Synthesis and Stereochemistry of 23,24-Dihydrocalysterol: Implications for Marine Sterols of a Unified Biosynthetic Scheme involving Protonated Cyclopropanes

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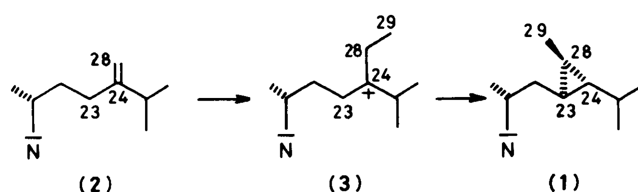
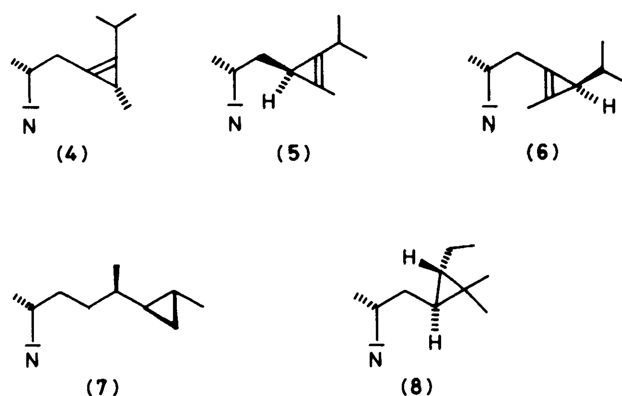
The partial synthesis of the marine cyclopropyl-sterol dihydrocalysterol (**1**), which has allowed the confirmation of the originally postulated absolute stereochemistry around the cyclopropane ring, is described. The firm knowledge of this stereochemistry and that of the other marine sterols petrosterol (**7**), dehydroaplysterol (**21**), ficisterol (**22**), nicasterol (**8**), and hebesterol (**23**) has allowed us to construct a unified biosynthetic scheme for these sterols of diverse side-chain structure. Our central postulate, that the intermediate in sterol side biomethylation may be described in terms of a protonated cyclopropane species [*i.e.* protonated dihydrocalysterol (**1**)], enables us to interrelate the absolute stereochemistries of the above sterols and predict the biosynthetic origin of the side-chain carbon atoms.

The cyclopropane-containing marine sterol 23,24-dihydrocalysterol (**1**)[1] was isolated in these laboratories as a trace component of the sterol mixture of the Mediterranean sponges *Calyx niceaensis* and *Petrosia ficiformis*.[2] Subsequent to its isolation, incorporation experiments showed it to be derived by biomethylation of 24-methylenecholesterol (**2**) in a process (Scheme 1) apparently involving a 1,2-hydride shift and ring closure between C-23 and C-28.[3] Based on radioactivity incorporation results,[3] dihydrocalysterol (**1**) is most likely a direct precursor of the cyclopropene sterols (**4**)—(**6**)[1,4] of *C. niceaensis*. We have also postulated[5] a protonated dihydrocalysterol species [formally equivalent to the carbonium ion (**3**)] to be a key enzyme-bound intermediate in the biosynthesis of petrosterol (**7**) and nicasterol (**8**).

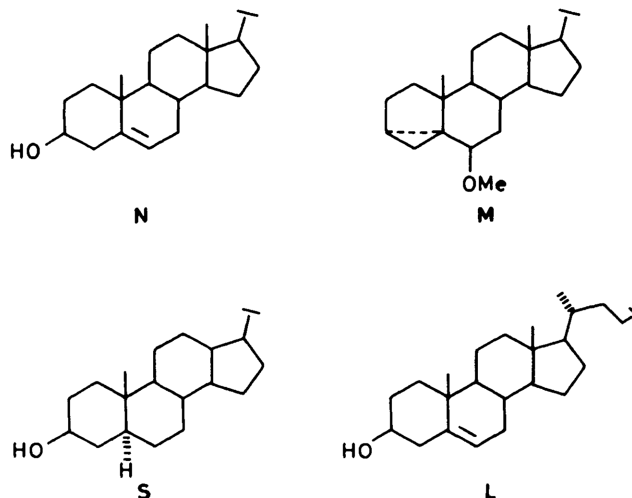


Scheme 1.

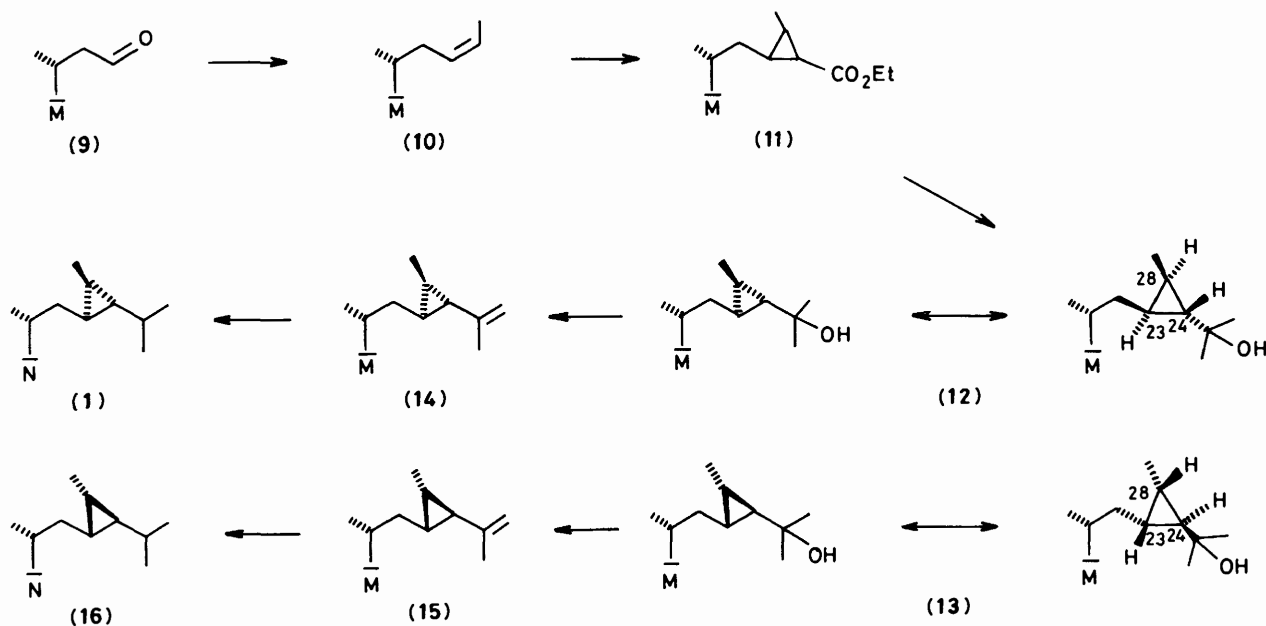
The relative stereochemistry of dihydrocalysterol was formulated,[1] on the basis of ^{1}H n.m.r. decoupling experiments, as either the 23*S*,24*S*,28*R*-isomer (**1**) or the 23*R*,24*R*,28*S*-isomer (**16**). The expressed preference for (**1**)[1] rested on a low-yield hydrogenolysis reaction which yielded (24*S*)-ethylcholestanol (**18**). In view of the central importance of the sterol (**1**) as a window on the details of the biomethylation of 24-methylenecholesterol (**2**), we sought a short efficient synthesis which would confirm the assigned stereochemistry and enable the facile introduction of radiolabels for later feeding experiments.



