Minor and Trace Sterols in Marine Invertebrates. 31.[1] Isolation and Structure Elucidation of 23*H*-Isocalysterol,[2] a Naturally Occurring Cyclopropene. Some Comparative Observations on the Course of Hydrogenolytic Ring Opening of Steroidal Cyclopropenes and Cyclopropanes

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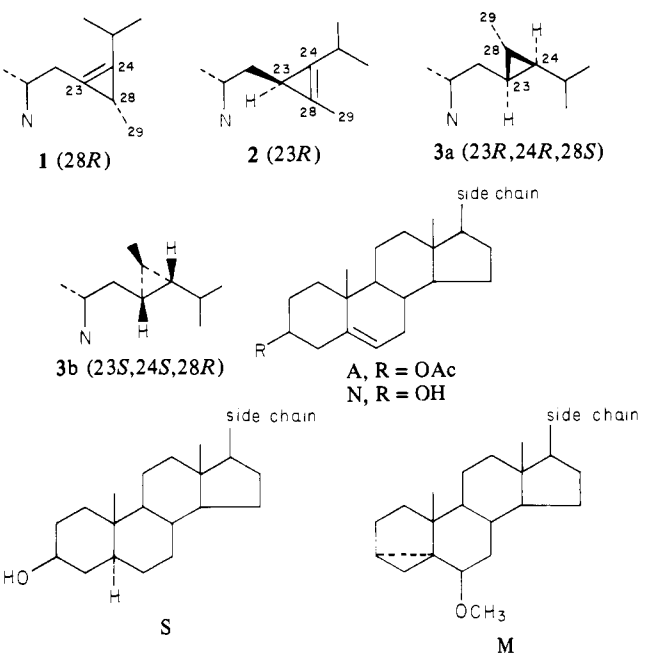
Abstract: A new naturally occurring cyclopropene, 23*H*-isocalysterol (**2**), and a novel steroidal cyclopropane, 23,24-dihydrocalysterol (**3**), were isolated from the sponge *Calyx niceaensis*. Structure elucidation was accomplished by both NMR and mass spectroscopic analyses. Catalytic hydrogenation and detailed NMR analysis of the products led to the determination of the absolute configuration of calysterol (**1**) (28*R*), 23*H*-isocalysterol (**2**) (23*R*), and 23,24-dihydrocalysterol (**3b**) (23*S*,24*S*,28*R*). Partial synthesis of several hydrogenolysis products as well as a synthetic approach to 23,24-dihydrocalysterol are also reported.

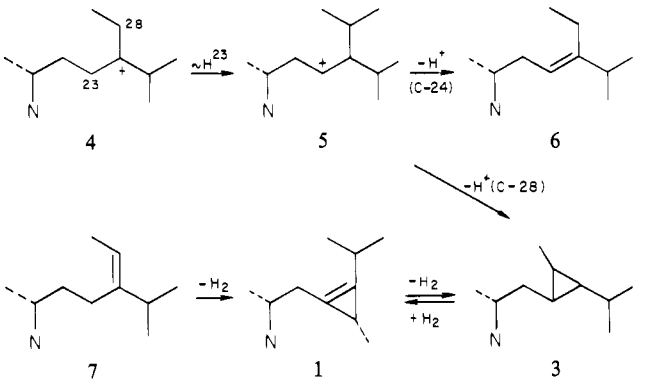
Among the great variety of marine sterols with unusual side-chain substitution patterns,[4] calysterol (**1**)[2,5] (Chart I)—the principal sterol component of the sponge *Calyx niceaensis*—contains one of the most intriguing functionalities since it represents the sole steroidal cyclopropene encountered in nature. Subsequent investigations led to the isolation of new sterols such as stigmasta-5,23-dien-3β-ol (**6**)[6] and to the significant observation[7] that the ubiquitous marine sterol fucosterol (**7**) (presumably a component of the sponge's diet) was an intermediate in the biosynthesis of calysterol (**1**). This finding is consistent with the biosynthetic sequence[8] outlined in Scheme I, which predicts that the hitherto unknown sterol 23,24-dihydrocalysterol (**3**) should also occur in nature. Given the continuous refinement of sterol separation techniques in our laboratory, we decided to reinvestigate the sterol mixture of *Calyx niceaensis* and to search for missing links that may be of biosynthetic significance and also shed light on the complicated food chain in the marine environment.

