Sterols in Marine Invertebrates. Part 57.[1] Stereostructure, Synthesis, and Acid-catalysed Isomerization of Hebesterol—A Biosynthetically Significant Cyclopropyl-containing Marine Sterol

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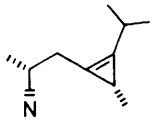
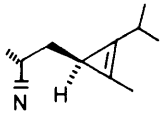
Three new cyclopropane-containing sterols hebesterol (**11**), petrostanol (**8**), and 23,24-dihydro-5α-calystanol (**10**) have been isolated from the sponge, *Petrosia hebes*, together with the principal sterol, petrosterol (**7**), and 21 known sterols. The structure elucidation of the new trace sterols was accomplished by spectroscopic methods and partial synthesis. Hebesterol (**11**) and its *trans*-diastereoisomers (**31**)—(**33**) have been synthesized and their acid-catalysed cyclopropane ring-opening studied. Each hebesterol isomer (**11**), (**31**)—(**33**) was correlated with the recently synthesized ficisterol isomers (**5**), (**53**), (**56**), and (**58**) of known absolute stereochemistry, thus leading to an unambiguous assignment of the absolute stereochemistry of hebesterol. The other products from the acid-catalysed isomerizations were characterized by spectroscopic methods, synthesis, and mechanistic considerations. Two of these products, (23*R*)-23-ethyldesmosterol (**51**) and (23*S*)-23-ethyldesmosterol (**59**), provided a relay to the absolute stereochemistry of the 23-ethylcholestanols, which had previously been synthesized without assignment of absolute stereochemistry. Hebesterol (**11**) is the key 'missing link' with the predicted stereochemistry in a proposed biosynthetic sequence encompassing the unusual marine sterols dihydrocalysterol (**9**), petrosterol (**7**), and ficisterol (**5**).

In recent years, lower marine organisms, notably sponges, have proven to be an extraordinarily rich source of unusual sterols.[2] One unique class is represented by marine sterols with a cyclopropane or cyclopropene ring in the side-chain. With the exception of the cyclopropene-containing calysterols (**1**)—(**3**),[3,4] all of the naturally occurring cyclopropanes have now been synthesized[5] and their stereochemistry established. Two outstanding questions remain. What is their biosynthetic origin and what, if any, biological role do they play? The same questions can be raised about another unique group of marine sterols, the 27-norergostanes [of type formula (**4**)][6], and especially the 23-ethyl-27-norergostane ficisterol (**5**),[7] which represents the only naturally occurring sterol with a 23-ethyl substituent.


(**1**)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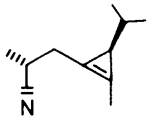
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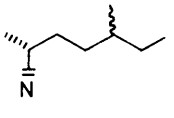
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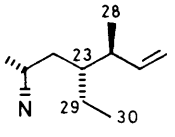
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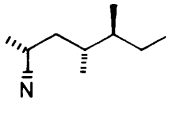
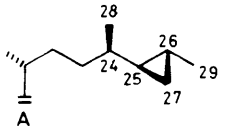
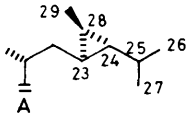
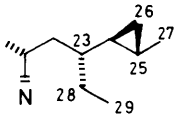

(**6**)


(**7**) A = N
(**8**) A = S

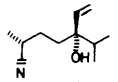
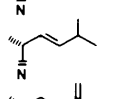
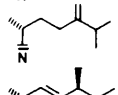
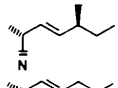
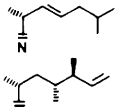
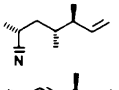
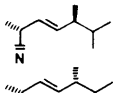
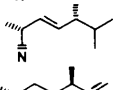
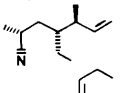
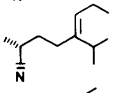
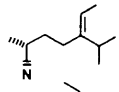
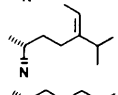
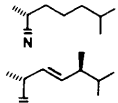
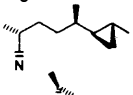
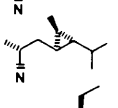
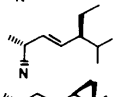
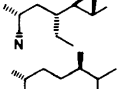
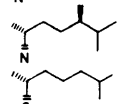
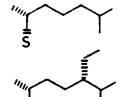
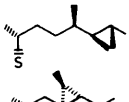
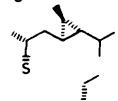
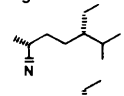
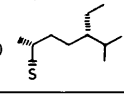

(**9**) A = N
(**10**) A = S


(**11**)

So far, no specific biological activity has been associated with marine sterols. However, we have proposed[8] that they may not just be metabolic end-products, but may also act as intermediates in bioalkylation processes. Even more likely is a functional role for those marine sterols that are present in significant amounts and which constitute the bulk of the sterol mixture. In those cases, it is extremely likely that such sterols replace cholesterol as a cell-membrane constituent[9] and work is currently underway in our laboratory to test this hypothesis with model membranes derived from synthetic sponge lipids.[10] Among cyclopropyl-containing sterols, petrosterol (**7**)[11] is the most likely candidate for a functioning cell-membrane component because it is the principal sterol[12] in the Mediterranean sponge *Petrosia ficiformis*. A detailed analysis[13] of the trace sterols accompanying petrosterol in that sponge proved to be productive in that a variety of unusual sterols, notably ficisterol (**5**) and its lower homologue norficisterol (**6**), were also isolated.[7] Consequently, we became interested in examining other *Petrosia* species. We now report our results with the New Zealand sponge *Petrosia hebes*, which led to some intriguing biosynthetic speculations.

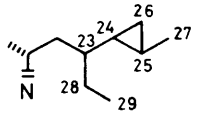
Column chromatography of the total sterol mixture of *Petrosia hebes*, followed by reverse-phase h.p.l.c. using different solvent systems, afforded 25 sterols as summarized in Table 1. The principal sterol, constituting over 50% of the total mixture, was again petrosterol (**7**). Dihydrocalysterol (**9**), which we believe to be the key biosynthetic intermediate,[14] was also present in significant amounts. Two new sterols, the saturated analogue 5α-petrostanol (**8**) and 23,24-dihydro-5α-calystanol (**10**), were also encountered. They were easily characterized through the diagnostic n.m.r. signals (Table 2), associated with their unique side-chain substitution patterns, and through the fact that they were identical with the previously described catalytic hydrogenation products of petrosterol (**7**)[1] and of 23,24-dihydrocalysterol (**9**).[3b] In addition, a new cyclopropane-containing sterol, now named hebesterol, was isolated whose structure elucidation, stereochemistry, synthesis, acid-catalysed isomerization, and potential key biosynthetic role constitute the subject of this paper.

Table 1. Sterols in *Petrosia hebes*

Structure	*M*	%	Rel. retent. times H.p.l.c.	G.c.
(12) 	428	1.2	0.30	
(13) 	370	0.4	0.68	0.65
(14) 	398	0.3	0.75	1.29
(15) 	384	1.0	0.80	0.86
(16) 	384	1.9	0.81	0.92
(6) 	398	0.3	0.85	1.25
(17) 	398	5.8	0.90	1.12
(18) 	398	0.3	0.93	1.12
(5) 	412	1.3	0.93	1.57
(19) 	426	1.3	0.97	1.97
(20) 	412	6.0	1.0	1.83
(21) 	412	2.1	1.0	1.75
(22) 	386	1.6	1.0	1.0
(23)	400	0.6	1.0	1.11
(7) 	412	50.6	1.06	1.57
(9) 	412	4.6	1.06	1.37
(24) 	412	0.3	1.06	1.37
(11) 	412	0.5	1.11	1.61
(25) 	400	4.5	1.11	1.30
(26) 	388	0.2	1.11	1.00
(27)	414	1.0	1.15	1.80
(8) 	414	2.6	1.15	1.53
(10) 	414	0.5	1.15	1.36
(28) 	414	10.7	1.22	1.58
(29) 	416	trace	1.38	1.55

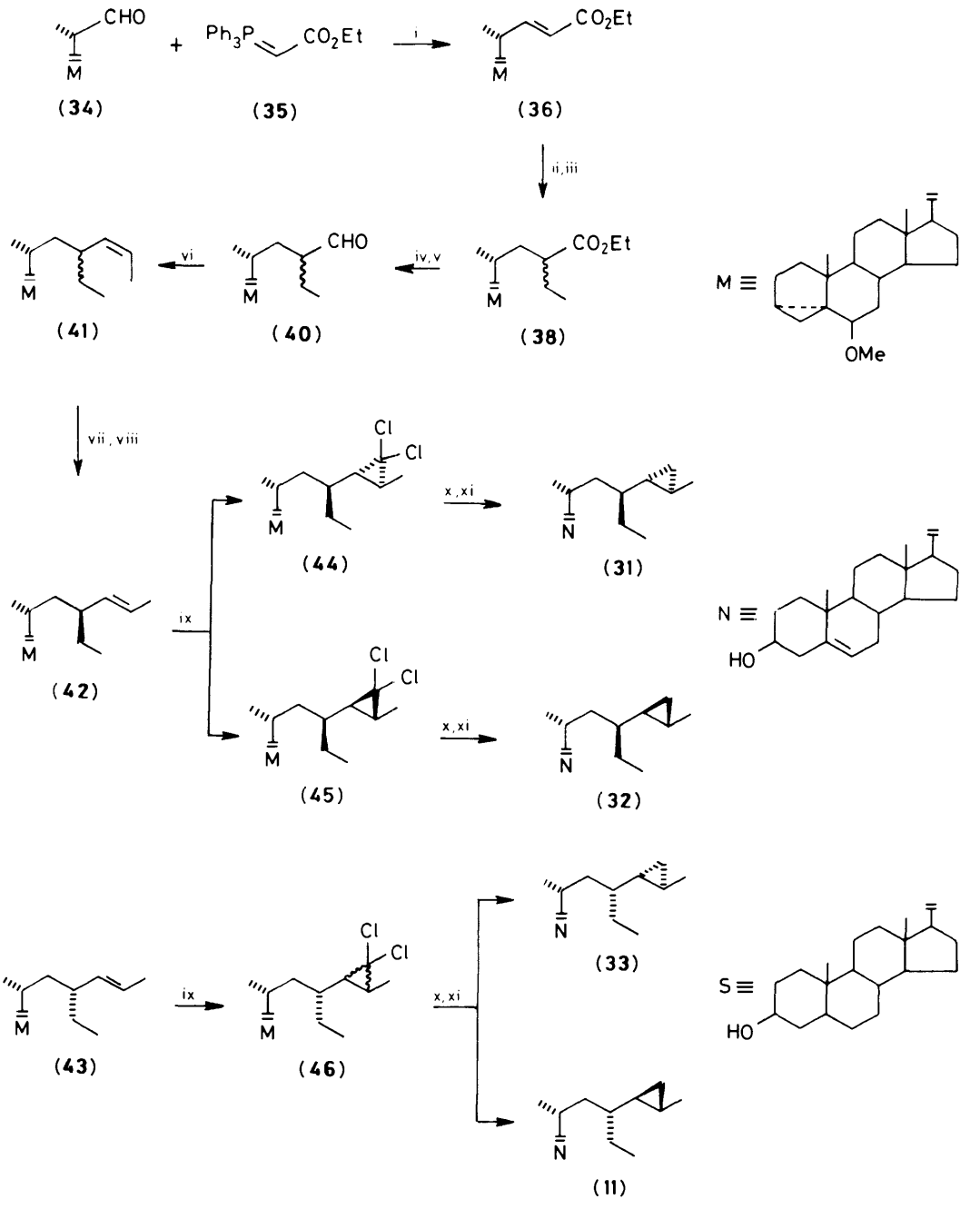
Structure Elucidation of Hebesterol (**11**).—Hebesterol (**11**) showed a molecular ion at *m/z* 412.3719 ($C_{29}H_{48}O$) and a fragmentation pattern typical[15] of a cholesterol nucleus (fragment ions at *m/z* 213, 231, 253, and 271). Diagnostic fragmentation peaks[16,17] at *m/z* 314 ($M - C_7H_{14}$) and 300 suggested the presence of unsaturation around C-24 in the side-chain. The 300 MHz ^{1}H n.m.r. spectrum (Table 2) of hebesterol (**11**) displayed two 2-proton resonances at highfield, indicative of a disubstituted cyclopropane ring system like petrosterol (**7**). Two doublets due to two secondary methyl groups (C-21, C-27), one triplet at 0.931 p.p.m. (*J* 7.4 Hz) associated with the ethyl group, and the 18- and 19-methyl substituents were also apparent.