Synthesis and Stereochemistry of Dihydrocalysterol.—The synthesis of dihydrocalysterol (**1**), which also yielded diastereoisomer (**16**), proceeded as shown in Scheme 2. The *cis*-olefin (**10**), available from the Wittig reaction of ethyltriphenylphosphorane with the aldehyde (**9**)[5] was treated with ethyl diazoacetate in the presence of the catalyst tris(trimethyl phosphite)copper(I) iodide.[6] Carbene attack may take place from either face of the double bond with the ethoxycarbonyl group occupying an *exo*-or an *endo*-position. *A priori*, no selectivity was expected in terms of frontside (**11a,b**) or backside (**11c,d**) attack (Figure 1). It was expected that within these two pairs, structure (**11a**) and (**11c**), resulting from an *exo*-orientation of the ethoxycarbonyl group, would predominate over (**11b**) and (**11d**) which possess a sterically crowded all-*cis*-cyclopropane ring system.[7] Both (**11a**) and (**11c**) possess the same relative stereochemistry around the cyclopropane ring as that proposed for dihydrocalysterol (**1**).[1]



In practice, the mixture of cyclopropanecarboxylic acid esters (**11**) obtained from the carbene reaction was treated with methyl-lithium and separation of the diastereoisomeric steroidal cyclopropanes was carried out at the stage of the



Scheme 2.

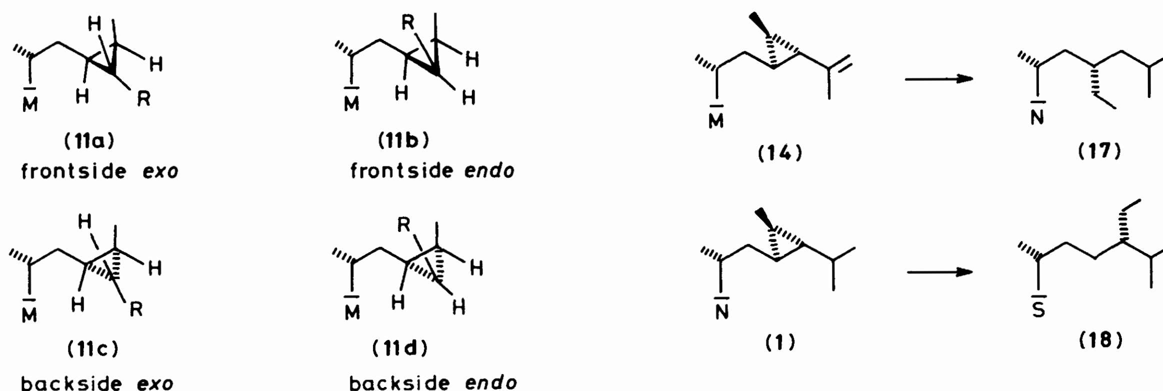


Figure 1.

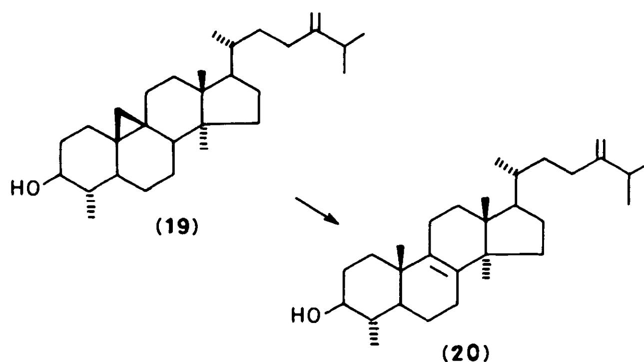
tertiary alcohols (**12**) and (**13**). Both sterols, (**12**) and (**13**), displayed one well resolved cyclopropyl proton signal (besides those due to the i-methyl ether protecting group M) which appeared as an apparent triplet in each case. Since the 23- and 28- would be expected to show dddd and ddq multiplicity respectively, the signal must be due to the 24-H. The apparent triplet multiplicity arises from equal couplings to adjacent 23-H and 28-H and the magnitude of these couplings, $J = 5.1$—5.3 Hz, is typical of *trans*-substituted cyclopropane-ring systems.[8] Thus 24-H is *trans* to 23-H and 28-H in compounds (**12**) and (**13**) and both, therefore, possess the expected relative stereochemistry. The synthetic pathway was completed, as shown in Scheme 2, by dehydration, catalytic hydrogenation, and cleavage of the i-methyl ether protecting group. One of the two isomers obtained was identical with the natural product (300 MHz ^{1}H n.m.r.; g.c.; h.p.l.c. co-injection).

The final hydrogenation of (**14**) and ring A deprotection reactions in Scheme 2 leading to dihydrocalysterol [*viz.* (**14**) ⟶ (**1**)] also gave a small amount of the cyclopropane hydrogenolysis [9a,*] product (23*S*)-ethylcholesterol (**17**). The absolute stereochemistry at C-23 of this sterol was determined by comparison of its 300 MHz ^{1}H n.m.r. spectrum with those of the corresponding 23-ethylcholestanols.[10] Particularly diagnostic of the absolute stereochemistry, determined to be as shown (23*S*), is the chemical shift of the ethyl group triplet which appears in the sterol (**17**) at δ 0.794 compared with δ 0.792 for (23*S*)-ethylcholestanol and δ 0.821 for the 23*R* isomer.[10] This stereochemistry at C-23 is consistent with the stereochemistry of 23,24-dihydrocalysterol as previously postulated.[1] The original hydrogenolysis reaction[1] which had given (24*S*)-ethylcholestanol (**18**) in very low yield (*ca.* 10%) was also repeated under improved conditions to give (**18**) in 90% yield and established unambiguously the originally assigned absolute stereochemistry of dihydrocalysterol (**1**).