In the present paper we report the isolation and the structure elucidation of two new three-membered-ring-containing sterols, 23*H*-isocalysterol (**2**) and the earlier predicted[8] 23,24-dihydrocalysterol (**3**) (Chart I). Furthermore, catalytic hydrogenation of the steroidal cyclopropenes **1** and **2** led, among other interesting results, to the determination of the side-chain stereochemistry that even in the case of calysterol (**1**) had not yet been fully elucidated. Additionally, we present all important spectroscopic properties, notably the NMR and mass spectroscopic data, and the synthesis of all compounds that were produced by reductive hydrogenolytic ring cleavage of **1-3**. Such ring cleavage is of intrinsic interest—outside the field of steroids—because relatively little is known about the stereochemical aspects of cyclopropene ring fission.

Chart I



Scheme I



(1) For part 30 in this series, see: Li, L. N.; Djerassi, C. *Tetrahedron Lett.* **1981**, 4639.

(2) These trivial names are derived from the cyclopropene-containing sterol calysterol (**1**), whose systematic IUPAC name is 23,28-cyclostigmasta-5,23-dien-3β-ol. 23*H*-Isocalysterol (**2**) and 23,24-dihydrocalysterol (**3**) may, therefore, also be named as 23,28-cyclostigmasta-5,24(28)-dien-3β-ol and 23,28-cyclostigmast-5-en-3β-ol, respectively.

(3) (a) Visiting investigator (1979-1981) from the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China. (b) Visiting investigator (1980-1982) on leave from Shanghai Institute of Pharmaceutical Industrial Research, Shanghai, China. (c) Swiss NSF postdoctoral fellow (1981-1982). Present address: Central Research Department, Ciba-Geigy AG, Basel, Switzerland. (d) Istituto di Chimica Organica dell'Universita di Napoli, Naples, Italy.

(4) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873-890 and references cited therein.

(5) Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C.; Sica, D. *Tetrahedron* **1975**, *31*, 1715-1716.

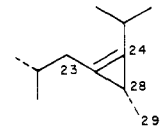
(6) Steiner, E.; Djerassi, C.; Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C.; Sica, D. *Helv. Chim. Acta* **1977**, *60*, 475-481.

(7) Minale, L.; Riccio, R.; Scalona, O.; Sodano, G.; Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C. *Experientia*, **1977**, *33*, 1550-1552.

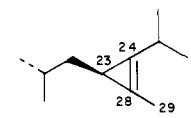
(8) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, *51*, 1815-1828.

0002-7863/82/1504-6726$01.25/0

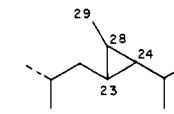
Table I. Physical and Selected Spectroscopic Data of 1-3



1



2



3

	1	2	3
molecular formula	$C_{29}H_{46}O$	$C_{29}H_{46}O$	$C_{29}H_{48}O$
M^+ (m/z)	410.355	410.354	412.370
mp, °C (solvent)	111-112 ($MeOH/Et_2O$)	115-116 ($MeOH/Et_2O$)	
$[\alpha]^{20}_{589}$, deg (c, solvent)	−30.0 (0.006, $CHCl_3$)	−47.3 (0.004, $CHCl_3$)	
t_R HPLC (ODS-2, calysterol = 1.00)	1.00	1.31	1.68
t_R GLC (OV-17, cholesterol = 1.00)	1.20	1.62	1.42
	Selected ^1H NMR (360 MHz, $CDCl_3$) Chemical Shifts[a]		
3H-C(18)	0.700	0.692	0.688
3H-C(19)	1.010	1.010	1.009
3H-C(21)	0.965 (6.6)	1.006 (6.0)	1.019 (6.9)
2H-C(23,28)			0.439
1H-C(25)	2.694 (6.8)	2.687 (6.4, 1.4)	not assigned
6H-C(26,27)	1.105 (6.8), 1.097 (6.8)	1.151 (6.8), 1.099 (6.8)	0.930 (6.0), 0.929 (6.0)
1H-C(28)	1.330 (4.1)		
3H-C(29)	0.999 (4.5)	1.994 (1.4)	0.990 (6.8)
	Main Mass Spectral Fragments[b]		
	410 (15)	410 (8)	412 (33)
	395 (10)	395 (2)	397 (6)
	392 (1)	314 (4)	394 (9)
	377 (2)	300 (11)	379 (6)
	367 (33)	271 (82)	356 (4)
	349 (6)	253 (7)	328 (7)
	300 (10)	231 (3)	314 (41)
	271 (87)	213 (6)	300 (25)
	253 (20)	96 (65)	273 (10)
	231 (8)	95 (100)	271 (100)
	213 (18)		255 (13)
	110 (100)		231 (7)
			213 (15)

