270 MHz in $CDCl_3$) spectra with that of TSL. The selected data are listed in the Table. The C-22 and C-25 stereochemistry of TSL was now unambiguously established to be 22*S*,25*R* on the basis of: (i) the coupling pattern (dd) for the 22-H resonance of TSL indicates a 22*S* configuration rather than 22*R*; (ii) the chemical shift (δ_H *ca.* 2.40) and coupling pattern (septet-like) of the 25-H resonance of TSL are similar to those of (22*S*,25*R*)- or (22*R*,25*S*)-isomers, rather than the other two isomers; (iii) the chemical shift (δ_H 1.30) for the C-27 methyl signal of TSL is in good agreement with those of (22*S*,25*R*)- and (22*R*,25*S*)-isomers.

Information on the conformation of these lactones was available by decoupling experiments (C-27 methyl signal was irradiated).[9] $J_{25\text{-H},24\text{-H}}$ Values are also included in the Table (see the column for 25-H). The *J* values (*ca.* 11.4 and 6.2 Hz) of compounds (**1**), (**7**), and (**9**) are quite reasonable for $J_{25\text{-H}_{ax},24\text{-H}_{ax}}$ and $J_{25\text{-H}_{ax},24\text{-H}_{eq}}$, respectively. On the other hand, the *J* values (*ca.* 7 Hz, triplet) of compounds (**8**) and (**10**) are understandable for $J_{25\text{-H}_{eq},24\text{-H}_{ax}}$ and $J_{25\text{-H}_{eq},24\text{-H}_{eq}}$, (the same values). It is therefore assumed that the lactone part of compounds (**1**), (**7**), and (**9**) exists in a half-chair conformation where the C-27 methyl group is located in an equatorial position as shown in structure (**I**), while that of compounds (**8**) and (**10**) exists in a half-chair conformation as well where the C-27 methyl group is in an axial position as shown in structure (**II**).‡



(**I**)



(**II**)

It follows that the C-25 stereochemical assignment deduced by the above mentioned conformational prediction supports the veracity of that obtained from equations (1) and (2).

Finally, ^{1}H n.m.r. data for the tetracyclic part [δ_H 3.45 (3-H_β), 3.83 (7-H_β), and 3.98 (12-H_β)] of TSL are in accord with a reference sample such as methyl cholate, thus confirming the previous structural assignment for the tetracyclic moiety.[2] In conclusion, the structure of TSL was established in the present study to be (**1**).

It is interesting to note that the bile acid of the bullfrog *Rana catesbeiana* contains both (25*R*)- and (25*S*)-isomers of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid with the former being predominant.[10] The stereochemistry of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid isolated from the frog *Bombina orientalis* has been reported to be (24*R*,25*S*).[10] In mammals it is reported that either the C-26 or C-27 methyl group is hydroxylated depending on the enzyme system (mitochondrial or microsomal fraction).[11] With respect to the C-22 position, the 22α-F (22*S*) stereochemistry is rather unusual because most of naturally occurring steroids bearing 22-hydroxy functionality such as 20,22-dihydroxycholesterol (an

* This type of hydrogenation [equation (2), R^1 = CH_2OAc, R^2 = Me] was first reported by Lavie *et al.*: D. Lavie, I. Kirson, E. Glotter, and G. Snatzke, *Tetrahedron*, 1970, **26**, 2221.

† Our preliminary work on the stereochemistry of these hydrogenations was presented at the 49th Chemical Society of Japan Spring meeting held in Tokyo, April, 1984; details on this subject will be published in due course.

‡ Compounds (**8**) and (**10**) contain the mirror images of structure (**I**) and (**II**).