Selective irradiation of the triplet at 0.931 p.p.m. changed none of the signals listed in Table 2, indicating that the ethyl substituent was separated from the cyclopropane ring and the two secondary methyl substituents. Irradiation of the doublet at 0.809 p.p.m. similarly changed none of the other signals, indicating that the methyl group associated with this doublet is separated from the cyclopropane ring and the ethyl group. Therefore, we attribute it to the C-21 methyl substituent. Irradiation of the multiplet (two cyclopropyl protons) at 0.55 p.p.m. simplified the methyl group doublet at 1.015 p.p.m. and the multiplet around 0.01 p.p.m., implying that one of these two cyclopropyl protons is adjacent to the methyl substituent. Finally, selective irradiation of the multiplet (two cyclopropyl protons) at 0.10 p.p.m. simplifed only the multiplet at 0.55 p.p.m., indicating that these two cyclopropyl protons correspond to C-26. These data, along with basic biosynthetic considerations[14,18] [*viz.*, its possible origin from 23,24-dihydrocalysterol (**9**)] led to the tentative formulation of hebesterol as 23-ethyl-24,25-cyclocholest-5-en-3β-ol (**30**) without any stereochemical assignmments. In view of the minute quantity (<1 mg) of the natural sterol, its synthesis was undertaken to confirm the proposed structure and to provide evidence for absolute configurational assignments.



(**30**)

Synthesis of Hebesterol.—The synthesis of hebesterol (**11**) and its diastereoisomers (**31**), (**32**), and (**33**) was accomplished as shown in Scheme 1. The stabilized phosphorane (**35**)[19] was subjected to a Wittig condensation with the known aldehyde (**34**)[20] to afford exclusively the *trans*-α,β-unsaturated ester (**36**) in 87% yield. Catalytic reduction and subsequent α-ethylation[21] using lithium di-isopropylamide and ethyl iodide in THF–10% hexamethylphosphoramide gave the 23-ethylated ester (**38**) as a diastereoisomeric mixture, which was converted into the α-ethyl aldehyde (**40**) (diastereoisomeric mixture) by lithium aluminium hydride reduction and pyridinium chlorochromate oxidation. A Wittig reaction of the isomeric aldehyde mixture (**40**) with ethylidenetriphenylphosphorane provided the *cis*-olefin mixture (**41**), which was epoxidized (*m*-chloroperbenzoic acid) and the resulting 24,25-epoxides deoxygenated[22] with lithium diphenylphosphide, followed by treatment with methyl iodide, to afford in 82% yield the *trans*-olefins (**42**) and (**43**) in a ratio of 10:7 with as yet unassigned stereochemistry at C-23. The diastereoisomer (**42**) underwent dichlorocarbene addition[23] to produce the two dichlorocyclopropanes (**44**) and (**45**) which were separated readily by reverse-phase h.p.l.c. Each was then transformed to the corresponding hebesterol isomer by reductive removal of the chlorine atoms *via* lithium–ammonia reduction, and regeneration of the Δ^5-3β-hydroxy group with toluene-*p*-sulphonic acid in dioxane–water. The other olefinic diastereoisomer (**43**) was



Scheme 1. Synthesis of four isomers of hebesterol. *Reagents and conditions:* i, toluene, reflux, 87%; ii, 10% Pd/C, EtOAc, 100%; iii, LDA, EtI, THF 10% HMPA, −70 °C ⟶ room temp., 85%; iv, $LiAlH_4$, ether, 88%; v, PCC, CH_2Cl_2, 92%; vi, $Ph_3P{=}CHCH_3$, THF, 94%; vii, MCPBA, CH_2Cl_2; viii, Ph_2PLi, THF, MeI, overall, vii, viii 78%; (**38**)/(**39**) = 10:7; ix, $CHCl_3$, 50% NaOH, BTEAC, (**40**), 39%, (**41**), 43% (**42**), 82%; x, Li/NH_3; xi, PTSA, dioxane–H_2O, overall x, xi 70%

subjected to the same transformation, and behaved similarly, except that the reverse-phase h.p.l.c. isomer separation did not succeed at the (**46**) stage, but only after removal of the chlorine atoms. Hydrolytic cleavage of the i-methyl ether protecting group in the nucleus then provided the other two pure hebesterol isomers (**33**) and (**11**). The ^1H n.m.r. spectra of the four hebesterol isomers (**11**), (**31**)—(**33**), one of which was identical with natural hebesterol, are summarized in Table 2.

Stereochemical Assignments through Cyclopropane Ring Openings.—Even though hebesterol (**11**) and its three *trans*-diastereoisomers (**31**)—(**33**) had now been synthesized, their absolute stereochemistry was still undetermined. In order to assign the absolute stereochemistry of hebesterol (**11**) and its diastereoisomers (**31**)—(**33**), *X*-ray crystallographic methods or chemical transformations into sterols of known absolute stereochemistry were necessary. As shown below in Scheme 7, the structure of hebesterol seemed to be a tempting biosynthetic precursor to that of ficisterol (**5**), whose absolute stereochemistry had been determined recently in our laboratory.[24] Therefore, we resorted to an examination of the acid-catalysed cyclopropane ring-opening of hebesterol (**11**) and its three isomers (**31**)—(**33**) in the hope that it would provide stereochemical information and at the same time constitute a biomimetic route to the unique side-chain of ficisterol (**5**).

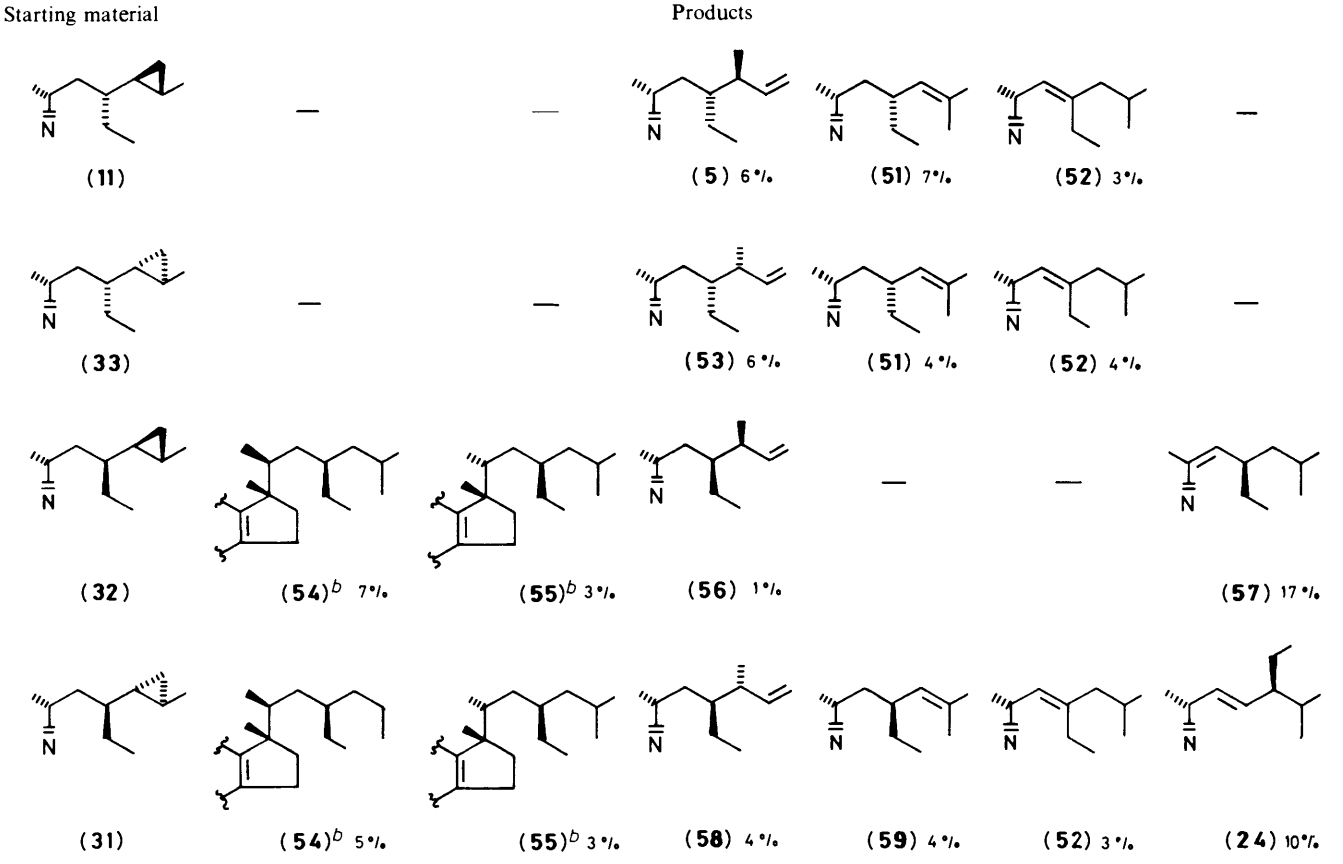
As reagent we selected 5% trifluoroacetic acid[25] in benzene,

Table 2. Selected 300 MHz ^{1}H n.m.r. data for the cyclopropyl sterols[a]

	C-18	C-19	C-21	C-27	C-29	Cyclopropane protons
(**8**)[b]	0.646	0.798	0.898 (d, 6.43)	0.887 (d, 6.33)	1.000 (d, 5.97)	0.06—0.20, 0.40—0.50, 0.55—0.65
(**8**)[c]	0.648	0.800	0.901 (d, 6.34)	0.883 (d, 6.54)[d]	0.997 (d, 5.92)	0.05—0.20, 0.40—0.50, 0.55—0.65
(**10**)[b]	0.656	0.802	1.003 (d, 6.94)	0.927/0.930[e]	0.986 (d, 5.74)	0.40—0.50, −0.18—(−0.07)
(**10**)[c]	0.655	0.802	1.003 (d, 6.20)	0.926[f]	0.985 (d, 5.71)	0.40—0.50, −0.18—(−0.07)
(**11**)[b]	0.678	1.005	0.809 (d, 6.44)	1.015 (d, 6.17)	0.931 (t, 7.37)	0.45—0.60, 0.05—0.15
(**11**)[c]	0.678	1.005	0.810 (d, 6.44)	1.015 (d, 6.24)	0.931 (t, 7.37)	0.45—0.60, 0.05—0.15
(**33**)[c]	0.683	1.006	0.830 (d, 6.44)	1.001 (d, 5.50)	0.897 (t, 7.40)	0.15—0.40, 0.50—0.65
(**31**)[c]	0.694	1.010	0.856 (d, 6.31)	1.008 (d, 5.53)	0.884 (t, 7.52)	−0.05—0.45, 0.50—0.65
(**32**)[c]	0.722	1.011	0.865 (d, 6.85)	1.015 (d, 5.44)	0.853 (t, 7.37)	−0.05—0.20, 0.40—0.57, 0.61—0.70

[a] Chemical shifts are given in p.p.m., J values (in parentheses) are in Hz. [b] Natural sterol. [c] Synthetic sterol. [d] Not C-27 but C-28. [e] J Value is 5.70 Hz. [f] The J value could not be determined, because of extensive peak broadness.