[a] Given as δ values; J values are given in parentheses in hertz. [b] Low-resolution data (MAT 711) obtained at 70 eV.

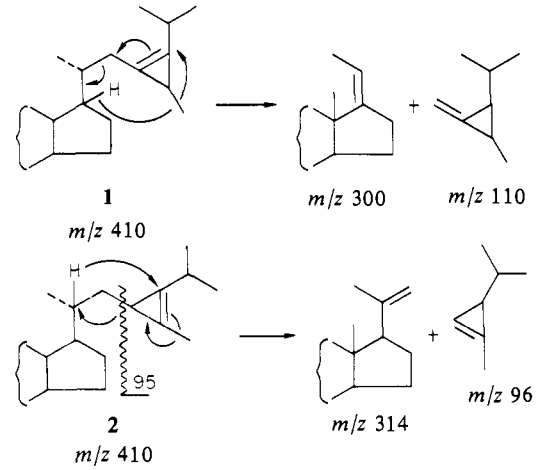
Results and Discussion

The crude sterol mixture of *Calyx niceaensis*[9] was crystallized from absolute methanol and subsequently separated by reverse-phase HPLC (ODS-2) to give 52% calysterol (**1**), 15% 23*H*-isocalysterol (**2**), and 1% 23,24-dihydrocalysterol (**3**). The most important physical and spectroscopic properties of these three sterols are summarized in Table I.

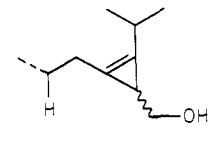
A comparison (cf. Table I) of the ^1H NMR data of calysterol (**1**) and 23*H*-isocalysterol (**2**) points to the C-29 proton resonances as the diagnostically most significant ones. The observed low-field shift in the spectrum of **2** is consistent with the presence of an olefinic methyl group. This conclusion is strongly supported by the magnitude of a ^4J(H,H) coupling constant of 1.42 Hz, which originiates from either H-C(23) or H-C(25). Irradiation experiments at 1.994 and 2.687 ppm show for the latter resonance a simultaneous decoupling of the C-26,27 methyl signals (1.151 and 1.009 ppm), thus pointing to H-C(25) as the actual coupling partner. In addition, the absence of any olefinic proton signal, aside from H-C(6) of the nuclear unsaturation, also indicates a tetrasubstituted side-chain double bond.

The structure elucidation of 23*H*-isocalysterol (**2**) was further supported by comparison of its mass spectroscopic fragmentation pattern with that of its stereoisomer **1** (Scheme II). The diagnostic peaks at m/z 271, 253, 231, and 213[10] indicate for both compounds two degrees of side-chain unsaturation in a 3β-hydroxy-Δ^5-sterol. A McLafferty type rearrangement,[11] which leads in calysterol (**1**) to the m/z 300 and 110 fragments, reflects itself in the spectrum of 23*H*-isocalysterol (**2**) by the m/z 314 and 96 fragments (Scheme II). The base peak fragment of mass 95 in **2** originates from an allylic cleavage of the 22,23 bond.