Table. Selected ^{1}H n.m.r. data for TSL (**1**) and model compounds (**7**)—(**10**)[a]

Compound	22-H	25-H	27-H_3	21-H_3	18-H_3	19-H_3
TSL (**1**)	4.42 (dd, 11.7 and 3.2)	2.40[b] (ddq,[c] 11.5, 6.2 and 6.8)	1.30 (d, 6.8)	1.11 (d, 6.5)	0.87 (s)	0.68 (s)
(22*S*,25*R*)-(**7**)	4.39 (dd, 11.7 and 3.2)	2.39 (ddq,[c] 11.2, 6.1, and 6.8)	1.30 (d, 6.8)	0.98 (d, 6.5)	1.02 (s)	0.74 (s)
(22*S*,25*S*)-(**8**)	4.38 (dd, 11.7 and 2.8)	2.60 (tq,[d] 6.6 and 6.7)	1.22 (d, 6.7)	1.01 (d, 6.5)	1.02 (s)	0.72 (s)
(22*R*,25*S*)-(**9**)	4.37 (dt, 10.9 and 3.6)	2.39 (ddq,[c] 11.4, 6.5, and 6.8)	1.30 (d, 6.8)	0.94 (d, 6.5)	1.10 (s)	0.74 (s)
(22*R*,25*R*)-(**10**)	4.34 (dt, 8.9 and 4.5)	2.61 (tq,[d] 7.2 and 6.8)	1.22 (d, 6.8)	0.97 (d, 6.5)	1.12 (s)	0.75 (s)

[a] Chemical shifts in p.p.m. and coupling constants in Hz. [b] Upper part of the signal overlapped with other hydrogen signals. [c] Septet-like. [d] Sextet-like.

intermediate of cholesterol side-chain cleavage in mammals), ecdysteroids, withanolides, and antheridiol have 22β-F (22*R*) configuration, although brassinolide has 22α-F (22*R*) stereochemistry.

The ^{1}H n.m.r. spectrum of TISL indicated that TISL also has a (22*S*,24*R*) lactone moiety. The full structure of TISL is currently under investigation.

Experimental

General Directions.— M.p.s were determined on a Yazawa hot-stage microscope and are uncorrected. ^{1}H N.m.r. spectra were recorded on a Hitachi R-24A (60 MHz) or JEOL JNM-GX-270 (270 MHz) spectrometer for $CDCl_3$ solution with tetramethylsilane (TMS) as the internal reference. In the ^{1}H n.m.r. spectrum of 6β-methoxy-3α,5-cyclo compounds, data for cyclopropane protons (δ_H 0.3—0.7, m, 4-H_2) are not given. Column chromatography was performed on Merck Kieselgel 60 (70—230 mesh) and p.l.c. on Merck Kieselgel 60 F_{254} precoated plates (0.5 mm thickness). R_F values reported were obtained on Merck Kieselgel 60 F_{254} precoated plates (0.25 mm thickness). H.p.l.c. was performed with a Shimadzu LC-4A instrument equipped with a u.v. detector (240 nm) using a Zorbax ODS reversed-phase column (4.6 mm × 25 cm) and commercial methanol as an eluant at a flow speed of 1.0 ml min^{-1}. High-resolution mass spectra were obtained with a Hitachi M-80 spectrometer. Routine m.s. were obtained with a Shimadzu 9020 DF spectrometer.

Sample of TSL (**1**).—The bile of *Amyda japonica* (turtle) was collected by extraction of six gall-bladders with ethanol. The extract was evaporated to dryness under reduced pressure. The residue was dissolved in water and extracted with ethyl acetate after acidification with 2M-HCl. The ethyl acetate layer was washed with 2% aqueous Na_2CO_3 to remove acidic material and then with water until the washings became neutral, dried over anhydrous Na_2SO_4, and concentrated to dryness under reduced pressure. The residue (149 mg) was chromatographed on a column of silica gel (10 g) with mixtures of ethyl acetate and acetone as the eluant. The fractions containing a major component [R_F 0.25; ethyl acetate–acetone (7:3)] were combined and the solvent was evaporated to dryness under reduced pressure. Recrystallization of the residue (107 mg) from ethyl acetate gave crystals (59 mg), m.p. 220 °C. The compound isolated here was identified as TSL on the basis of comparison of the t.l.c. and g.l.c. behaviour, and of the i.r. and ^{1}H n.m.r. spectra with Amimoto's sample (m.p. 212 °C),[2] which was contaminated with a small amount of a less polar compound, as judged by t.l.c.