Table 3. Acid-catalysed isomerization of hebesterol (**11**), (**31**), (**32**), and (**33**)[a]



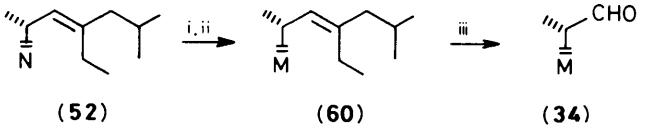
[a] Recovered starting material 30—40%; unidentified isomerization products 5—10%; the remainder of the reaction mixture consisted of hydroxy sterols which were not investigated. [b] The assignments may be interchanged.

which had served earlier in the acid-catalysed opening of 22,23-methylenecholesterol[25a] and petrosterol.[25b] Since the products were trifluoroacetates, they were re-converted to the free sterols by reaction with $LiAlH_4$ prior to separation and characterization. After 3—6 days at room temperature, 20—30% yields of isomerization products and 30—40% of recovered starting materials were encountered. The remainder consisted of products derived from addition of trifluoroacetic acid to the cyclopropane ring. The olefins, separated by reverse-phase h.p.l.c., and structurally characterized by spectroscopic, degradative and/or synthetic methods, are listed in Table 3.

Starting with hebesterol (**11**), three isomerization products were isolated and characterized. One of these was ficisterol (**5**), which arose by loss of the C-27 proton exactly as expected by analogy to the acid-catalysed ring-opening of petrosterol (**7**).[25b,26] Therefore, the absolute stereochemistry of natural hebesterol (**11**) can now be defined as 23*R*, 24*S*, 25*S*. The second product, (**51**), is derived directly from the carbonium ion at C-24 produced by Markownikoff cleavage of the C(24)—C(26) bond. Although hitherto unknown, the sterol (**51**) could be assigned the stereostructure (23*R*)-23-ethyldesmosterol based on n.m.r. and mass spectra. The ^{1}H n.m.r. spectra displayed a broad doublet (1 H) in the olefinic region (4.86 p.p.m.), two vinylic methyl groups (1.684 and 1.599 p.p.m.), quite similar to those of desmosterol, a doublet (0.921 p.p.m.) assignable to the C-21 methyl group, and an apparent triplet (J 7.3 Hz) at 0.784 p.p.m.

which arose from the 23-ethyl group. Diagnostic mass spectral fragmentation peaks at *m/z* 314 and 300 are consistent with a McLafferty rearrangement which is very common in desmosterol derivatives.[16,17]

The third product of this isomerization of hebesterol (**11**) was assigned the structure of (22*E*) 23-ethylcholesta-5,22-dien-3β-ol (**52**), which arose from a 1,2-hydride shift. Its n.m.r. and mass spectra were consistent with those of (22*E*)-23-methylcholesta-5,22-dien-3β-ol.[27] The unsaturation position of (**52**) was confirmed by the degradation outlined in Scheme 2. This sterol



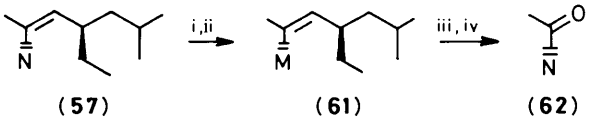
(**52**) (**60**) (**34**)

Scheme 2. i, TsCl, pyridine; ii, $MeCO_2K$, MeOH; iii, O_3, Me_2S

(**52**) was converted into the i-methyl ether (**60**) by a standard method and subsequent ozonolysis gave the known (20*S*)-6β-methoxy-3α,5-cyclo-5α-pregnane-20-carbaldehyde (**34**).

The hebesterol isomer (**33**) behaved identically to natural hebesterol (**11**): the products were shown by ^1H n.m.r. and mass spectral measurements to be the olefins (**51**) and (**52**) together with (23*R*,24*R*)-ficisterol (**53**).[24] The latter's established stereochemistry proves that the hebesterol isomer (**33**) must possess the 23*R*,24*R*,25*R* stereochemistry.

The isomerization of the hebesterol isomer (**32**) showed major differences. The only similarity was the generation of (23*S*,24*S*)-ficisterol (**56**), whose established stereochemistry[24] proves the 23*S*,24*S*,25*S* stereochemistry in the starting material (**32**). The n.m.r. spectral data of the sterol (**57**) arising from a 1,3-hydride shift, displayed different patterns by comparison with those of normal sterols, notably the highfield position of the 0.575 p.p.m. singlet of its 18-methyl group compared to the 'usual' range (see Table 2). Such an upfield shift of the 18-methyl group is quite common in $\Delta^{20(22)}$ sterols,[25a,27] and is further supported by the



(**57**) (**61**) (**62**)

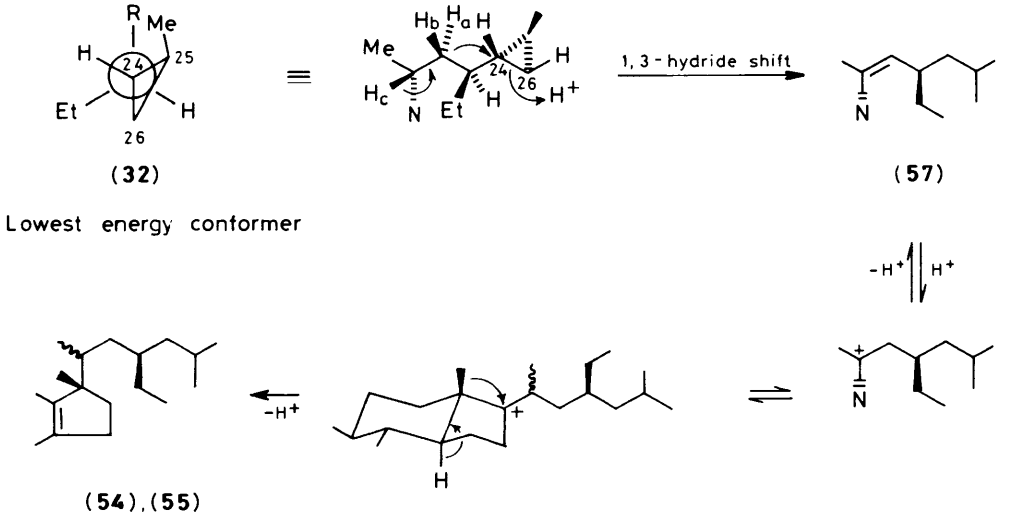
Scheme 3. i, TsCl, pyridine; ii, $MeCO_2K$, MeOH; iii, O_3, Me_2S; iv, PTSA, dioxane–H_2O

downfield shift of the 21-methyl group (doublet, *J* 0.9 Hz at 1.611 p.p.m.). Structure (**57**) was proved unambiguously by degradation (Scheme 3) to be 3β-hydroxypregn-5-en-20-one (**62**). Because the vinylic methyl group[25b] of *Z*-olefins consistently appears below 1.600 p.p.m., a doublet at 1.611 p.p.m. suggests the *Z*-configuration for the double bond of (**57**). This is also consistent with the Newman projection (Scheme 4) of the lowest-energy conformer of the hebesterol isomer (**32**), in which the C(24)–C(26) bond, H_a, and H_c retain a *trans*-antiperiplanar relationship, which is the optimum conformation for the generation of a (*Z*)-$\Delta^{20(22)}$ double bond.

The two remaining olefins, (**54**) and (**55**) were unexpected backbone rearrangement products of the 18-nor-methyl sterol type. Presumably they arose from (**57**) (Scheme 4) by the classical Kagi-Miescher rearrangement,[29] since exposure of the olefin (**57**) to the same acidic condition provided 29% of (**54**) and 38% of (**55**) in addition to 33% of recovered starting material. The n.m.r. spectra of these two isomers were quite similar to those of other 18-nor-methyl sterols,[30] in that they lacked olefinic proton (except for C-6) or 18-methyl signals. The mass spectra of (**54**) and (**55**) (see Experimental section) were also consistent with those of other 18-nor-methyl sterols.[25a,31]

The hebesterol isomer (**31**) was the most resistant one to acid isomerization and required 6 days, whereupon six major isomers were isolated (see Table 3). The sterols (**52**), (**54**), and (**55**) were characterized by comparison of their mass and n.m.r. spectra with those of the corresponding isomerization products of the hebesterol isomers (**33**) and (**32**). Isolation of (23*S*,24*R*)-ficisterol (**58**)[24] showed that this hebesterol isomer (**31**) possesses the 23*S*,24*R*,25*R* stereochemistry. Of particular interest and stereochemical relevance was the isolation of stigmasterol (**24**), which presumably arose by a 1,2-ethyl shift (Scheme 5). A Newman projection along the C(23)–C(24) bond shows that in the lowest-energy conformer, the 23-ethyl group is *trans*-antiperiplanar to the C(24)–C(26) cyclopropane bond, and thus ideally situated for a stereospecific 1,2-ethyl shift.

The sixth product (Table 3) was identified as (23*S*)-23-ethyldesmosterol (**59**) by the close similarity of its mass and n.m.r. spectra with those of (23*R*)-23-ethyldesmosterol (**51**). The assigned structures of the isomeric 23-ethyldesmosterols (**51**) and (**59**) were confirmed by synthesis as shown in Scheme 6. The aldehyde (**40**) was condensed with isopropylidenetriphenylphosphorane to yield, after reverse-phase h.p.l.c. separation, two isomeric 23-ethyldesmosterol i-methyl ethers in a ratio of 2:5. Subsequent hydrolysis of each pure i-methyl ether gave (23*S*)-

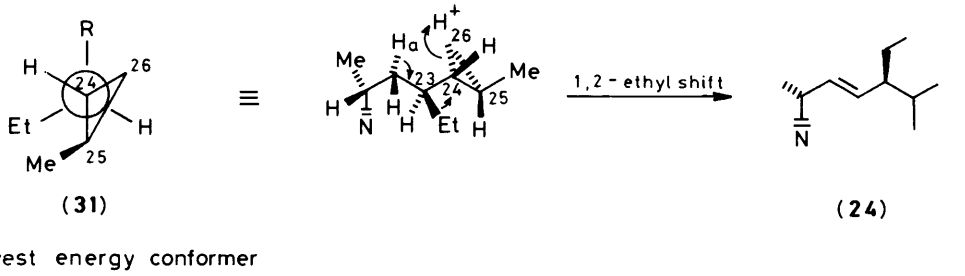


(**32**) (**57**)

Lowest energy conformer

(**54**), (**55**)

Scheme 4.

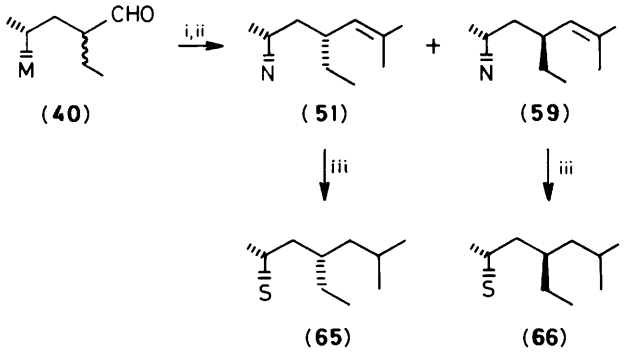


(31)

Lowest energy conformer

(24)

Scheme 5.