Scheme II



While these ^1H NMR and mass spectroscopic interpretations unraveled the side-chain structures of both steroidal cyclopropenes **1** and **2**, the absolute configuration at C-28 in calysterol (**1**) and at C-23 in 23*H*-isocalysterol (**2**) remained to be determined. Since an earlier synthetic approach[6] to calysterol (**1**) ended at the stage of the homoallylic alcohol **8**, indirect proof of the stereochemistry



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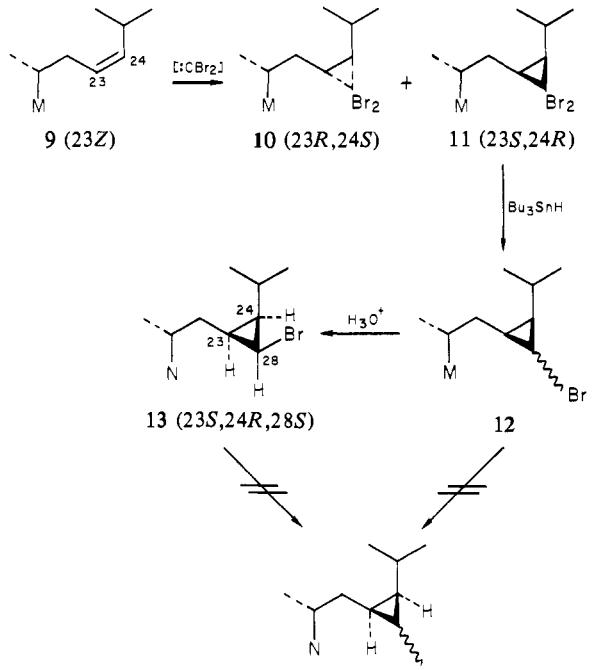
(9) The sponge *Calyx niceaensis* from which the sterol extract was derived was collected in the Bay of Toronto.

(10) Wyllie, S. G.; Amos, B. A.; Tokes, L. *J. Org. Chem.* **1977**, *42*, 725-731.

(11) (a) Massey, I. J.; Djerassi, C. *J. Org. Chem.* **1979**, *44*, 2448-2456. (b) Wyllie, S. G.; Djerassi, C. *Ibid.* **1968**, *33*, 305-313.

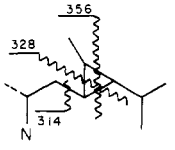
Scheme III



in question was attempted by hydrogenolytic reduction of the cyclopropene moiety to its saturated analogues. It was hoped that the spectroscopic data of the resulting cyclopropanes could be related to those of known diastereoisomerically pure steroidal cyclopropanes (vide infra).

The constitution of the side chain of the minor component 23,24-dihydrocalysterol (**3**) was primarily established by ^{1}H NMR spectroscopic analysis (cf. Table I).[12] Irradiation experiments at 0.990 and 0.439 ppm led to the assignment of both the C-29 methyl group and H–C(28). The remaining 1 H moiety of δ 0.439 multiplet could be ascribed to H–C(23). The remarkably high-field shifted three-membered ring hydrogen (d × d × d) resonance signal (−0.126 ppm) with a larger 8-Hz and two smaller 4.5-Hz coupling constants can only be linked to the C-24 position. On the assumption that the smaller 4.5-Hz couplings are due to the vicinity of H-C(23) and H-C(28), their positions on the three-membered ring have to be trans to H-C(24).[13] Indeed, such a cyclopropane alkyl substitution pattern, in which the bulky isopropyl group is situated trans to the sterically less demanding methyl and *n*-alkyl groups, is also from the stereochemical viewpoint the most favorable one. This, in turn, leads to the two possible diastereoisomeric formulations (23*R*,24*R*,28*S*)-**3a** and (23*S*,24*S*,28*R*)-**3b** in Chart I as the only possible ones for naturally occurring 23,24-dihydrocalysterol, whose eventual isolation had been predicted earlier.[8]