Ethyl (24E,22S)-22-*Hydroxy*-6β-*methoxy*-3α,5-*cyclo*-5α-*cholest*-24-*en*-26-*oate* (**3**) *and its* (22R)-*Epimer* (**4**).—HMPA (1.8 ml, 10.3 mmol) was added to a solution of LDA prepared from di-isopropylamine (1.4 ml, 9.96 mmol) and 1.4M-Bu^{n}Li–hexane (7.8 ml, 11.0 mmol) in THF (26 ml) at −78 °C. Then ethyl tiglate [ethyl (*E*)-2-methylbut-2-enoate] (1.2 ml, 8.64 mmol) was added and the mixture was stirred at the same temperature for 1 h. A solution of the aldehyde (**2**)[3] (1.58 g, 4.57 mmol) in THF (7 ml) was added and the mixture was stirred for 30 min at −78 °C and then warmed to room temperature during 2 h. Saturated aqueous NH_4Cl and ether were added, and the mixture was extracted with more ether. Chromatography of the residual oil (obtained on evaporation of the extract) afforded the *less polar product* (**3**) [972 mg, 45%; eluted with hexane-ethyl acetate (6:1)] as an oil, and the *more polar product* (**4**) [361 mg, 17%; eluted with hexane–ethyl acetate (5:1)] as an oil.

For compound (**3**) (Found: M^+, 472.3577. $C_{30}H_{48}O_4$ requires M, 472.3554); m/z 472 (M^+), 457, 432, 417, 400, 377, 313, 255, and 128 (base peak); δ_H (*inter alia*) 0.73 (3 H, s, 19-H_3), 0.955 (3 H, d, J 6 Hz, 21-H_3), 1.026 (3 H, s, 19-H_3), 1.302 (3 H, t, J 7 Hz, OCH_2CH_3), 1.87 (3 H, s, 27-H_3), 2.78 (1 H, m, 6-H), 3.34 (3 H, s, OMe), 3.839 (1 H, dd, J 8.5 and 5 Hz, 22-H), 4.195 (2 H, q, J 7 Hz, OCH_2CH_3), and 6.81 (1 H, t, J 7 Hz, 24-H).

For compound (**4**) (Found: M^+, 472.3591); the mass spectrum was the same as for (**3**); δ_H (*inter alia*) 0.77 (3 H, s, 18-H_3), 0.987 (3H, d, J 6.5 Hz, 21-H_3), 1.028 (3 H, s, 19-H_3), 1.300 (3 H, t, J 7 Hz, OCH_2CH_3), 1.86 (3 H, s, 27-H_3), 2.78 (1 H, m, 6-H), 3.33 (3 H, s, OMe), 3.82 (1 H, dt, J 6.5 and 3.5 Hz, 22-H), 4.195 (2 H, q, J 7 Hz, OCH_2CH_3), and 6.85 (1 H, t, J 7 Hz, 24-H).

H.p.l.c. analysis of the crude products showed that the ratio of compounds (**3**) and (**4**) was 3.2:1 [retention times for (**3**) and (**4**) were 7.5 and 9.6 min, respectively].

Ethyl (22S,25ξ)-22-*Hydroxy*-6β-*methoxy*-3α,5-*cyclo*-5α-*cholestan*-26-*oate* (**5**).—A mixture of the unsaturated ester (**3**) (822 mg, 1.74 mmol) and 10% Pd–C (300 mg) in ethyl acetate (35 ml) was stirred under hydrogen overnight. The catalyst was removed by passage through a short column of silica gel, and evaporation of the filtrate gave the *saturated ester* (**5**) (720 mg, 87%) as an oil (Found: M^+, 474.3670. $C_{30}H_{50}O_4$ requires M, 474.3711); m/z 474 (M^+), 459, 442, 419, 403, 398, 377, 284, 255, and 253; δ_H (*inter alia*) 0.73 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.25 (2 H, t, J 6.5 Hz, OCH_2CH_3), 2.73 (1 H, m, 6-H), 3.31 (3 H, s, OMe), 4.13 (2 H, 2 q with slightly different chemical shifts, J *ca.* 6.5 Hz, OCH_2CH_3).