(40) (51) (59) (65) (66)

Scheme 6. Synthesis of 23-ethyldesmosterol (**51**), (**59**). i, $Ph_3P{=}CMe_2$, THF, 30 min, 95%; ii, PTSA, dioxane/H_2O, 99%; iii, PtO_2, H_2, EtOAc–$MeCO_2H$, 95%

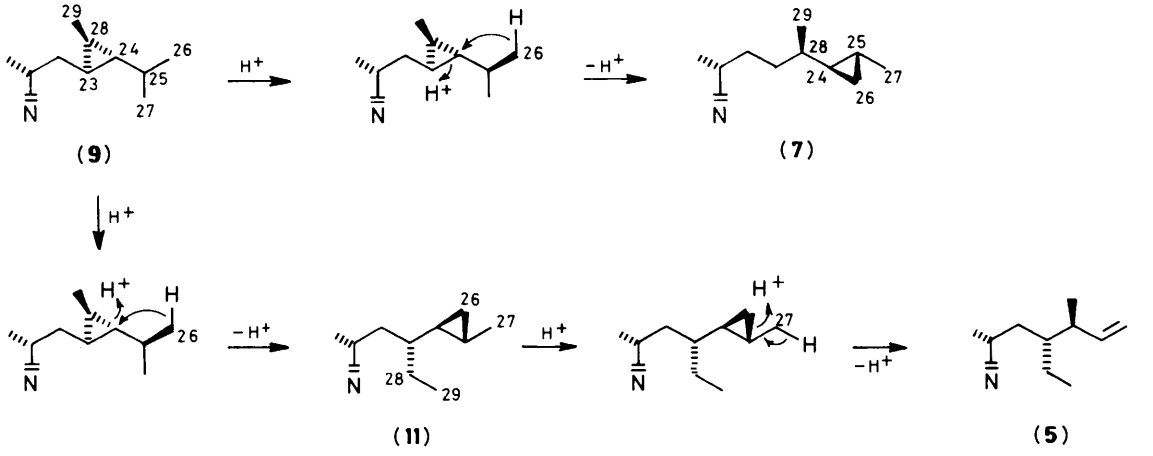
(**59**) and (23*R*)-23-ethyldesmosterol (**51**) in quantitative yield.

In addition, catalytic hydrogenation of the pure 23-ethyldesmosterols (**51**) and (**59**) produced in 95% yield their respective tetrahydro analogues (**65**) and (**66**). Although these 23-ethylcholestanols (**65**) and (**66**) had been synthesized[3b] previously in our laboratory, the absolute stereochemistry of the 23-ethyl group was not determined at that time. It is now possible to do so, since the established absolute stereochemistry of the 23-ethyl group of the starting materials (**51**) annd (**59**) is maintained during the catalytic hydrogenation.

Potential Biosynthetic Significance of Hebesterol.—Hebesterol (**11**) is both structurally and biosynthetically one of the most interesting marine sterols encountered to date. It is one of the few sterols[32] to carry a cyclopropane ring between C-24 and C-25 and is only the second example of a 23-ethylated sterol[7] from any source. Since the absolute stereochemistry of 23,24-dihydrocalysterol (**9**),[14] hebesterol (**11**), and ficisterol (**5**)[24] has now been shown by us to be identical and since all of these sterols co-occur in the same sponge, it is not unreasonable to postulate that they are biosynthetically related and derived, as outlined in the preceding paper,[14] from dihydrocalysterol (**9**). A common biosynthetic scheme for all of these unusual sterols, including petrosterol (**7**), is summarized in Scheme 7. In this scheme hebesterol (**11**) plays a key role as the presumed missing link in the biosynthesis of ficisterol (**5**). Successful incorporation of radioactively labelled dihydrocalysterol (**9**) or hebesterol (**11**) would be necessary to confirm these attractive postulates.

Experimental

General Methods.—High performance liquid chromatography (h.p.l.c.), used for preparative-scale separation of diastereoisomeric sterol mixtures as well as for monitoring of product purification, was carried out by using a Waters Associates HPLC system (M 6000 pump, R403 differential refractometer). For reverse-phase chromatography, we employed Altex Ultrasphere ODS 5 μm (25 cm × 10 mm i.d., two columns in series) with methanol as the mobile phase. The flow rate was 3.0 ml/min. Cholesterol was used as the standard for relative retention time (rel. R_t) in g.c. and h.p.l.c. ^1H N.m.r. spectra were recorded on a Nicolet 300 MHz instrument. All n.m.r. spectra were recorded in $CDCl_3$ with the solvent peak ($CHCl_3$, 7.259 p.p.m.) as an internal standard. Analytical gas-liquid chromatography (g.l.c.) was performed at 260 °C on a U-shaped column (1.8 m × 2 mm i.d.) packed with 3% OV-17. The column was mounted in a Hewlett Packard 402 high-efficiency gas chromatograph equipped with a flame ionization detector. Low-resolution mass spectra were recorded on a Finnigan MAT-44 GLC/MS system at 70 eV using a 3% OV-17 column as well as on a Hewlett Packard 5970 Series Mass system with a 5890A g.c. for sample introduction and a Hewlett Packard 9133 for data acquisition. High-resolution mass spectra were recorded



(**9**) (**7**) (**11**) (**5**)

Scheme 7. Biosynthetic pathways of cyclopropyl sterols

on a Finnigan MAT-711 double-focussing mass spectrometer with a direct-inlet system for sample introduction and a PDP-11/45 computer for data acquisition and reduction. M.p.s were determined on a Koffler hot-stage apparatus and are uncorrected. Specific rotations were recorded in chloroform at 20 °C on a Rudolph Research Autopol III automatic polarimeter equipped with a thermostatted 1.00-dm microcell. Commercial reagents and solvents were analytical grade or were purified by standard procedures[33] prior to use.

Extraction, Sterol Isolation, and Fractionation.—An air-dried sample (263 g) of the deep-sea cave sponge *Petrosia hebes* was collected near the northeastern coast of New Zealand in July of 1983, and extracted according to the method of Bligh and Dyer[34] with $CHCl_3$–MeOH–H_2O. The organic layer was concentrated under reduced pressure and the total extract was fractionated on an open silica-gel column (eluant: hexane–ether, 3:1). The sterol fractions (R_F, cholesterol by t.l.c.) were combined and evaporated under reduced pressure. The yield of free sterols was 1.7 g (0.6%). The sterol mixture was initially fractionated by h.p.l.c. on an ODS-2 column (mobile phase, MeOH) which yielded fraction A (10% of the total sterols, rel.-R_t 0.62—0.90 on ODS-2) and fraction B (90%, rel.-R_t 0.90—1.40). Repeated reverse-phase h.p.l.c. of fraction A (2 × Altex Ultrasphere, mobile phase MeOH) afforded six known (**13**)—(**17**) sterols. Further fractionation of fraction B by reverse-phase h.p.l.c. (2 × Altex Ultrasphere, mobile phase, MeOH or MeCN–EtOAc–MeOH = 22:9:9) eventually provided, along with 15 known (**5**), (**7**), (**9**), (**18**)—(**29**) sterols, three new cyclopropane-containing sterols, 5α-petrostanol (**8**), (23*S*,24*S*,-28*R*)-23,24-dihydro-5α-calystanol (**10**), and hebesterol (**11**).

5α-*Petrostanol* (**8**).—G.c. relative retention times (rel.-R_t) *vs.* cholesterol and h.p.l.c. rel.-R_t *vs.* cholesterol (Altex Ultrasphere ODS 5, MeOH) are presented in Table 1. ^{1}H N.m.r. (300 MHz) data are reported in Table 2; high-resolution mass spectrum: *m/z* (relative intensity) 414.3876 (M^+, 45, calc. for $C_{29}H_{50}O$ 414.3862), 399.3626 ($C_{28}H_{47}O$, 8), 357.3184 ($C_{25}H_{41}O$, 10), 316.2780 ($C_{22}H_{36}O$, 30), 301.2538 ($C_{21}H_{33}O$, 18), 273.2213 ($C_{19}H_{29}O$, 100), 233.1913 ($C_{16}H_{25}O$, 18), and 215.1800 ($C_{16}H_{23}$, 25). There was insufficient material to determine the melting point or optical rotation of natural petrostanol.

(23S,24S,28R)-5α-*Dihydrocalystanol* (**10**).—For ^{1}H n.m.r. (300 MHz) data, see Table 2; *m/z* (relative intensity) 414.3882 (M^+, 25. Calc. for $C_{29}H_{50}O$: 414.3862), 399.3650 ($C_{28}H_{47}O$, 5), 316.2766 ($C_{22}H_{36}O$, 25), 302.2608 ($C_{21}H_{34}O$, 13), 301.2545 ($C_{21}H_{33}O$, 21), 285.2576 ($C_{21}H_{33}$, 15), 273.2215 ($C_{19}H_{29}O$, 100), 255.2098 ($C_{19}H_{27}$, 14), 233.1903 ($C_{16}H_{25}O$, 13), and 215.1798 ($C_{16}H_{23}$, 19). G.c. and h.p.l.c. rel.-R_t are recorded in Table 1.

Hebesterol (**11**).—^{1}H N.m.r. (300 MHz) data are presented in Table 2; *m/z* (relative intensity) 412.3719 (M^+, 31, calc. for $C_{29}H_{48}O$ 412.3705), 397.3491 ($C_{28}H_{45}O$, 6), 394.3591 ($C_{29}H_{46}$, 7), 379.3337 ($C_{28}H_{43}$, 12), 314.2629 ($C_{22}H_{34}O$, 11), 301.2557 ($C_{21}H_{33}O$, 18), 300.2468 ($C_{21}H_{32}O$, 40), 283.2414 ($C_{21}H_{31}$, 15), 272.2125 ($C_{19}H_{28}O$, 31), 271.2070 ($C_{19}H_{27}O$, 100), 255.2103 ($C_{19}H_{27}$, 28), 253.1956 ($C_{19}H_{25}$, 18), 231.1747 ($C_{16}H_{23}O$, 13), 215.1783 ($C_{16}H_{23}$, 14), and 213.1632 ($C_{16}H_{21}$, 37). There was insufficient material to record either a melting point or an optical rotation of the natural hebesterol. The relevant data were, therefore, secured with synthetic material. G.c. and h.p.l.c. rel.-R_t data are summarized in Table 1.

General Procedure for the Hydrogenation of Petrosterol (**7**) *and* 23,24-*Dihydrocalysterol* (**9**).—A solution of the sterol (1—5 mg) in methanol (5 ml) was hydrogenated over platinum oxide (20 mg) for 3 days. Filtration and evaporation of the solvents gave the crude product which was purified by h.p.l.c. (mobile phase, MeOH); yield 60—70% based on the h.p.l.c. traces.

From (24*R*,25*R*,26*R*)-petrosterol (**7**) was obtained (24*R*,-25*R*,26*R*)-5α-petrostanol (**8**), m.p. 122—123 °C (MeOH); δ (300 MHz) data are presented in Table 2; *m/z* (relative intensity) 414.5 (M^+, 26), 394.4 (5), 316.3 (18), 301.4(13), 274.2 (30), 273.2 (100), 272.2 (23), 255.2 (23), 233.3 (19), 231.2 (11), 229.3 (10), 215.3 (42), and 213.2 (15).

From (23*S*,24*S*,28*R*)-23,24-dihydrocalysterol (**9**) was obtained (23*S*,24*S*,28*R*)-5α-dihydrocalystanol (**10**), m.p. 119—120 °C (MeOH); δ data are recorded in Table 2; *m/z* (relative intensity) 414.45 (M^+, 6), 399.30 (5), 317.20 (7), 316.20 (30), 303.20 (6), 302.20 (16), 301.20 (19), 285.20 (19), 223.20 (100), 257.20 (15), 255.20 (11), and 215.15 (15).

(22E)-*Ethyl* 6β-*Methoxy*-3α,5-*cyclochol*-22-*enate* (**36**).—A solution of the aldehyde (**34**)[20] (2.4 g, 7.0 mmol) and triphenylethoxycarbonylmethylenephosphorane (**35**) (5.2 g, 14.9 mmol) in toluene was heated under reflux in an argon atmosphere for 15 h. The reaction mixture was cooled and evaporated. Column chromatography over silica gel (eluant, hexane–ether, 12:1) gave the *trans*-α,β-unsaturated ester (**36**) (2.5 g) in 87% yield; δ (300 MHz) 6.826 (1 H, d, d, *J* 15.6, 9.0 Hz, 22-H), 5.728 (1 H, d, d, *J* 15.6, 0.6 Hz, 23-H), 4.170 (2 H, q, *J* 7.1 Hz, OCH_2CH_3), 3.317 (3 H, s, OCH_3), 1.280 (3 H, t, *J* 7.2 Hz, OCH_2CH_3), 1.082 (3 H, d, *J* 6.6 Hz, 21-H), 1.021 (3 H, s, 19-H), 0.749 (3 H, s, 18-H); *m/z* (relative intensity) 414.3151 (M^+, 28, calc. for $C_{27}H_{42}O_3$, 414.3123), 399.2911 ($C_{26}H_{39}O_3$, 52), 382.2880 ($C_{26}H_{38}O_2$, 71), 367.2623 ($C_{25}H_{35}O_2$, 11), 359.2577 ($C_{23}H_{35}O_3$, 100), 356.2694 ($C_{24}H_{36}O_2$, 16), 255.2103 ($C_{19}H_{27}$, 36), 253.1942 ($C_{19}H_{25}$, 13), and 213.1646 ($C_{16}H_{21}$, 12).