As shown below, a secure knowledge of the absolute configuration is a key element in our biosynthetic proposals.

Biosynthetic Implications of the Absolute Stereochemistry of 23,24-Dihydrocalysterol (**1**).—The natural occurrence of sterols containing the cyclopropyl group in the side-chain has raised speculation as to their biological role. Before the isolation of any such sterol, Lederer had proposed a cyclopropane species as a discrete intermediate leading to a saturated methyl group.[11] Incorporation experiments, however, failed to support this idea.[11b] Experimental results from this laboratory have tended to support the alternative hypothesis[12] that cyclopropanes may be precursors to an allylic methyl group *via* an enzymatically

* We have also noted an identical hydrogenolysis in which cyclopropane cleavage is directed by an adjacent double bond.[9b]

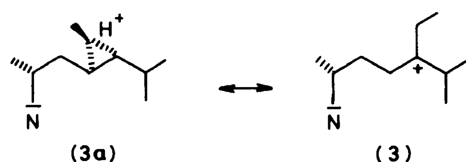


(19) (20)

induced isomerization reaction which, although receiving considerable support from *in vitro* acid-catalysed experiments,[13,9b] has been demonstrated *in vivo* only in the conversion of cycloeucalenol (**19**) into obtusifoliol (**20**).[14]

Below, we present an alternative view of the importance of marine cyclopropyl sterols. We propose that these sterols, whether or not they are discrete intermediates in the biomethylation sequence, give important insights into the processes occurring during this reaction.

Our hypothesis was prompted by the co-occurrence [1,2] of dihydrocalysterol (**1**) with petrosterol (**7**),[15] 26-dehydroaplysterol (**21**),[16] and ficisterol (**22**)[17] in *P. ficiformis*; with nicasterol (**8**)[5] in *C. niceaensis*; and with hebesterol (**23**)[10b] in *P. hebes*; and the question as to whether there is a biosynthetic relationship among these unusual sterols. Since dihydrocalysterol (**1**) is a common denominator, our analysis started with this sterol. In particular, we considered its protonated form (**3a**) which is formally equivalent to the carbonium ion (**3**)* the usual representation of the biomethylation intermediates (see Scheme 1). As far as we are aware, there has been only one such representation of a biomethylation intermediate in sterol side-chain biosynthetic studies.[19] The existence, albeit transient, of protonated cyclopropanes is well documented[20] and it is not unreasonable to suppose that the final step in the biosynthesis of the sterol (**1**) is deprotonation of (**3a**). The singular advantage of representation (**3a**) over (**3**) is the existence and possibility of manipulating three asymmetric centres.



(3a) (3)

The subsequent analysis rests on the following postulates:† (1) that the key enzyme-bound intermediate in the biosynthesis of the marine sterols (**1**), (**7**), (**8**), (**21**), (**22**), and (**23**) with their unique side-chains, may be represented as (**3a**); (2) that the proton may activate the C(23)–C(24), C(23)–C(28), or C(24)–C(28) bonds towards nucleophilic attack by C-25, C-26, or C-27;‡·§ and (3) that such nucleophilic attack will result in inversion of stereochemistry at the centre attacked.[23]

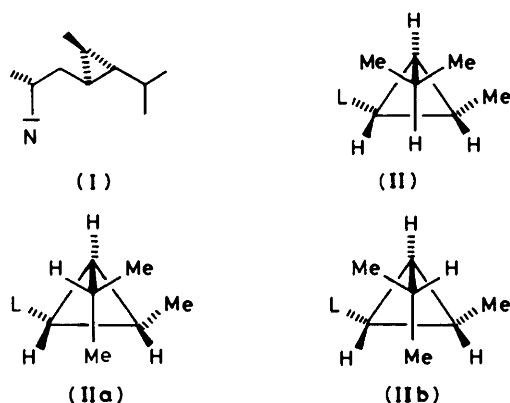
* Protonated cyclopropanes have long been invoked to explain various aspects of carbonium ion chemistry.[18]

† The postulates are well precedented by the biogenetic isoprene rule as applied to the biosynthesis of triterpenes and sterols.[21]

‡ The stereochemistry of proton position on the three-membered ring is not speculated upon in this analysis. It is, in principle, possible to distinguish whether there is retention or inversion of stereochemistry at the site of proton addition by incorporation experiments involving suitably labelled precursors followed by determination of isotope distribution and stereochemistry.

§ For studies on the stereochemistry of electrophilic attack on cyclopropanes see ref. 22.

The course of these isomerization reactions, in each case, will be depicted in two ways: one involving a straightforward representation of the sterol side-chain as usually drawn (**I**) and the other involving a Newman projection (**II**) along the C(24)–C(25) bond.



(I) (II) (IIa) (IIb)

The choice of (**II**) over (**IIa**) and (**IIb**) as the reacting conformer was made on the basis of its being the conformer of lowest potential energy. While we recognized that this conformation might not have been that existing at the enzyme active site we were gratified that it indeed provided the basis for interrelating the structures and absolute stereochemistries of the sterols (**1**), (**7**), (**8**), (**21**), (**22**), and (**23**). It should be noted that invoking conformer (**IIa**) in Scheme 3 or conformer (**IIb**) in Scheme 5 would result in cyclopropanes having a *cis* substituted ring system.