Ethyl (22R,25ξ)-22-*Hydroxy*-6β-*methoxy*-3α,5-*cyclo*-5α-*cholestan*-26-*oate* (**6**).—The unsaturated ester (**4**) (334 mg, 0.705 mmol) was hydrogenated in the same manner as described for compound (**3**) to give the *saturated ester* (**6**) (289 mg, 86%) as an oil (Found: M^+, 474.3656); the mass spectrum was the same as for compound (**5**); δ_H 0.73 (3 H, s, 18-H_3), 1.03 (3 H, s, 19-H_3), 1.25 (3 H, t, J 6.5 Hz, OCH_2CH_3), 2.73 (1 H, m, 6-H), 3.31 (3 H, s, OMe), and 4.10 (2 H, 2 q with slightly different chemical shifts, J *ca.* 6.5 Hz, OCH_2CH_3).

(22S,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*cholestano*-26,22-*lactone* (**7**) *and its* (22S,25S)-*Isomer* (**8**).—A mixture of the saturated ester (**5**) (550 mg, 1.16 mmol) and 10% KOH–methanol (4 ml) was heated at *ca.* 50 °C for 30 min, and was then cooled to room temperature. 2M-HCl (6 ml) and ethyl acetate (10 ml) were added and the mixture was stirred for 5 min. The mixture was extracted with ethyl acetate, washed with brine, dried over $MgSO_4$, and evaporated to give an oily residue. The oil was dissolved in ethyl acetate containing a catalytic amount of *p*-TsOH·H_2O, and the solution was concentrated on a rotary evaporator. This operation was repeated twice more. The resulting residue was chromatographed on silica gel to give a lactone fraction [182 mg, 37%; eluted with hexane–ethyl acetate (7:1); partly solidified], which contained the *less polar lactone* (**7**) [R_F 0.315, hexane–ethyl acetate 4:1)] and the *more polar lactone* (**8**) [R_F 0.28, hexane–ethyl acetate (4.:1)]. The two components were separated by p.l.c. to afford 3 mg of each from 7 mg of the lactone mixture.

Compound (**7**) had m.p. 152—153 °C with sparkling at *ca.* 140 °C (from methanol) (Found: M^+, 428.3269. $C_{28}H_{44}O_3$ requires *M*, 428.3292); *m/z* 428 (M^+), 413, 396, 373, and 43 (base peak); ^{1}H n.m.r. data are listed in the Table. Additional signals were: δ_H 2.75 (1 H, m, 6-H) and 3.31 (3 H, s, OMe). Since these two signals were essentially the same for the lactone isomers (**7**)—(**10**), the data are not given in the respective columns in the Table.

Compound (**8**) was an oil (Found: M^+, 428.3300); the mass spectrum was the same as for compound (**7**).

(22R,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*cholestano*-26,22-*lactone* (**9**) *and its* (22R,25R)-*Isomer*.—The saturated ester (**6**) (300 mg, 0.632 mmol) was hydrolysed and cyclized in the same manner as described for compound (**5**) to give a lactone fraction (95 mg, 35%) which contained the *less polar* lactone [R_F 0.28, hexane–ethyl acetate (4:1)] and the *more polar lactone* (**10**) [R_F 0.24, hexane–ethyl acetate (4:1)]. The two isomers were separated by p.l.c.

Compound (**9**) was an oil (Found: M^+, 428.3305. $C_{28}H_{44}O_3$ requires M^+, 428.3292).

Compound (**10**) was an oil (Found: M^+, 428.3279). The mass spectra of compounds (**9**) and (**10**) were the same as for compound (**7**).

(22S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*cholest*-24-*eno*-26,22-*lactone* (**13**).—A solution of the (22*S*)-lactones (**7**) and (**8**) (50 mg, 0.117 mmol) in dry THF (1 ml) was added to a solution of LDA [prepared from di-isopropylamine (60 μl, 0.428 mmol) and 1.5M-Bu^{n}Li–hexane (0.30 ml, 0.45 mmol)] in dry THF (1 ml) at −70 °C. After the mixture had been stirred at the same temperature for 1 h, a solution of diphenyl disulphide (100 mg, 0.32 mmol) in dry THF (1 ml) and HMPA (0.10 ml) was added. The mixture was stirred at −70 °C for 30 min and then at room temperature for 30 min. Addition of aqueous NH_4Cl, followed by extractive (ethyl acetate) work-up, gave the crude product which was purified by silica gel chromatography [eluted with hexane–ethyl acetate (10:1)] to give the sulphide (**11**) (*inter alia*) (92 mg, 75%), δ_H 0.72 (3 H, s, 18-H_3), 1.02 (3 H, s, 19-H_3), 1.45 (3 H, s, 27-H_3), 2.76 (1 H, m, 6-H), 3.32 (3 H, s, OMe), 4.87 (1 H, m, 22-H), and 7.40 (5 H, m, Ph).