Ethyl 6β-*Methoxy*-3α,5-*cyclocholanate* (**37**).—A solution of the *trans*-α,β-unsaturated ester (**36**) (583 mg, 1.4 mmol) in ethyl acetate (30 ml) was hydrogenated over 10% Pd–C (77 mg) for 2 h. Filtration and evaporation of the solvents gave the crude product which was purified by silica gel column chromatography; yield 557 mg (95%); δ (300 MHz) δ 4.120 (2 H, q, *J* 7.2 Hz, OCH_2CH_3), 3.320 (3 H, s, OCH_3), 1.253 (3 H, t, *J* 7.2 Hz, OCH_2CH_3), 1.019 (3 H, s, 19-H), 0.922 (3 H, d, *J* 6.4 Hz, 21-H), and 0.716 (3 H, s, 18-H); *m/z* (relative intensity) 416.35 (M^+, 16), 402.20 (9), 401.20 (30), 385.20 (8), 384.20 (26), 369.20 (7), 362.20 (11), 361.20 (40), 358.20 (7), 339.20 (6), 255.20 (12), 213.05 (13), and 55.10 (100) (Found: M^+, 416.3293. $C_{27}H_{44}O_3$ requires M^+, 416, 3279).

Ethyl 23ξ-*Ethyl*-6β-*methoxy*-3α,5-*cyclocholanate* (**38**).—To a solution of di-isopropylamine (163 μl, 1.2 mmol) in dry tetrahydrofuran (THF) (2 ml) at 0 °C under an atmosphere of dry argon was added 1.6M butyl-lithium (731 μl, 1.2 mmol). After being stirred for 15 min, the solution was cooled to −70 °C and the ester (**37**) (485 mg, 1.2 mmol) in THF (1 ml) and hexamethylphosphoramide (HMPA) (0.8 ml) was added and stirred for 30 min. Ethyl iodide (138 μl, 1.6 mmol) was added to the reaction mixture which was then maintained at −70 °C for 10 min. The solution was warmed to room temperature over the course of 15 min after which the reaction was quenched by addition of water. Excess of water was removed with anhydrous magnesium sulphate, the slurry was filtered, and the solvents evaporated under reduced pressure to give the crude α-ethyl ester (**38**), which was purified by silica-gel column chromatography (eluant, hexane–ether 10:1); yield 440 mg (85%); δ (300 MHz) 4.128 (2 H, q, *J* 7.1 Hz, OCH_2CH_3), 3.320 (3 H, s, OCH_3), 1.255 (3 H, t, *J* 7.1 Hz, OCH_2CH_3), 1.017 (3 H, s, 19-H), 0.907 (3 H, d, *J* 6.2 Hz, 21-H), 0.874 (3 H, t, *J* 7.4 Hz, 23-CH_2CH_3), and 0.719 (3 H, s, 18-H); *m/z* (relative intensity) 444.3599 (M^+, 62; calc. for $C_{29}H_{48}O_3$, 444.3591),

429.3364 ($C_{28}H_{45}O_3$, 57), 412.3343 ($C_{28}H_{44}O_2$, 72), 397.3121 ($C_{27}H_{41}O_2$, 14), 390.3106 ($C_{25}H_{42}O_3$, 28), 389.3077 ($C_{25}H_{41}O_3$, 100), 386.3192 ($C_{26}H_{42}O_2$, 26), 367.2984 ($C_{26}H_{39}O$, 20), 361.2741 ($C_{23}H_{37}O_3$, 13), 297.2592 ($C_{22}H_{33}$, 25), 291.2327 ($C_{19}H_{31}O_2$, 15), 255.2112 ($C_{19}H_{27}$, 33), 245.1913 ($C_{17}H_{25}O$, 14), and 229.1951 ($C_{17}H_{25}$, 15).

23ξ-*Ethyl*-6β-*methoxy*-3α,5-*cyclocholan*-24-*ol* (**39**).—To the α-ethyl ester (**38**) (440 mg, 1.0 mmol) in dry ether (15 ml) was added lithium aluminium hydride (140 mg, 3.3 mmol). The reaction mixture was stirred at room temperature for 30 min after which the excess of lithium aluminium hydride was destroyed by addition of ethyl acetate and water. Filtration and evaporation of the solvent at reduced pressure gave the crude product which was purified by column chromatography over silica gel (eluant, hexane–ether, 5:1) to give the alcohol (**39**) (354 mg, 88%); δ (300 MHz) 3.322 (6 H, s, OCH_3), 1.017 (6 H, s, 19-H), 0.917 (6 H, d, *J* 6.7 Hz, 21-H), 0.900 (3 H, t, *J* 6.0 Hz, 26-H), 0.874 (3 H, t, *J* 7.3 Hz, 26-H), 0.731, and 0.721 (6 H, s, 18-H); *m/z* (relative intensity) 402.30 (M^+, 20), 388.20 (9), 387,20 (34), 370.20 (28), 348.20 (11), 347.20 (46), 344.20 (8), 255.20 (11), 213.05 (10), 105.05 (39), 79.10 (42), and 55.10 (100).

23ξ-*Ethyl*-6β-*methoxy*-3α,5-*cyclocholan*-24-*al* (**40**).—To a solution of the alcohol (**39**) (402 mg, 1.0 mmol) in methylene dichloride (10 ml) was added pyridinium chlorochromate (647 mg, 3.0 mmol). The reaction mixture was stirred for 1 h. Filtration, evaporation, and column chromatography over SiO_2 (eluant, hexane–ether, 10:1) gave the aldehyde (**40**) (368 mg, 92%); δ (300 MHz) 9.589 (1 H, d, *J* 2.6 Hz, CHO), 9.474 (1 H, d, *J* 4.1 Hz, CHO), 3.324 (3 H, s, OCH_3), 3.319 (3 H, s, OCH_3), 1.017 (3 H, s, 19-H), 1.011 (3 H, s, 19-H), 0.908 (6 H, d, *J* 7.0 Hz, 21-H), 0.907 (3 H, t, *J* 7.4 Hz, 26-H), 0.896 (3 H, t, *J* 7.8 Hz, 26-H), 0.726 (3 H, s, 18-H), and 0.687 (3 H, s, 18-H); *m/z* (relative intensity) 400.30 (M^+, 25), 386.30 (14), 385.20 (49), 369.20 (11), 368.30 (35), 346.30 (16), 345.20 (63), 229.15 (12), 213.15 (13), 105.10 (47), and 43.05 (100) (Found: M^+, 400.3341. $C_{27}H_{44}O_2$ requires M^+, 400.3330).

(24Z)-23ξ-*Ethyl*-6β-*methoxy*-3α,5-*cyclo*-5α,27-*norcholest*-24-*ene* (**41**).—To a suspension of ethyltriphenylphosphonium bromide (557 mg, 1.5 mmol) in dry THF (8 ml) under an argon atmosphere at 0 °C was added dropwise butyl-lithium (1.6M in hexane; 0.94 ml, 1.5 mmol). The resultant solution was stirred at 0 °C for 30 min. This phosphorane solution was then added, *via* a syringe, to a stirred solution of the aldehyde (**40**) (300 mg, 0.75 mmol) in dry THF (2 ml) at 0 °C under argon. After being stirred for 1 h, the reaction mixture was quenched with methanol and evaporatd under reduced pressure. Fractionation of the crude mixture over silica gel (eluant, hexane–ether, 10:1) gave the *cis* olefin (**41**) (270 mg, 94%); δ (300 MHz) 5.48, 5.40 (2 H, m, 25-H), 5.15, 4.95 (2 H, m, 24-H), 3.321 (6 H, s, OCH_3), 1.603 (6 H, d, *J* 6.8 Hz, 26-H), 1.014 (6 H, s, 19-H), 0.932 (3 H, d, *J* 6.5 Hz, 21-H), 0.874 (3 H, d, *J* 6.5 Hz, 21-H), 0.830 (6 H, t, *J* 6.8 Hz, 29-H), 0.720, and 0.686 (6 H, s, 18-H); *m/z* (relative intensity) 412.40 (M^+, 16), 398.30 (10), 397.30 (33), 380.30 (19), 365.30 (5), 358.30 (13), 357.30 (49), 314.30 (7), 253.15 (21), 213.15 (13), 191.15 (7), 145.10 (24), and 55.05 (100) (Found: M^+, 412.3714. $C_{29}H_{48}O$ requires M^+, 412.3693).

(24E)-23-*Ethyl*-6β-*methoxy*-33α,5-*cyclo*-5α,27-*norcholest*-24-*ene* (**42**) *and* (**43**).—The *cis* olefin (**41**) (270 mg, 0.66 mmol) and *m*-chloroperbenzoic acid (226 mg, 1.31 mmol) in methylene dichloride (10 ml) were stirred at ambient temperature for 7 h. The reaction mixture was then diluted with brine and extracted with ether. The resultant ether layer was washed with saturated aqueous potassium carbonate, dried ($MgSO_4$), and concentrated under reduced pressure to give the crude epoxide, which was purified by silica gel column chromatography (eluant, hexane–ether 10:1), yield 266 mg (95%). To a solution of lithium diphenylphosphide (5-fold excess, 3.1 mmol) in dry THF (8 ml) was added the epoxide (260 mg, 0.61 mmol) in dry THF (2 ml) and the resultant solution was stirred at room temperature for 10 h. Excess of methyl iodide (1 ml) was added and the mixture maintained at room temperature for 30 min. The reaction mixture was then poured into water and the resulting mixture extracted with ether. The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give the diastereoisomeric mixture of the *trans* olefins (**42**) and (**43**), which were purified by silica gel column chromatography (eluant, hexane–ether, 10:1); yield 205 mg (82%). Fractionation of the diastereoisomeric mixture (**42**), (**43**) by reverse-phase h.p.l.c. (mobile phase, MeOH) gave the (23S)-*trans* olefin (**42**) and (23R)-*trans* olefin (**43**) in a ratio of 10:7.

Fraction 1. (24*E*,23*S*)-23-Ethyl-6β-methoxy-3α,5-cyclo-5α,27-norcholest-24-ene (**42**); δ (300 MHz) 5.43 (1 H, m, 25-H), 5.01 (1 H, m, 24-H), 3.327 (3 H, s, OCH_3), 1.657 (3 H, m, 26-H), 1.020 (3 H, s, 19-H), 0.852 (3 H, d, *J* 6.4 Hz, 21-H), 0.817 (3 H, t, *J* 7.4 Hz, 29-H), and 0.697 (3 H, s, 18-H); *m/z* (relative intensity) 412.3689 (M^+, 34; calc. for $C_{29}H_{48}O$ 412.3693), 397.3487 ($C_{28}H_{45}O$, 43), 380.3440 ($C_{28}H_{44}$, 38), 365.3214 ($C_{27}H_{41}$, 11), 357.3152 ($C_{25}H_{41}O$, 84), 314.2601 ($C_{22}H_{34}O$, 44), 298.2323 ($C_{21}H_{30}O$, 14), 282.2346 ($C_{21}H_{30}$, 28), 275.2368 ($C_{19}H_{31}O$, 10), 259.2416 ($C_{19}H_{31}$, 12), 255.2111 ($C_{19}H_{27}$, 19), 253.1956 ($C_{19}H_{25}$, 45), 241.1966 ($C_{18}H_{25}$, 10), 229.1956 ($C_{17}H_{25}$, 12), 227.1796 ($C_{17}H_{23}$, 12), 213.1633 ($C_{16}H_{21}$, 26), 205.1599 ($C_{14}H_{21}O$, 31), and 201.1658 ($C_{15}H_{21}$, 14).