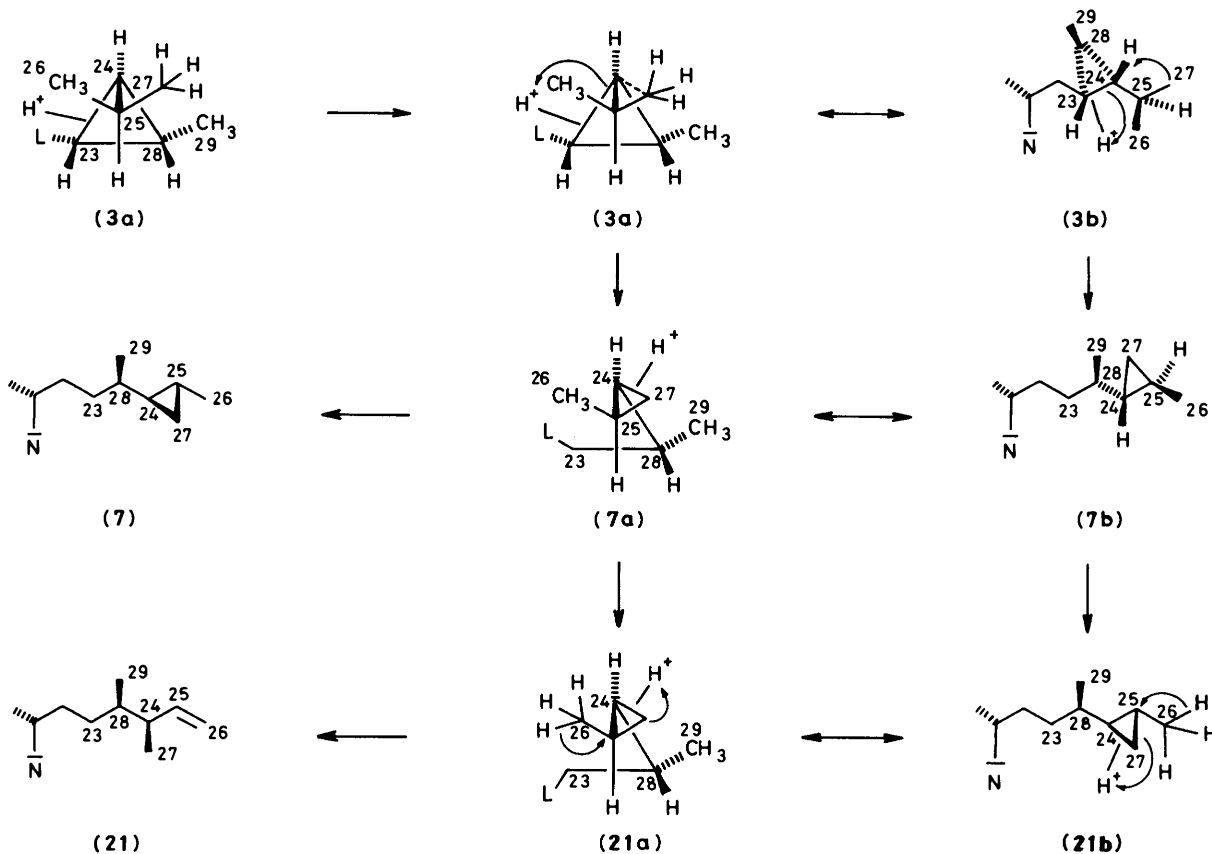
Solid (▬) and broken (⦙⦙⦙⦙⦙) lines bear conventional stereochemical implications while new bond formation is indicated by a dashed (– – – –) line. The bond labilized is indicated by a line drawn from the proton [*cf.* (**3a**) and (**3b**) in Scheme 3]. No implication is made as to whether edge or corner protonated cyclopropane intermediates are involved. Either representation will lead to the same results.

The biosynthesis of petrosterol (**7**) and dehydroaplysterol (**21**) is postulated to proceed from the protonated dihydrocalysterol species (**3a**),(**3b**) as shown in Scheme 3. Cleavage of the C(23)–C(24) bond with concomitant bond formation between C-24 and C-27 as depicted gives rise to the protonated petrosterol species (**7a**),(**7b**), which can lose a proton to give petrosterol (**7**) or undergo further isomerization, as shown, to the dehydroaplysterol derivative (**21**), *via* (**21a**),(**21b**).

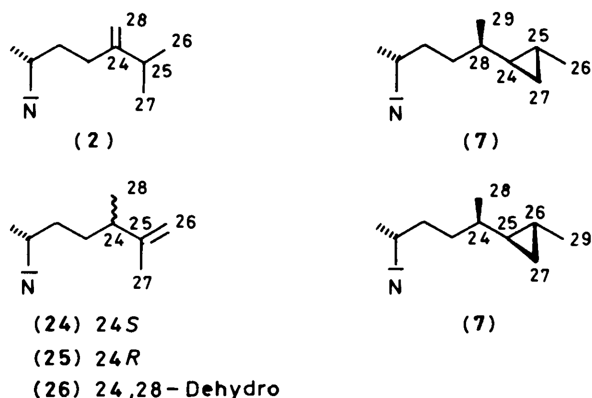
Scheme 3 has several implications in regard to petrosterol biosynthesis. First, 24-methylenecholesterol (**2**), rather than epicodisterol (**24**), codisterol (**25**), or the diene (**26**), should be the immediate biosynthetic precursor of (**7**) (Scheme 4).[24] Second, this mechanism predicts an extensive and specific rearrangement of the cholesterol side-chain carbon atoms [note side-chain carbon numbering in (**7**), Scheme 4]—a prediction which is testable by the feeding of specifically labelled precursors.¶

Scheme 5 depicts our proposed biosynthesis of ficisterol (**22**)[17] and the recently isolated hebesterol (**23**).[10b] It follows a course similar to that of Scheme 3 except that, now, the proton labilizes the C(24)–C(28) bond and nucleophilic attack occurs from C-26 [see (**3c**),(**3d**) Scheme 5]. Further izomerization can

¶ We have shown that this is the case in petrosterol (**7**) biosynthesis and that the predicted rearrangement of carbon atoms occurs.[24] The same implications apply to sterol (**21**) but since it is only a minor component of the *Petrosia* sterol mixture it is unlikely that they will be tested until a sponge is found which contains larger amounts of this rare sterol.



Scheme 3.



Scheme 4.

lead to ficisterol (**22**) while immediate proton loss gives hebesterol (**23**). In Scheme 3 the absolute stereochemistry of the product sterols (**7**) and (**21**) was known. In this instance, at the time of development of this hypothesis, the stereochemistry of ficisterol (**22**) and hebesterol (**23**) had not been determined. The rationale in Scheme 5 was used to predict these absolute stereochemistries and subsequent stereocontrolled partial syntheses and chemical correlations proved these predictions to be correct.[10b,17c] The scheme contains the assumption that the cyclopropane ring is *trans* substituted (subsequently proven to be correct), which is based on the observation that all the 1,2-disubstituted marine cyclopropylsterols isolated to date.[10b,15,25] have had the least congested substitution pattern around the cyclopropane ring.