A mixture of compound (**11**) (66 mg, 0.123 mmol) and MCPBA (70% purity; 32 mg, 0.13 mmol) in $CHCl_3$ (10 ml) was stirred for 30 min and then aqueous $NaHCO_3$ was added. Extractive (ethyl acetate) work-up gave a crude sulphoxide, which was heated (no solvent) at 100 °C for 10 min. Chromatographic purification [eluted with hexane–ethyl acetate (7:1)] of the crude product gave the *unsaturated lactone* (**13**) (19 mg, 40%), δ_H (*inter alia*) 0.71 (3 H, s, 18-H_3), 1.01 (3 H, s, 19-H_3), 1.88 (3 H, br s, 27-H_3), 2.73 (1 H, m, 6-H), 3.30 (3 H, s, OMe), 4.45 (1 H, dd, *J* 12 and 4 Hz, 22-H), and 6.58 (1 H, m, 24-H) (Found: M^+, 426.3174. $C_{28}H_{42}O_3$ requires M^+, 426.3136); *m/z* 426 (M^+), 411, 394, 379, and 371 (base peak); R_F 0.46 [hexane–ethyl acetate (7:1); developed four times].

(22R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*cholest*-24-*eno*-26,22-*lactone* (**14**).—When a mixture of the (22*R*)-lactones (**9**) and (**10**) (59 mg) was treated in the same manner as described above, the *unsaturated lactone* (**14**) (34 mg, 48%) was obtained as an oil (Found: M^+, 426.3116); δ_H (*inter alia*) 0.73 (3 H, s, 18-H_3), 0.97 (3 H, d, *J* 7 Hz, 21-H_3), 1.01 (3 H, s, 19-H_3), 1.88 (3 H, br s, 27-H_3), 2.73 (1 H, m, 6-H), 3.31 (3 H, m, OMe), 4.33 (1 H, dt, *J* 12 and 4 Hz, 22-H), and 6.58 (1 H, m, 24-H); R_F 0.43 [hexane–ethyl acetate (7:1); developed four times]. The mass spectrum of compound (**14**) was the same as for its epimer (**13**).

The ^{1}H n.m.r. data for the intermediate (**12**) were (*inter alia*) δ_H 0.74 (3 H, s, 18-H_3), 0.93 (3 H, d, *J* 7 Hz, 21-H_3), 1.01 (3 H, s, 19-H_3), 1.45 (3 H, s, 27-H_3), 2.73 (1 H, m, 6-H), 3.30 (3 H, s, OMe), 4.78 (1 H, m, 22-H), and 7.38 (5 H, m, Ph).

Hydrogenation of the (22S)-*Unsaturated Lactone* (**13**).—A mixture of compound (**13**) (19 mg), $NaHCO_3$ (13 mg), and 10% Pd–C (10 mg) in dioxane (2.0 ml) was stirred under hydrogen overnight. Removal of the catalyst through a short column of silica gel and evaporation of the filtrate afforded an oily saturated lactone (18 mg, 98%). The t.l.c. mobility and ^{1}H n.m.r. spectrum of the product were identical with those of the more polar lactone (**8**). Very minor formation of the less polar lactone (**7**). (*ca.* 5%) was also noted (t.l.c.).

Hydrogenation of the (22R)-*Unsaturated Lactone* (**14**).—The unsaturated lactone (**14**) (26 mg) was similarly hydrogenated to give a saturated lactone (27 mg, 100%), which was identified as the more polar lactone (**10**) by t.l.c. and ^{1}H n.m.r. spectroscopy. Very minor formation of the less polar lactone (**9**) (*ca.* 5%) was indicated by t.l.c.

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