Fraction 2. (24*E*,23*R*)-23-Ethyl-6β-methoxy-3α,5-cyclo-5α,27-norcholest-24-ene (**43**); δ (300 MHz) 5.35 (1 H, m, 25-H), 5.20 (1 H, m, 24-H), 3.326 (3 H, s, OCH_3), 1.649 (3 H, m, 26-H), 1.020 (3 H, s, 19-H), 0.896 (3 H, d, *J* 6.5 Hz, 21-H), 0.802 (3 H, t, *J* 7.3 Hz, 29-H), and 0.720 (3 H, s, 18-H); *m/z* (relative intensity) 412.40 (M^+, 10), 398.30 (7), 397.20 (19), 381.30 (5), 380.30 (15), 358.30 (11), 357.30 (41), 285.15 (6), 282.15 (10), 253.15 (17), and 55.05 (100) (Found: M^+, 412.3694. $C_{29}H_{48}O$ requires M^+, 412.3693).

Dichlorocarbene Addition to Compounds (**42**) and (**43**).—To a vigorously stirred solution of the *trans* olefin (**42**) (106 mg, 0.25 mmol)/(**43**) (73 mg, 0.17 mmol) and of benzyltriethylammonium chloride [BTEAC; 100 mg, 0.44 mmol for (**42**), 70 mg for (**43**)] in chloroform (10 ml) was added slowly an aqueous solution of sodium hydroxide [50%; 3.4 ml for (**42**) 2.6 ml for (**43**)] at 0 °C. The reaction mixture was stirred for 36 h at room temperature and then diluted with water and extracted with chloroform. The combined extracts were washed with brine, dried (K_2CO_3), and evaporated under reduced pressure. After purification of the crude product by column chromatography (eluant, hexane–ether, 10:1), further fractionation was accomplished by reverse phase h.p.l.c. (Altex, MeOH).

From (**42**), two major fractions (83% based on h.p.l.c. traces) were obtained in a 1:1 ratio.

Fraction 1. (23*S*,24*S*,25*S*)-24,25-(Dichloromethylene)-23-ethyl-6β-methoxy-3α,5-cyclocholestane (**44**); δ (300 MHz) 3.319 (3 H, s, OCH_3), 1.263 (3 H, d, *J* 7.9 Hz, 27-H), 1.023 (3 H, s, 19-H), 0.962 (3 H, d, *J* 6.3 Hz, 21-H), 0.881 (3 H, t, *J* 7.3 Hz, 29-H), and 0.789 (3 H, s, 18-H); *m/z* (relative intensity) 498.25 (M^+4, 4), 496.35 (M^+2, 18), 494.35 (M^+, 25), 482.25 (10), 481.35 (31), 480.35 (15), 479.25 (46), 464.35 (27), 463.35 (14), 462.35 (37), 447.25 (12), 443.25 (12), 442.25 (16), 441.20 (59), 440.30 (28), 439.30 (87), 438.30 (12), 436.30 (15), 341.10 (14), 285.25 (14), 255.15 (31), 253.15 (40), 213.15 (24), and 55.05 (100) (Found: M^+, 494.3074. $C_{30}H_{48}Cl_2O$ requires M^+, 494.3071).

Fraction 2. (23*S*,24*R*,25*R*)-24,25-(Dichloromethylene)-23-ethyl-6β-methoxy-3α,5-cyclocholestane (**45**); δ (300 MHz) 3.322 (3 H, s, OCH_3), 1.274 (3 H, d, *J* 1.8 Hz, 27-H), 1.021 (3 H, s, 19-H), 0.947 (3 H, t, *J* 7.3 Hz, 29-H), 0.878 (3 H, d, *J* 6.4 Hz, 21-H),

and 0.727 (3 H, s, 18-H); *m/z* (relative intensity) 498.35 (M^+ 4, 3), 496.35 (M^+ 2, 13), 494.35 (M^+, 22), 482.35 (10), 481.35 (33), 480.35 (17), 479.35 (50), 464.35 (23), 463.35 (11), 462.35 (35), 447.35 (12), 443.35 (14), 442.35 (18), 441.30 (63), 440.30 (30), 439.30 (97), 436.30 (10), 254.15 (19), 253.15 (78), 227.15 (14), 213.15 (25), and 55.05 (100) (Found: M^+, 494.3072. $C_{30}H_{48}Cl_2O$ requires M^+, 494.3071).

From (**43**) one major fraction (83%) was obtained as a diastereoisomeric mixture of (23*R*,24ξ,25ξ)-24,25-(dichloromethylene)-23-ethyl-6β-methoxy-3α,5-cyclocholestane (**46**); δ (300 MHz) 3.327 (6 H, OCH_3), 1.257 (6 H, d, *J* 5 Hz, 27-H), 1.019 (6 H, s, 19-H), 0.726 (6 H, s, 18-H), with the other peaks overlapping and therefore not assigned; *m/z* (relative intensity) 498.35 (M^+ 4, 4), 496.35 (M^+ 2, 19), 494.35 (M^+, 27), 482.35 (11), 481.35 (34), 480.35 (14), 479.35 (48), 464.35 (30), 463.35 (16), 462.35 (42), 447.35 (13), 443.35 (13), 442.35 (18), 441.30 (63), 440.30 (31), 439.20 (100), 285.15 (11), 255.15 (24), 254.15 (12), 253.15 (48), 229.15 (17), and 213.15 (23) (Found: M^+, 494.3066. $C_{30}H_{48}Cl_2O$ requires M^+, 494.3071).

General Procedure for Dechlorination of Dichlorocarbenes (**44**)—(**46**).—To a solution of lithium (100 mg) in liquid ammonia (20 ml) at −70 °C was added the dichlorocyclopropane in dry ether (2 ml). The mixture was stirred for 5 h at 70 °C, and then quenched with ethanol–ether (1:2). After evaporation of the ammonia and dilution with water, the reaction mixture was extracted with ether and the combined extracts dried (K_2CO_3). Filtration and evaporation of the solvents gave the crude product which was purified by column chromatography (eluant, hexane–ether, 10:1) and further fractionated by reverse-phase h.p.l.c. (Altex, MeOH); yield 98%.

From (23*S*,24*S*,25*S*)-dichlorocyclopropane (**44**) was obtained (23*S*,24*R*-25*R*)-23-ethyl-6β-methoxy-3α,5:24,26-dicyclocholestane (**47**); δ (300 MHz) 3.327 (3 H, s, OCH_3), 1.021 (3 H, s, 19-H), 1.011 (3 H, d, *J* 6.6 Hz, 27-H), 0.884 (3 H, t, *J* 7.4 Hz, 29-H), 0.847 (3 H, d, *J* 6.6 Hz, 21-H), and 0.727 (3 H, s, 18-H); *m/z* (relative intensity) 426.35 (M^+, 11), 412.35 (6), 411.35 (20), 394.25 (12), 372.20 (9), 371.20 (32), 285.20 (7), 253.20 (16), and 55.10 (100) (Found: M^+, 426.3860. $C_{30}H_{50}O$ requires M^+, 426.3849).

From (23*S*,24*R*,25*R*)-dichlorocyclopropane (**45**) was obtained (23*S*,24*S*,25*S*)-23-ethyl-6β-methoxy-3α,5:24,26-dicyclocholestane (**48**); δ (300 MHz) 3.324 (3 H, s, OCH_3), 1.026 (3 H, s, 19-H), 1.017 (3 H, d, *J* (5.4 Hz, 27-H), 0.860 (3 H, d, *J* 6.3 Hz, 21-H), 0.855 (3 H, t, *J* 7.4 Hz, 29-H), and 0.756 (3 H, s, 18-H); *m/z* (relative intensity) 426.35 (M^+, 33), 412.35 (14), 411.35 (45), 395.25 (11), 394.35 (32), 372.20 (19), 371.20 (73), 369.20 (10), 368.20 (10), 255.20 (10), 253.20 (14), 213.15 (11), 97.15 (35), and 55.10 (100) (Found: M^+, 426.3871. $C_{30}H_{50}O$ requires M^+, 426.3849).

From the (23*R*,24ξ,25ξ)-dichlorocyclopropane mixture (**46**) were obtained two diastereoisomers (1:1 ratio) which were separated by h.p.l.c.

Fraction 1. (23*R*,24*R*,25*R*)-23-Ethyl-6β-methoxy-3α,5:24,26-dicyclocholestane (**49**); δ (300 MHz) 3.325 (3 H, s, OCH_3), 1.019 (3 H, s, 19-H), 1.008 (3 H, d, *J* 7.0 Hz, 27-H), 0.898 (3 H, t, *J* 7.4 Hz, 29-H), 0.824 (3 H, d, *J* 6.4 Hz, 21-H), and 0.718 (3 H, s, 18-H); *m/z* (relative intensity) 426.35 (M^+, 14), 412.35 (6), 411.35 (19), 394.35 (12), 372.20 (8), 371.20 (30), 255.20 (5), 253.20 (9), 213.05 (5), 97.15 (23), and 55.10 (100) (Found: M^+, 426.3862. $C_{30}H_{50}O$ requires M^+, 426.3849).

Fraction 2. (23*R*,24*S*,25*S*)-23-Ethyl-6β-methoxy-3α,5:24,26-dicyclocholestane (**50**); δ (300 MHz) 3.324 (3 H, s, OCH_3), 1.017 (3 H, d, *J* 5.9 Hz, 27-H), 1.017 (3 H, s, 19-H), 0.931 (3 H, t, *J* 7.3 Hz, 29-H), 0.803 (3 H, d, *J* 6.5 Hz, 21-H), 0.714 (3 H, s, 18-H); *m/z* (relative intensity) 426.35 (M^+, 16), 412.35 (11), 411.35 (36), 395.25 (7), 394.25 (20), 379.20 (5), 372.20 (18), 371.20 (64), 285.20 (18), 255.20 (12), 253.20 (61), 227.05 (12), 213.05 (12), 97.15 (35), and 55.10 (100) (Found: M^+, 426.3864. $C_{30}H_{50}O$ requires M^+, 426.3849).

Hebesterol Isomers (**11**), (**31**), (**32**), and (**33**).—Each cyclopropyl i-methyl ether (20—30 mg) was heated under reflux in dioxane (4 ml) and water (1 ml) containing toluene-*p*-sulphonic acid (PTSA; 1 mg) for 1 h in an atmosphere of argon. The reaction mixture was extracted with ether, and dried (K_2CO_3). Filtration and evaporation of the solvents gave the crude product which was purified by column chromatography (eluant, hexane–ether, 3:1), and further purified by reverse-phase h.p.l.c. (Altex, MeOH), yield (quantitative based on h.p.l.c. traces).

From (**47**) was obtained (23*S*,24*R*,25*R*)-23-ethyl-24,26-cyclocholest-5-en-3β-ol (**31**): m.p. 149—150 °C (CH_3CN); $[\alpha]_D^{20}$ −28.5°; g.c. rel.-R_t 1.50, h.p.l.c. rel.-R_t 1.10; for δ (300 MHz) data see Table 2; *m/z* (relative intensity) 412.3679 (M^+, 23; calc. for $C_{29}H_{48}O$ 412.3705), 397.3484 ($C_{28}H_{45}O$, 10), 314.2624 ($C_{22}H_{34}O$, 16), 300.2458 ($C_{21}H_{32}O$, 54), 285.2239 ($C_{20}H_{29}O$, 13), 283.2432 ($C_{21}H_{31}$, 17), 271.2070 ($C_{19}H_{27}O$, 100), 267.2118 ($C_{20}H_{27}$, 19), 255.2124 ($C_{19}H_{27}$, 15), 253.1977 ($C_{19}H_{25}$, 17), 231.1729 ($C_{16}H_{23}O$, 11), 227.1788 ($C_{17}H_{23}$, 10), 215.1805 ($C_{16}H_{23}$, 15), and 213.1646 ($C_{16}H_{21}$, 24).