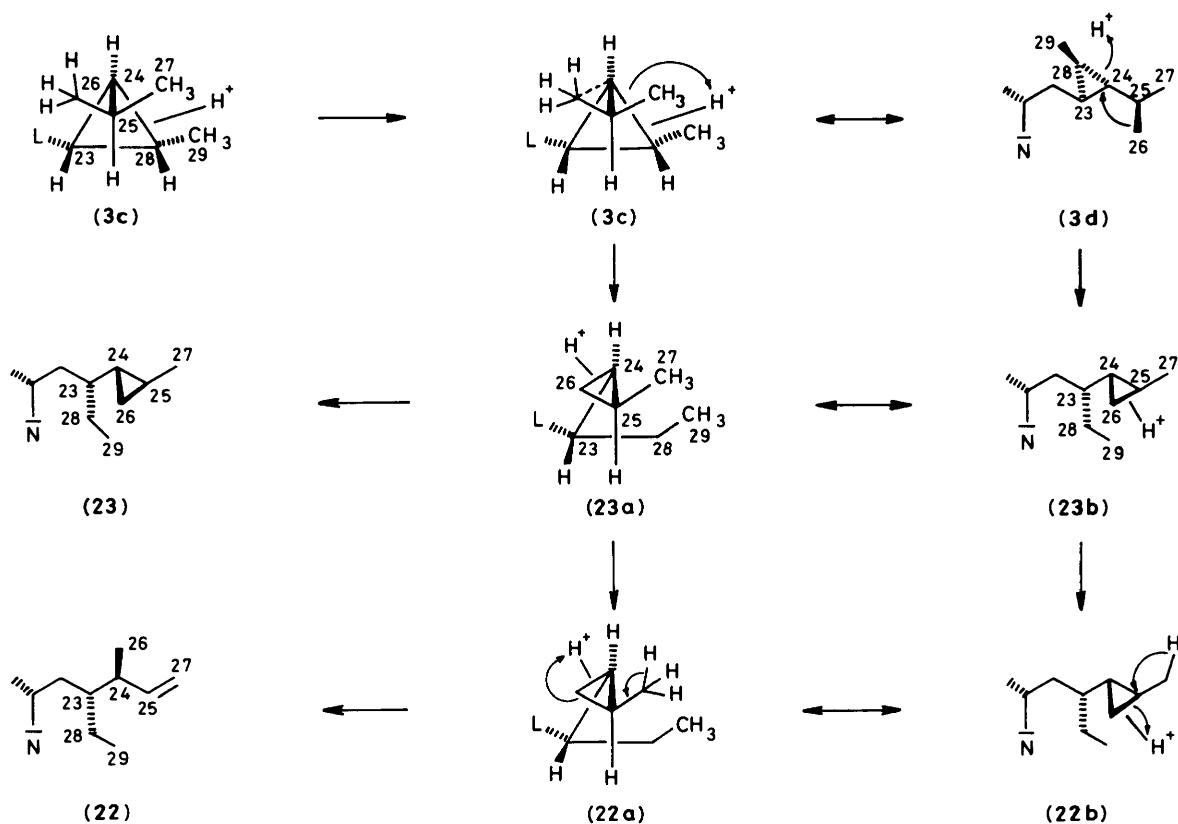
Such a biosynthesis (Scheme 5) for the 23-ethyl sterols (**22**) and (**23**) differs fundamentally from that previously hypothesized[12] (Scheme 6), which involved several reactions (biomethylation, reduction, demethylation) on the unknown 23-methylene-24-methylcholesterol (**27**).[26]

The biosynthesis of nicasterol (**8**)[5] may be depicted as in Scheme 7 with the tertiary carbon atom at C-25, and not the primary methyl carbons at C-26 and C-27, involved in cyclopropane formation. The proton labilizes the C(23)–C(28) bond and a new bond is formed between C-23 and C-25. As in all previous cases the derived absolute stereochemistry corresponds to that of the natural sterol.

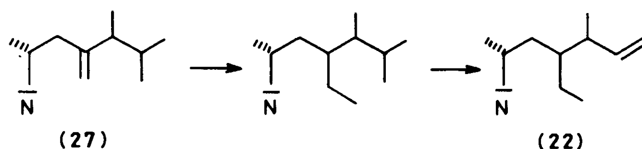
The biosynthetic outline presented in Schemes 3, 5, and 7 receives support from the co-occurrence of the various sterols, their stereochemical interrelationships, and the incorporation experiments which have thus far been carried out.[24] It may also be extended to encompass the biosynthesis of other marine sterols.

The structural and stereochemical relationship between dihydrocalysterol (**1**) and ficisterol (**22**) is reflected by 23,24-methylenecholesterol (**28**)[9b] and norficisterol (**29**).[17] A scheme similar to Scheme 5 could be drawn postulating a biosynthetic relationship which would also include the as yet unknown 29-norhebesterol (**30**).

Returning to the biosynthesis of the unusual sterols (**1**), (**7**), (**21**), (**22**), (**8**), and (**23**), we are faced with two fundamental questions. First, although our biosynthetic scheme can explain inductively the absolute stereochemistries of these diverse sterols and the origin of the side-chain carbon atoms of petrosterol (**7**) in terms of the protonated dihydrocalysterol species (**3a**), we are still ignorant of the mechanism which produces this species. In particular, we would like to know the

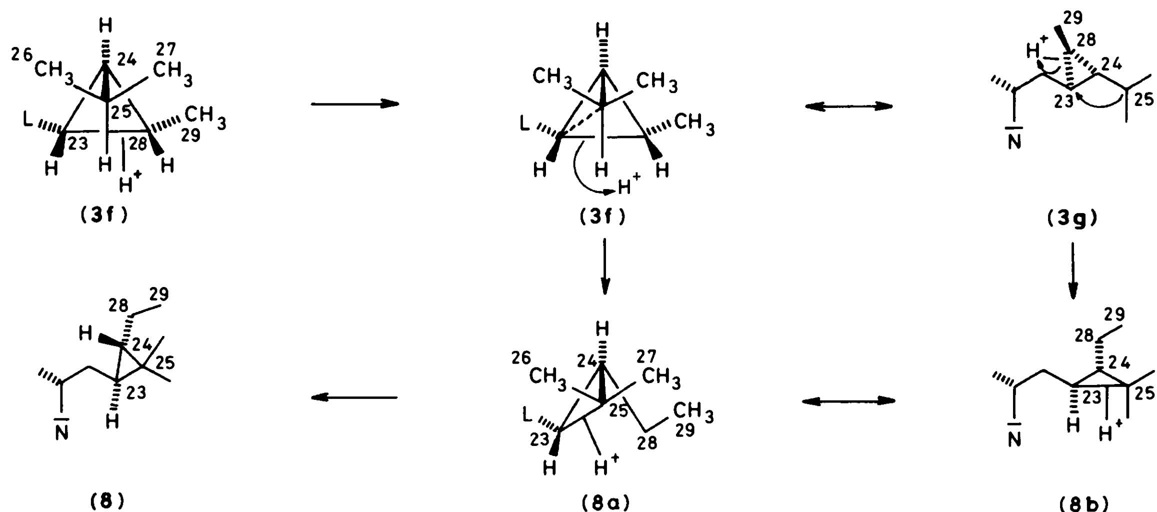


Scheme 5.