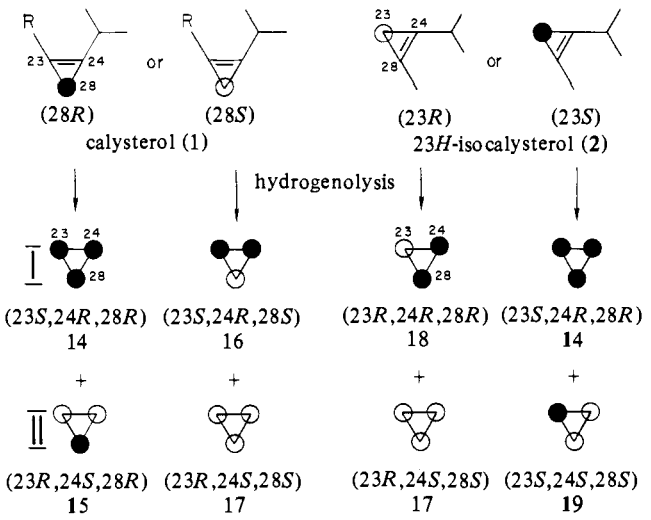
The mass spectrum of 23,24-dihydrocalysterol (**3**) shows besides



3

the typical fragments of a 3β-hydroxy-Δ^5-sterol (vide supra) three diagnostic peaks at *m/z* 356, 328, and 314, which confirm the cyclopropane-containing side chain of **3**.

Chart II[a]



[a] (•) Hydrogen above the plane; (○) hydrogen below the plane; (I) topside attack of H_2; (II) bottom side attack of H_2.

Further attempts at a conclusive assignment of the absolute configuration of the naturally occurring 23,24-dihydrocalysterol (**3**) were made by synthesis of authentic material with known absolute configuration. According to our most recent[14] synthesis of diastereoisomerically pure steroidal cyclopropanes, a synthetic approach to a particular diastereoisomer (23*R*,24*S*,28*R* and/or 28*S*) of **3** was envisaged (Scheme III). Dibromocarbene addition to (23*Z*)-6β-methoxy-3α,5-cyclo-5α-cholest-23-ene (**9**)[14] led to an easily separable (reverse-phase HPLC, ODS-2) mixture of **10** and **11** in addition to recovered **9**. Assignment of the absolute configuration of these dibromocyclopropanes was made by correlation of the ^{1}H NMR chemical shifts with those of the 23,24-dichloromethylene analogues.[14] Selective debromination[15] by using tributyltin hydride furnished the monobromo *i*-methyl ether **12**, whose 3β-hydroxy-Δ^5 system was recovered by acid-catalyzed hydrolysis to yield pure (23*S*,24*R*,28*S*)-23,24-bromomethylenecholest-5-en-3β-ol (**13**). The assigned cyclopropane substitution pattern was based on the H–C(28) resonance signal which for this particular isomer shows a d × d at 3.25 ppm and two coupling constants of 7.9 Hz. Such rather larger couplings are consistent with an all-cis hydrogen-substituted[16] three-membered ring. Several attempts were made to synthesize a 23,24-dihydrocalysterol (**3**) isomer from either **12** or **13**, but none were successful. The main difficulty was the generation of a cyclopropyllithium intermediate with either lithium metal,[17] methyllithium, or *n*-butyllithium[18] in ether or tetrahydrofuran as the solvents (even at elevated temperatures). Quenching with dimethyl sulfate or methyl iodide gave only the recovered monobromide and none of the desired product **3**.

Some Observations on the Catalytic Hydrogenolysis of Steroidal Cyclopropenes (1 and 2) and Cyclopropanes (3). It is well-known from the literature[19] that catalytic hydrogenation of cyclopropenes—in contrast to cyclopropanes—already takes place under very mild conditions at or below room temperature in the presence of palladium, platinum, or Raney nickel as catalysts. As expected, the main product is always the saturated cyclopropane, but ring opening[19,20] may also occur to yield a variety of isomeric hydro-

(12) ^{1}H NMR (360 MHz, C_6Dc_6) δ 1.083 (d, J = 6.12 Hz, 3 H, C-29, s upon irradiation at 0.486), 1.147 (d, J = 6.67 Hz, 3 H, C-21), 1.049 (d, J = 6.07 Hz, 3 H) and 1.004 (d, J = 5.85 Hz, 3 H, C-26 and C-27), 0.943 (s, 3 H, C-19), 0.705 (s, 3 H, C-18), 0.554 (m, 1 H, C-23), 0.486 (m, 1 H, C-28), −0.066 (ddd, J = 4.5, 8.0, 4.5 Hz, 1 H, C-24).