From (**48**) was obtained (23*S*,24*S*,25*S*)-23-ethyl-24,26-cyclocholest-5-en-3β-ol (**32**), m.p. 134—135 °C (MeCN); $[\alpha]_D^{20}$ −5.8°; g.c. rel.-R_t 1.47, h.p.l.c. rel.-R_t 1.07; for δ (300 MHz) data, see Table 2; *m/z* (relative intensity) 412.3701 (M^+, 31; calc. for $C_{29}H_{48}O$ 412.3705), 397.3483 ($C_{28}H_{45}O$, 11), 394.3613 ($C_{29}H_{46}$, 14), 379.3367 ($C_{28}H_{43}$, 11), 370.3257 ($C_{26}H_{42}O$, 11), 300.2454 ($C_{21}H_{32}O$, 100), 283.2438 ($C_{21}H_{31}$, 37), 271.2077 ($C_{19}H_{27}O$, 84), 267.2101 ($C_{20}H_{27}$, 17), 255.2109 ($C_{19}H_{27}$, 23), 253.1963 ($C_{19}H_{25}$, 14), 231.1749 ($C_{16}H_{23}O$, 22), 227.1803 ($C_{17}H_{23}$, 11), 215.1806 ($C_{16}H_{23}$, 18), and 213.1644 ($C_{16}H_{21}$, 35).

From (**49**) was obtained (23*R*,24*R*,25*R*)-23-ethyl-24,26-cyclocholest-5-en-3β-ol (**33**), m.p. 135—136 °C (MeCN); $[\alpha]_D^{20}$ −40.5°; g.c. rel.-R_t 1.60, h.p.l.c. R_t 1.05; for δ (300 MHz) data, see Table 2; *m/z* (relative intensity) 412.3710 (M^+, 26; calc. for $C_{29}H_{48}O$ 412.3705), 394.3606 ($C_{29}H_{46}$, 15), 379.3366 ($C_{28}H_{43}$, 13), 314.2631 ($C_{22}H_{34}O$, 300.2473 ($C_{21}H_{32}O$, 100), 285.2230 ($C_{20}H_{29}O$, 21), 283.2449 ($C_{21}H_{31}$, 33), 271.2068 ($C_{19}H_{27}O$, 81), 267.2092 ($C_{20}H_{27}$, 18), 255.2114 ($C_{19}H_{27}$, 26), 253.1946 ($C_{19}H_{25}$, 17), 241.1948 ($C_{18}H_{25}$, 16), 231.1750 ($C_{16}H_{23}O$, 15), 227.1798 ($C_{17}H_{23}$, 12), 215.1805 ($C_{16}H_{23}$, 21), and 213.1643 ($C_{16}H_{21}$, 38).

From (**50**) was obtained (23*R*,24*S*,25*S*)-23-ethyl-24,26-cyclocholest-5-en-3β-ol (**11**) (hebesterol), m.p. 133—134 °C (MeCN); $[\alpha]_D^{20}$ −2.1°; g.c. rel.-R_t 1.60, h.p.l.c. rel.-R_t 1.11; for δ (300 MHz) data, see Table 2; *m/z* (relative intensity) 412.3693 (M^+, 31; calc. for $C_{29}H_{48}O$ 412.3705), 397.3469 ($C_{28}H_{45}O$, 6), 394.3621 ($C_{29}H_{46}$, 7), 379.3363 ($C_{28}H_{43}$, 9), 314.2601 ($C_{22}H_{34}O$, 9), 301.2550 ($C_{21}H_{33}O$, 16), 300.2443 ($C_{21}H_{32}O$, 38), 299.2381 ($C_{21}H_{31}O$, 15), 283.2431 ($C_{21}H_{31}$, 14), 271.2049 ($C_{19}H_{27}O$, 100), 255.2094 ($C_{19}H_{27}$, 19), 253.1960 ($C_{19}H_{25}$, 14), 231.1730 ($C_{16}H_{23}O$, 11), and 213.1643 ($C_{16}H_{21}$, 23).

General Procedure for Hebesterol Isomerization Reactions.—The free sterol (7 mg) was dissolved in a 5% trifluoroacetic acid–benzene solution (5 ml) and maintained for 3—6 days at room temperature. Evaporation of the solvents gave the crude products (as trifluoroacetates), which were treated with an excess of lithium aluminium hydride in dry ether. The excess of reagent was destroyed by the addition of ethyl acetate and water. Filtration and evaporation of the solvents gave the crude free sterols, which were fractionated by reverse-phase h.p.l.c. (Altex, MeOH).

The isomerization of (23*R*,24*S*,25*S*)-hebesterol (**11**) gave the following fractions in order of elution.

Fraction 1. (23*R*)-23-Ethylcholesta-5,24-dien-3β-ol (**51**); δ (300 MHz) 4.86 (1 H, br d, 24-H), 1.684 (3 H, d, *J* 0.8 Hz, 26- or 27-H), 1.599 (3 H, d, *J* 1.1 Hz, 27- or 26-H), 1.004 (3 H, s, 19-H), 0.921 (3 H, d, *J* 6.4 Hz, 21-H), 0.784 (3 H, t, *J* 7.3 Hz, 29-H), and 0.679 (3 H, s, 18-H); *m/z* (relative intensity) 412.35 (M^+, 22), 379.20 (6), 314.20 (16), 301.20 (13), 300.20 (50), 299.20 (17), 285.20 (11), 271.20 (32), 213.05 (12), 97.15 (100), and 55.10 (100).

Fraction 2. (23*R*,24*S*)-23-Ethyl-24-methyl-27-norcholesta-5,25-dien-3β-ol (**5**) (natural ficisterol); [7,24] δ (300 MHz) 5.70—5.85 (1 H, m, 25-H), 4.90—5.00 (2 H, m, 26-H), 1.005 (3 H, s, 19-H), 0.874 (3 H, d, *J* 6.8 Hz, 28-H), 0.864 (3 H, d, *J* 6.3 Hz, 21-H), 0.848 (3 H, t, *J* 7.5 Hz, 30-H), and 0.686 (3 H, s, 18-H); *m/z* (relative intensity) 412.45 (M^+, 20), 379.20 (6), 301.20 (7), 300.20 (26), 299.20 (10), 285.20 (6), 272.10 (7), 271.20 (17), 258.20 (5), 253.20 (5), 213.05 (7), 97.15 (89), and 55.10 (100).

Fraction 3. (*E*)-23-Ethylcholesta-5,22-dien-3β-ol (**52**); δ (300 MHz) 4.82 (1 H, d, *J* 9.8 Hz, 24-H), 1.011 (3 H, s, 19-H), 0.955 (3 H, d, *J* 6.2 Hz, 21-H) 0.945 (3 H, t, *J* 7.6 Hz, 29-H), 0.842 (3 H, d, *J* 6.7 Hz, 26- or 27-H), 0.820 (3 H, d, *J* 6.4 Hz, 27- or 26-H), and 0.714 (3 H, s, 18-H); *m/z* (relative intensity) 412.35 (M^+, 41), 379.20 (8), 369.20 (9), 351.20 (19), 314.20 (10), 301.20 (11), 300.20 (30), 299.20 (11), 273.10 (11), 272.20 (23), 221.10 (44), 270.10 (11), 258.10 (10), 256.20 (11), 255.20 (44), 229.05 (11), 215.05 (10), 213.05 (20), and 55.10 (100).

Fraction 4. Recovered starting material (**11**).

The isomerization of (23*R*,24*R*,25*R*)-hebesterol (**33**) gave the following fractions in order of elution.

Fraction 1. (23*R*,24*R*)-23-Ethyl-24-methyl-27-norcholesta-5,25-dien-3β-ol (**53**); [24] δ (300 MHz) 5.60—5.72 (1 H, m, 25-H), 4.9—5.0 (2 H, m, 26-H), 1.004 (3 H, s, 19-H), 0.967 (3 H, d, *J* 6.9 Hz, 28-H), 0.874 (3 H, d, *J* 6.3 Hz, 21-H), 0.843 (3 H, t, *J* 7.1 Hz, 30-H), and 0.685 (3 H, s, 18-H); *m/z* (relative intensity) 412.35 (M^+, 7), 394.25 (5), 379.20 (6), 339.30 (11), 314.20 (11), 300.10 (5), 281.00 (9), 273.10 (6), 272.10 (9), 271.10 (27), 255.20 (14), 213.05 (15), 55.00 (68), and 41.10 (100).

Fraction 2. (23*R*)-23-Ethylcholesta-5,24-dien-3β-ol (**51**). For spectral data, see above.

Fraction 3. (*E*)-23-Ethylcholesta-5,22-dien-3β-ol (**52**). For spectral data, see above.

Fraction 4. Recovered starting material (**33**).

The isomerization of (23*S*,24*S*,25*S*)-hebesterol (**32**) gave the following five fractions in order of elution.

Fraction 1. (17*S*,20*S*,23*S*)-23-Ethyl-17-methyl-18-norcholesta-5,13(14)-dien-3β-ol (**54**); δ (300 MHz) 0.995 (3 H, s, 19-H), 0.989 (3 H, s, 17-H), 0.865 (3 H, d, *J* 6.7 Hz, 26- or 27-H), 0.843 (3 H, d, *J* 6.7 Hz, 21-H), 0.829 (3 H, t, *J* 7.5 Hz, 29-H), and 0.810 (3 H, d, *J* 6.9 Hz, 27- or 26-H); *m/z* (relative intensity) 412.00 (M^+, 0.1), 272.00 (21), 271.00 (100), and 253.00 (11).

Fraction 2. (17*S*,20*R*,23*S*)-23-Ethyl-17-methyl-18-norcholesta-5,13(14)-dien-3β-ol (**55**); δ (300 MHz) 1.000 (3 H, s, 19-H), 0.985 (3 H, s, 17-H), 0.865 (3 H, t, *J* 7.2 Hz, 29-H), 0.859 (3 H, d, 21-H), 0.712 (3 H, d, *J* 6.8 Hz, 26- or 27-H), and 0.671 (3 H, d, *J* 6.7 Hz, 27- or 26-H); *m/z* (relative intensity) no M^+ ion peak, 272.0 (29), 271.0 (100), 270.0 (7), 254.0 (4), and 253.0 (18).

Fraction 3. (23*R*)-23-Ethylcholesta-5,20(22)-dien-3β-ol (**57**); δ (300 MHz) 4.937 (1 H, d, *J* 9.8 Hz, 22-H), 1.611 (3 H, d, *J* 0.9 Hz, 21-H), 0.850 (3 H, d, *J* 6.9 Hz, 26- or 27-H), 0.827 (3 H, d, *J* 6.4 Hz, 27- or 26-H), 0.793 (3 H, t, *J* 7.4 Hz, 29-H), and 0.575 (3 H, s, 18-H); *m/z* (relative intensity) 412.0 (M^+, 16), 355.0 (7), 272.0 (20), 271.0 (100), 256.0 (11), 255.0 (44), 253.0 (7), and 213.0 (10).

Fraction 4. (23*S*,24*S*)-23-Ethyl-24-methyl-27-norcholesta-5,25-dien-3β-ol (**56**); [24] δ (300 MHz) 5.75—5.90 (1 H, m, 25-H), 4.90—5.00 (2 H, m, 26-H), 1.008 (3 H, s, 19-H), 0.905 (3 H, d, *J* 6.7 Hz, 21-H), 0.868 (3 H, d, *J* 7.0 Hz, 28-H), 0.832 (3 H, t, *J* 7.0 Hz, 30-H), and 0.690 (3 H, s, 18-H); *m/z* (relative intensity) 412.45 (M^+, 14), 394.25 (8), 379.20 (8), 339.20 (15), 314.20 (19), 300.20 (8), 299.20 (8), 272.20 (12), 271.20 (43), 256.20 (10), 255.20 (21), 231.15 (9), 215.15 (8), 213.15 (19), and 55.10 (100).