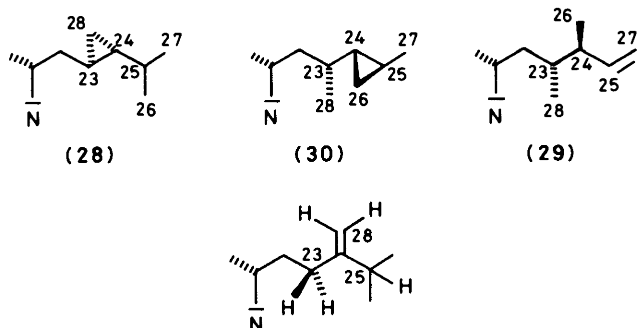


Scheme 6.

origin of the hydrogen atom at C-24 of dihydrocalysterol (**1**), *i.e.* whether it originally was the *E*-or *Z*–C(28)–H, the pro-*R* or pro-*S* C(23)–H or the C(25)–H of 24-methylenecholesterol (**2**). The elucidation of the further details of this mechanism rests on this knowledge and preliminary incorporation experiments towards this end are currently under way. Second, we may ask why, in the sponges *P. ficiformis*, *P. hebes* and *C. niceaensis*, the biomethylation of 24-methylenecholesterol produces such unprecedented structures rather than sterols of the more commonly encountered 24-ethyl or ethylidene type. This is related to the first question in that it concerns the details of the biomethylation-enzyme mechanism. Two alternatives seem to be possible. The basic residue which finally abstracts the proton may be located in such a position as to render unfavourable proton loss from C-28 or C-25 or the particular conformation of the sterol on the active site might achieve the same end.



Scheme 7.



Conclusions

We suggest that the biosyntheses of a number of unique marine sterols can be integrated into a unified biosynthetic scheme involving a protonated dihydrocalysterol species. This scheme has specific stereochemical implications, some of which have now been verified, thus strengthening the likelihood that we have a description of the processes actually operating at the enzymatic level. At present, the question is still open as to whether the structures depicted in this scheme represent distinct intermediate species or different descriptions of a stereochemically well defined transition state. The present synthesis of the sterol (**1**) (Scheme 2), opening the way to a variety of labelled dihydrocalysterols for incorporation experiments, should help to shed light on this question.

Experimental

High performance liquid chromatography (h.p.l.c.) was carried out on a Waters Associates HPLC system (M6000 pump, R403 differential refractometer). Two Altex Ultrasphere ODS 5 μm columns (10 mm i.d. × 25 cm) were used in series with methanol as the mobile phase. Analytical gas-liquid chromatography was performed on a Hewlett-Packard 402A gas chromatograph with a flame ionization detector and a U-shaped glass column (2 mm i.d. × 1.8 m) packed with 3% OV-17 on Gas Chrom Q. The oven temperature was 260 °C with helium as the carrier gas. Fourier transform ^{1}H n.m.r. spectra were recorded on a Nicolet Magnetics Corporation NMC-300 spectrometer equipped with a 1 280 data system and operating at 300 MHz. Spectra were referenced to either $CHCl_3$ (7.26 p.p.m.) or C_6H_6 (7.15 p.p.m.). Low resolution mass spectra were obtained with a Hewlett-Packard 5 995 spectrometer in either g.c.–m.s. or d.i. modes. High resolution mass spectra were determined on an MS-50 instrument at the University of California at Berkeley. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter.

(Z)-6β-*Methoxy*-3α,5-*cyclo*-26,27-*dinorcholest*-23-*ene* (**10**).—To a suspension of ethyltriphenylphosphonium bromide (680 mg, 1.83 mmol) in dry THF (10 ml) at 0 °C under argon was added butyl-lithium (1.3M in hexane; 1.4 ml). The mixture was stirred at 0 °C for 0.5 h. This solution was then added dropwise, *via* a syringe, to a solution of aldehyde (**9**)[5] (280 mg, 0.78 mmol) in dry THF (10 ml) at 0 °C under argon. After 1 h the reaction was quenched by the addition of methanol (0.5 ml) and the reaction mixture was evaporated to dryness. Fractionation of the crude mixture over silica (eluant CH_2Cl_2–hexane, 2:3) gave the olefinic fraction (219 mg, 75%) as a 4:1 mixture of *Z*:*E* isomers (^{1}H n.m.r. and g.c. analysis). Purification of the *Z*-isomer could be accomplished by argentic t.l.c. with toluene–hexane (3:2) as developer; ^{1}H n.m.r. (300 MHz; $CDCl_3$) δ 5.32—5.58 (2 H, complex, 23-and 24-H), 3.323 (3 H, s, OCH_3), 1.595 (3 H, d, *J* 6.1 Hz, 25-H), 1.021 (3 H, s, 19-H), 0.910 (3 H, d, *J* 6.5 Hz, 21-H), and 0.724 (3 H, s, 18-H); *m/z* (assignment, relative intensity) 370.3239 (M^+, 40, $C_{26}H_{42}O$ requires 370.3235), 355.2993 ($C_{25}H_{39}O$, 42), 338.2964 ($C_{25}H_{38}$, 68), 323.2750 ($C_{24}H_{35}$, 12), 315.2717 ($C_{22}H_{35}O$, 100), 283.2437 ($C_{21}H_{31}$, 44), 255.2128 ($C_{19}H_{27}$, 13), 253.1979 ($C_{19}H_{25}$, 13), 229.1951 ($C_{17}H_{25}$, 13), 227.1803 ($C_{17}H_{23}$, 14), 217.1968 ($C_{16}H_{25}$, 31), 215.1791 ($C_{16}H_{23}$, 17), 213.1654 ($C_{16}H_{21}$, 16), and 201.1633 ($C_{15}H_{21}$, 17).

The *E*-isomer (**10a**) possessed the following spectral characteristics: δ (300 MHz; $CDCl_3$) δ 5.38 (2 H, complex, 23- and 24-H), 3.321 (3 H, s, OCH_3), 1.657 (3 H, d, *J* 3.3 Hz, 25-H), 1.016 (3 H, s, 19-H), 0.897 (3 H, d, *J* 6.7 Hz, 21-H), and 0.712 (3 H, s, 18-H); *m/z* (relative intensity) 370 (M^+, 45), 355 (65), 338 (50), 323 (12), 315 (100), 283 (36), 255 (7), 253 (10), 229 (5), 227 (12), 217 (11), 215 (11), 213 (14), and 201 (15) (Found: M^+, 370.3237. $C_{26}H_{42}O$ requires M^+, 370.3235).