Fraction 5. Recovered starting material (**32**).

The isomerization of (23*S*,24*R*,25*R*)-hebesterol (**31**) gave the following six fractions in order of elution.

Fraction 1. (17*S*,20*S*,23*S*)-23-Ethyl-17-methyl-18-norcholesta-5,13(14)-dien-3β-ol (**54**); for spectral data, see above.

Fraction 2. (17*S*,20*R*,23*S*)-23-Ethyl-17-methyl-18-norcholesta-5,13(14)-dien-3β-ol (**55**); for spectral data, see above.

Fraction 3. (23*S*)-23-Ethylcholesta-5,24-dien-3β-ol (**59**); δ (300 MHz) 4.7 (1 H, br d, 24-H), 1.699 (3 H, d, *J* 1.1 Hz, 26- or 27-H), 1.594 (3 H, d, *J* 1.2 Hz, 27- or 26-H), 1.002 (3 H, s, 19-H), 0.858 (3 H, d, *J* 6.5 Hz, 21-H), 0.800 (3 H, t, *J* 7.4 Hz, 29-H), and 0.645 (3 H, s, 18-H); *m/z* (relative intensity) 412.45 (M^+, 17), 379.20 (7), 314.20 (12), 301.20 (18), 300.20 (85), 299.20 (22), 285.20 (16), 282.20 (12), 272.20 (15), 271.10 (32), 267.10 (14), 253.10 (11), 97.15 (97), and 55.10 (100) (Found: M^+, 412.3708. $C_{29}H_{48}O$ requires M^+, 412.3705).

Fraction 4. (23*S*,24*R*)-23-Ethyl-24-methyl-27-norcholest-5,25-dien-3β-ol (**58**); [24] δ (300 MHz) 5.65—5.76 (1 H, m, 25-H), 4.9—5.0 (2 H, m, 26-H), 1.008 (3 H, s, 19-H), 0.900 (3 H, d, *J* 6.9 Hz, 28-H), 0.889 (3 H, d, *J* 6.5 Hz, 21-H), 0.850 (3 H, t, *J* 6.3 Hz, 30-H), and 0.689 (3 H, s, 18-H); *m/z* (relative intensity) 412.35 (M^+, 15), 394.35 (8), 379.20 (9), 339.20 (21), 327.20 (8), 315.20 (7), 314.20 (27), 273.20 (8), 272.10 (13), 271.10 (40), 255.20 (21), 231.15 (14), and 213.05 (25).

Fraction 5. (*E*)-23-Ethylcholesta-5,22-dien-3β-ol (**52**); for spectral data, see above.

Fraction 6. (22*E*,24*S*)-24-Ethylcholesta-5,22-dien-3β-ol (**24**) (stigmasterol); δ (300 MHz) 4.95—5.20 (2 H, m, 22-, 23-H), 1.021 (3 H, d, *J* 6.4 Hz, 21-H), 1.010 (3 H, s, 19-H), 0.844 (3 H, d, *J* 6.4 Hz, 26-or 27-H), 0.803 (3 H, t, *J* 7.3 Hz, 30-H), 0.793 (3 H, d, *J* 6.0 Hz, 27- or 26-H), and 0.697 (3 H, s, 18-H); *m/z* (relative intensity) 412.45 (M^+, 34), 351.20 (18), 314.20 (11), 301.20 (11), 300.20 (30), 299.20 (11), 273.10 (12), 272.20 (25), 271.10 (47), 270.20 (10), 229.05 (11), 215.05 (10), 213.05 (21), and 55.10 (100).

General Procedure for Degradation of Compounds (**52**) *and* (**57**).—To a pyridine solution of each sterol (0.5—1 mg) was added toluene-*p*-sulphonyl chloride (20 mg); after 24 h at room temperature, the resultant mixture was distributed between aqueous cupric sulphate and hexane. The combined hexane layer was concentrated and purified by short-column chromatography (eluant, hexane–ether, 10:1) to afford the corresponding tosylate. To a methanol (10 ml) solution of the tolsylate was added potassium acetate (50 mg); after being heated under reflux for 3 h, the reaction mixture was cooled to room temperature and purified by short-column chromatography (silica gel, hexane–ether, 10:1) to afford the corresponding i-methyl ether. A saturated solution of ozone in CH_2Cl_2 (5 ml) at −70 °C was transferred to the i-methyl ether −70 °C and stirred at the same temperature for 5 min. Excess of ozone was destroyed by the addition of methyl sulphide. Concentration and separation by column chromatography gave the corresponding compound.

From (**52**) was obtained (20*S*)-6β-methoxy-3α,5-cyclo-5α-pregnane-20-carbaldehyde (**34**); δ (300 MHz) 9.588 (1 H, d, *J* 3.6 Hz, CHO), 3.326 (3 H, s, OCH_3), 1.125 (3 H, d, *J* 6.9 Hz, 21-H), 1.105 (3 H, s, 19-H), and 0.771 (3 H, s, 18-H); *m/z* (relative intensity) 344.15 (M^+, 18), 330.15 (13), 329.05 (54), 313.15 (12), 312.15 (44), 297.15 (11), 290.15 (20), 289.05 (95), 286.15 (15), and 41.10 100).

From (**57**), after deprotection and purification in the previously described manner, there was obtained 3β-hydroxypregn-5-en-20-one (**62**), which was shown to be identical with authentic material by chromatographic retention time and ^1H n.m.r. and mass spectral characteristics; g.c. rel.-R_t 0.55; δ (300 MHz) 2.124 (3 H, s, 21-H), 1.012 (3 H, s, 19-H), 0.636 (3 H, s, 18-H); *m/z* (relative intensity) 316.20 (M^+, 40), 298.15 (26), 283.15 (23), 255.15 (9), 231.15 (24), 213.15 (14), and 43.05 (100).

Acid-catalysed Isomerization of (**57**).—Isomerization of (**53**) (1.2 mg) was carried out in the manner previously described for the hebesterol isomers except that the reaction time was shortened to 2 days. Fractionation by reverse-phase h.p.l.c. afforded three fractions in a ratio of 29:38:33.

Fraction 1. (17*S*,20*S*,23*S*)-23-Ethyl-17-methyl-18-norcholesta-5,13(14)-dien-3β-ol (**54**). For spectral data, see above.

Fraction 2. (17*S*,20*R*,23*S*)-23-Ethyl-17-methyl-18-norcholesta-5,13(14)-dien-3β-ol (**55**). For spectral data, see above.

Fraction 3. Recovered starting material (**57**).

Synthesis of 23-*Ethyldesmosterol* (**51**) *and* (**59**).—To a suspension of isopropyltriphenylphosphonium bromide (144 mg, 0.37 mmol) in dry THF (2 ml) under argon at 0 °C was added dropwise butyl-lithium (1.7M in hexane; 0.23 ml, 0.37 mmol). The resultant solution was stirred at 0 °C for 30 min and then added, *via* a syringe, to a stirred THF solution (1 ml) of the aldehyde (**40**) (42 mg, 0.11 mmol) at 0 °C under argon. After being stirred for 1 h the reaction mixture was quenched with methanol and evaporated under reduced pressure. Fractionation of the crude mixture over silica gel (eluant, hexane–ether, 10:1) gave the diastereoisomeric mixture of 23-ethyldesmosterol i-methyl ethers in 95% yield. Further fractionation by reverse-phase h.p.l.c. (Altex, MeOH) afforded (**63**) and (**64**) in a 5:2 ratio.

Fraction 1. (23*S*)-23-Ethyl-6β-methoxy-3α,5-cyclocholestane (**64**); δ (300 MHz) 4.69 (1 H, br d, 24-H), 3.321 (3 H, OCH_3), 1.703 (3 H, d, *J* 1.1 Hz, 26- or 27-H), 1.595 (3 H, d, *J* 1.4 Hz, 27- or 26-H), 1.017 (3 H, s, 19-H), 0.932 (3 H, d, *J* 6.5 Hz, 21-H), 0.807 (3 H, t, *J* 7.3 Hz, 29-H), and 0.721 (3 H, s, 18-H).

Fraction 2. (23*R*)-23-6β-Methoxy-3α,5-cyclocholestane (**63**); δ (300 MHz) 4.86 (1 H, br d, 24-H), 3.320 (3 H, OCH_3), 1.683 (3 H, d, *J* 1.0 Hz, 26- or 27-H), 1.600 (3 H, d, *J* 1.0 Hz, 27- or 26-H), 1.015 (3 H, s, 19-H), 0.914 (3 H, d, *J* 6.5 Hz, 21-H), 0.783 (3 H, t, *J* 7.3 Hz, 29-H), and 0.715 (3 H, s, 18-H).

Each 23-ethyldesmosterol i-methyl ether was converted in quantitative yield into the free sterol by the same method used in the preparation of hebesterol isomers.

From (**63**) was obtained (23*R*)-23-ethylcholesta-5,24-dien-3β-ol (**51**); for δ (300 MHz) data see above; *m/z* (relative intensity) 412.3683 (M^+, 49; calc. for $C_{29}H_{48}O$ 412.3705), 379.3353 ($C_{28}H_{43}$, 10), 314.2588 ($C_{22}H_{34}O$, 22), 300.2464 ($C_{21}H_{32}O$, 75), 285.2226 ($C_{20}H_{29}O$, 12), 283.2433 ($C_{21}H_{31}$, 10), 271.2081 ($C_{19}H_{27}O$, 46), 267.2128 ($C_{20}H_{27}$, 10), 255.2127 ($C_{19}H_{27}$, 13), 213.1639 ($C_{16}H_{21}$, 16), and 97.1006 (C_7H_{13}, 100).

From (**64**) was obtained (23*S*)-23-ethylcholesta-5,24-dien-3β-ol (**59**); for spectral data, see above.

Catalytic Hydrogenation of 23-*Ethyldesmosterol* (**51**), (**59**).—Each sterol (2—5 mg) in ethyl acetate–acetic acid (1:1; 5 ml) was hydrogenated for 5 h at room temperature with platinum oxide (20 mg). Filtration and evaporation of the solvents gave the crude product which was purified by reverse phase h.p.l.c. (Altex, MeOH); yield 95%.

From (**51**) was obtained (23*S*)-23-ethylcholestan-3β-ol (**65**);[3b] δ (300 MHz) 0.869 (3 H, d, *J* 6.4 Hz, 21-H), 0.859 (3 H, d, *J* 6.6 Hz, 26- or 27-H), 0.832 (3 H, d, *J* 6.6 Hz, 27 or 26-H), 0.798 (3 H, s, 19-H), 0.789 (3 H, t, *J* 7.4 Hz, 29-H), 0.657 (3 H, s, 18-H); *m/z* (relative intensity) 416.45 (M^+, 48), 402.35 (10), 401.35 (29), 383.35 (14), 290.30 (13), 234.15 (58), 233.15 (85), 232.15 (10), 231.15 (14), 219.15 (11), 217.15 (24), 216.15 (36), 215.15 (77), and 43.05 (100).

From (**59**) was obtained (23*R*)-23-ethylcholestan-3β-ol (**66**);[3b] δ (300 MHz) 0.871 (3 H, d, *J* 6.5 Hz, 21-H), 0.865 (3 H, d, *J* 6.4 Hz, 26- or 27-H), 0.828 (3 H, d, *J* 6.7 Hz, 27- or 26-H), 0.817 (3 H, t, *J* 6.7 Hz, 29-H), 0.802 (3 H, s, 19-H), and 0.662 (3 H, s, 18-H); *m/z* (relative intensity) 416.45 (M^+, 50), 402.35 (10), 401.35 (30), 383.35 (14), 290.30 (12), 234.15 (64), 233.15 (82), 231.15 (12), 217.15 (23), 216.15 (32), 215.15 (76), and 57.10 (100).

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