Carbene Addition to (Z)-6β-*Methoxy*-3α,5-*cyclo*-26,27-*dinorcholest*-23-*ene* (**10**).—To the neat olefin (**10**) (28 mg, 0.075 mmol) was added tris(trimethyl phosphite)copper(I) iodide (10 mg). Ethyl diazoacetate was added to this mixture with vigorous stirring (1 drop/5 min) until no further starting material was consumed (t.l.c. monitoring). The crude reaction was filtered through a short silica plug (eluant CH_2Cl_2) and evaporated under reduced pressure to remove the volatile materials. Chromatography over silica gel (eluant hexane–CH_2Cl_2) gave, in order of elution, recovered starting material (**10**) (3 mg, 11%) and the mixture of esters (**11**) (27 mg, 74%); *m/z* (relative intensity) 456 (M^+, 36), 441 (50), 424 (50), 409 (15), 401 (100), 398 (19), 303 (12), 285 (11), 283 (11), 255 (56), 253 (29), 229 (27), 215 (15), 213 (39), and 201 (19) (Found: M^+, 456.3595. $C_{30}H_{48}O_3$ requires M^+, 456.3591).

The mixture of esters obtained was used directly in the next reaction.

Synthesis of the Tertiary Alcohols (**12**) *and* (**13**).—To the ester fraction (**11**) (27 mg, 0.059 mmol) in dry THF (2 ml) was added methyl-lithium (1.5M in ether; 2 ml, 50 equiv.). The reaction mixture was allowed to come to room temperature over 2 h, when t.l.c. monitoring showed that no starting material remained. The reaction was quenched by addition of ethyl acetate and the organic phase was washed with water, dried ($MgSO_4$), filtered, and evaporated. Chromatography over silica (eluant CH_2Cl_2–hexane gradient) gave three fractions.

Fraction 1 was a mixture of all-*cis*-cyclopropanes (5.1 mg, 20%), which was not investigated further.

Fraction 2 gave (23S,24S,28R)-24-*ethyl*-25-*hydroxy*-6β-*methoxy*-3α,5:23,25-*dicyclocholestane* (**12**) (6.7 mg, 26%); δ (300 MHz; $CDCl_3$) δ 3.322 (3 H, s, OCH_3), 1.178 (3 H, s, 26- or 27-H), 1.168 (3 H, s, 26- or 27-H), 1.011 (6 H, 2 × d, *J* not available, 21- and 29-H), 1.013 (3 H, s, 19-H), 0.724 (3 H, s, 18-H), and 0.353 (1 H, dd, *J* 5.2 Hz, 5.2 Hz, 25-H); *m/z* (assignment, relative intensity) 442.3805 (M^+, 10, $C_{30}H_{50}O_2$ requires 442.3798), 427.3561 ($C_{29}H_{47}O_2$, 7), 424.3693 ($C_{30}H_{48}O$, 6), 410.3555 ($C_{29}H_{46}O$, 5), 392.3450 ($C_{29}H_{44}$, 9), 387.3257 ($C_{26}H_{42}O_2$, 14), 310.2645 ($C_{23}H_{34}$, 5), 283.2425 ($C_{21}H_{31}$, 8), 255.2096 ($C_{19}H_{27}$, 10), 253.1965 ($C_{19}H_{25}$, 100), 227.1795 ($C_{17}H_{23}$, 11), 213.1642 ($C_{16}H_{21}$, 8), and 199.1490 ($C_{15}H_{19}$, 5).

Fraction 3 gave (23R,24R,28S)-24-*ethyl*-25-*hydroxy*-6β-*methoxy*-3α,5:23,28-*dicyclocholestane* (**13**) (7.9 mg, 30%); δ (300 MHz; $CDCl_3$) δ 3.325 (3 H, s, OCH_3), 1.186 (3 H, s, 26- or 27-H), 1.163 (3 H, s, 26- or 27-H), 1.024 (3 H, s, 19-H), 1.014 (6 H, d, *J* 6.6 Hz, 21- and 29-H), 0.729 (3 H, s, 18-H), and 0.261 (1 H, dd, *J* 5.1 Hz, 5.1 Hz, 25-H); *m/z* (relative intensity) 442 (M^+, 6), 427 (5), 424 (3), 410 (4), 392 (2), 387 (10), 310 (4), 285 (14), 283 (5), 255 (9), 253 (32), 227 (14), 213 (9), 199 (7), and 43 (100) (Found: M^+, 442.3806. $C_{30}H_{50}O_2$ requires M^+, 442.3798).

Typical Procedure for the Dehydration of the Alcohols (**12**) *and* (**13**).—The alcohol (7 mg, 0.0178 mmol) was dissolved in dry pyridine (0.5 ml) and phosphorus oxychloride (0.15 ml, 1.6 mmol, 100 equiv.) was added. The mixture was left undisturbed at room temperature for 12 h and then quenched by the cautious addition of water. Ethyl acetate was added and the organic phase was worked up in the usual manner. Chromatography over silica (eluant CH_2Cl_2) gave the olefin (5.6 mg, 83%) and recovered starting material (1.1 mg, 15%).

(23S,24S,28R)-24-*Ethyl-25-dehydro-6β-methoxy-3α,5:23,25-dicyclocholestane* (**14**). This showed δ (300 MHz; $CDCl_3$) 4.596 (2 H, br s, 26-H), 3.321 (3 H, s, OCH_3), 1.619 (3 H, s, 27-H), 1.057 (3 H, d, *J* 5.9 Hz, 28-H), 1.019 (3 H, s, 19-H), 1.001 (3 H, d, *J* 6.9 Hz, 21-H), and 0.725 (3 H, s, 18-H); *m/z* (relative intensity) 424 (M^+, 2), 409 (3), 392 (11), 377 (6), 369 (6), 327 (3), 311 (5), 310 (5), 287 (6), 285 (6), 283 (6), 281 (4), 253 (100), 227 (8), 213 (10), and 201 (6).

(23R,24R,28S)-24-*Ethyl-25-dehydro-6β-methoxy-3α,5:23,28-dicyclocholestane* (**15**). This showed δ (300 MHz; $CDCl_3$) 4.593 (2 H, br s, 26-H), 3.322 (3 H, s, OCH_3), 1.164 (3 H, s, 27-H), 1.045 (3 H, d, *J* 5.2 Hz, 28-H), 1.019 (3 H, s, 19-H), 0.992 (3 H, d, *J* 6.5 Hz, 21-H), and 0.727 (3 H, s, 18-H); *m/z* (relative intensity) 424 (M^+, 2), 409 (5), 392 (5), 377 (3), 369 (8), 327 (4), 311 (3), 310 (3), 287 (6), 285 (5), 283 (4), 281 (5), 253 (100), 227 (6), 213 (6), and 201 (3).

Conversion of the Olefins (**14**) *and* (**15**) *into Dihydrocalysterol* (**1**) *and Its Epimer* (**16**).—The olefin (5 mg, 0.01 mmol), dissolved in propanol (1 ml), was stirred under H_2 in the presence of 5% Rh/C catalyst (2 mg) for 2 h. The reaction mixture was filtered and evaporated. Hydrolysis of the i-methyl ether protecting group was accomplished the usual manner. The free sterols obtained were purified by reverse phase h.p.l.c. (mobile phase; methanol).

(23S,24S,28R)-24-*Ethyl-23,28-cyclocholest-5-en-3β-ol* (**1**) (dihydrocalysterol). This had m.p. 159—161 °C; $[\alpha]_D^{20}$ −56° (*c* 0.8, CH_2Cl_2); δ (300 MHz; $CDCl_3$ data are given in the Table; *m/z* (relative intensity) 412 (M^+, 12), 397 (2), 394 (2), 379 (7), 356 (2), 328 (4), 314 (24), 300 (12), 285 (5), 283 (10), 281 (8), 273 (9), 271 (100), 255 (12), 253 (13), 243 (8), 231 (13), 229 (15), 217 (14), 215 (18), 213 (30), and 201 (10) (Found: M^+, 412.3714. $C_{29}H_{48}O$ requires M^+, 412.3705).

(23R,24R,28S)-24-*Ethyl-23,28-cyclocholest-5-en-3β-ol* (**16**). This showed δ (300 MHz; $CDCl_3$) 1.016 (3 H, d, *J* 6.2 Hz, 21-H), 1.013 (3 H, s, 19-H), 0.967 (3 H, d, *J* 5.9 Hz, 24-H), 0.928 (6 H, br s, 26- and 27-H), 0.696 (3 H, s, 18-H), 0.483 (2 H, complex, 23- and 28-H), −0.216 (2 H, complex, 24-H); *m/z* (relative intensity) 412 (M^+, 17), 397 (10), 394 (10), 356 (10), 328 (21), 314 (25), 300 (46), 285 (14), 283 (24), 281 (19), 273 (14), 271 (100), 255 (19), 253 (15), 243 (13), 231 (14), 229 (15), 217 (9), 213 (26), and 201 (8) (Found: M^+, 412.3716. $C_{29}H_{48}O$ requires M^+, 412.3705).

The purification of dihydrocalysterol (**1**) by h.p.l.c. also yielded a small amount [*ca.* 5% relative to (**1**)] of (23S)-*ethylcholest-5-en-3β-ol* (**17**); δ (300 MHz; $CDCl_3$) 1.006 (3 H, s, 19-H), 0.886 (3 H, d, *J* 7.0 Hz, 21-H), 0.863 (3 H, d, *J* 6.9 Hz, 26- or 27-H), 0.834 (3 H, d, *J* 6.6 Hz, 26- or 27-H), 0.794 (3 H, t, *J* 7.2 Hz, 29-H), and 0.689 (3 H, s, 18-H); *m/z* (relative intensity) 414 (M^+, 100), 399 (44), 396 (53), 381 (36), 357 (6), 354 (10), 342 (5), 329 (68), 303 (46), 273 (46), 255 (46), 231 (33), 229 (18), and 213 (59) (Found: M^+, 414.3857. $C_{29}H_{50}O$ requires M^+, 414.3849).

Hydrogenolysis of Dihydrocalysterol (**1**).—Dihydrocalysterol (**1**) (1.5 mg) was stirred under H_2 in acetic acid–ethyl acetate (1:1; 1 ml) in the presence of PtO_2 for 24 h. The reaction mixture was filtered, evaporated, and fractionated by reverse-phase h.p.l.c. (mobile phase; methanol) to give two fractions in 1:1 ratio. Fraction 1, the more polar, was identified as dihydrocalystanol,[1] which was recycled. Fraction 2 was identified as (24*S*)-ethylcholestan-3β-ol (**18**) by comparison with literature data[1,27] (overall yield 90% by h.p.l.c. traces after recycling of dihydrocalystanol); δ (300 MHz; $CDCl_3$) 0.903 (3 H, d, *J* 6.3 Hz, 21-H), 0.846 (3 H, t, *J* 7.4 Hz, 29-H), 0.824 (3 H, d, *J* 6.5 Hz, 26- or 27-H), 0.803 (3 H, d, *J* 6.4 Hz, 26- or 27-H), 0.798 (3 H, s, 19-H), and 0.643 (3 H, s, 18-H); *m/z* (relative intensity) 416 (M^+, 43), 401 (25), 398 (3), 383 (11), 359 (5), 318 (3), 316 (4), 303 (4), 300 (4), 290 (13), 285 (4), 281 (3), 271 (6), 257 (6), 255 (5), 248 (9), 233 (98), 231 (11), 219 (12), 215 (100), 206 (6), and 201 (13) (Found: M^+, 416.4025. $C_{29}H_{52}O$ requires M^+, 416.4005).

Table. Comparison of the ^1H n.m.r. data of natural (360 MHz) and synthetic (300 MHz) dihydrocalysterol (**1**)

C–H	Natural (**1**)[a]		Synthetic (**1**)	
Signal	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
18	0.688	0.705	0.688	0.704
19	1.009	0.943	1.009	0.943
21	1.019, 6.9	1.147, 6.7	1.021[c]	1.146, 6.6
23, 28	0.439	0.544, 0.486	0.440	0.511
26[b]	0.930	1.049, 6.1	0.935	1.048, 5.9
27[b]	0.929	1.004, 5.9	0.922	1.003, 5.6
29	0.990	1.083, 6.1	0.990	1.081, 6.1
24	−0.126	−0.066	−0.127	−0.066

[a] Natural material was isolated from *C. niceaensis*;[1] differences to the spectra reported in ref. 1 are due to misassignment of peak multiplicity in this reference. The 26- and 27-methyl groups, for the spectrum recorded in $CDCl_3$, appear as broad singlets, and not as overlapping doublets as originally reported. This occurs because the methyl groups coincidentally absorb at the same frequency as 25-H—an effect probably due to a combination of the propinquity of the cyclopropane ring, and the solvent. When the spectrum is recorded in C_6D_6 the signals are well separated and appear as doublets. [b] Assignments are interchangeable. [c] Coupling constant not available.

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