(13) Becker, E. D. "High Resolution NMR"; Academic Press: New York, 1980; p 95.

(14) Lang, R. W.; Djerassi, C. *J. Org. Chem.* **1982**, *47*, 625–633.

(15) (a) Seyferth, D.; Yamazaki, H.; Alleston, D. L. *J. Org. Chem.* **1963**, *28*, 703–706. (b) Kuivila, H. G. *Synthesis* **1970**, 499–509.

(16) Brugel, W. "Handbook of NMR Spectral Parameters"; Heyden: London, 1979; Vol. I, p 251.

(17) DePuy, C. H.; Funfschilling, P. C.; Andrist, A. H.; Olson, J. M. *J. Am. Chem. Soc.* **1977**, *99*, 6297–6303.

(18) Walborsky, H. M.; Impasato, F. J.; Young, A. E. *J. Am. Chem. Soc.* **1964**, *86*, 3283–3288.

(19) (a) Charton, M. In "The Chemistry of Alkenes"; Zabicky, J., Ed.; Interscience: London 1970; Vol. 2, 511. (b) Wendisch, D. *Methoden Org. Chem. (Houben-Weyl) 4th Ed.* **1971**, *3*, 1. (c) De Meijere, V. *Angew. Chem.* **1979**, *91*, 867–950.

Table II. Catalytic Hydrogenolysis of Calysterol (**1**), 23*H*-Isocalysterol (**2**), and 23,24-Dihydrocalysterol (**3**)

		HPLC,[a] t_R	GLC,[b] t_R	M$^+$, *m/z*	substrate, % 1[c]	2[d]	3[e]
	1.85	1.27	414	17		
	15 (23*R*,24*S*,28*R*)[g]	1.91	1.91	414	58		
	14 (23*S*,24*R*,28*R*)	2.02	1.91	414	7	58	
	17 (23*R*,24*S*,28*S*)	1.89	1.38	414		17	
	18 (23*R*,24*R*,28*R*)	1.93	1.42	414			86
	20 (23*S*,24*S*,28*R*)						
	21a (24*R*)	2.16	1.59	416	5[f]	8[f]	
	21b (24*S*)	2.16	1.59	416	3[f]	4[f]	14
	22a,b (23*R/S*)	2.07 1.94	1.33 1.28	416 416	4 2		
	23a,b (24*R/S*)	2.09 2.11	1.44 1.44	416 416	3 1	6 7	

[a] Altex Ultrasphere ODS; methanol/water (97.5:2.5) as the eluent; calysterol t_R 1.00. [b] OV-17; cholesterol t_R 1.00. [c] Pd/C (2%); ethanol, 2 atm, 24 h at room temperature. [d] Pd/C (2%), ethanol, 2 atm, 36 h at room temperature. [e] Pd/C (10%), ethanol, 3 atm, 100 h at 65 °C. [f] Ratio assigned according to ^{1}H NMR shift differences (see Experimental Section). [g] (•) Hydrogen above the plane; (○) hydrogen below the plane.

carbons depending on the original cyclopropene substitution pattern.

In order to get more information about the as yet unknown absolute configuration at C-28 in calysterol (**1**) and at C-23 in 23*H*-isocalysterol (**2**), we subjected the naturally occurring steroidal cyclopropenes **1**[21] and **2** to catalytic hydrogenation. Assuming syn hydrogenation to be operative on both sides of the double bond of the two (*R* and *S*) C-28 epimers of calysterol (**1**) as well as of the two possible C-23 epimers of 23*H*-isocalysterol (**2**), one may encounter six possible diastereoisomeric cyclopropanes, **14**–**19** (cf. Chart II). On the assumption that steric factors (hydrogen attack opposite to the C-28 methyl group in calysterol (**1**) and the C-23 alkyl in 23*H*-isocalysterol (**2**)) would play the dominant stereochemical role,[20] we may also draw conclusions about any expected major or minor reduction product. Thus, the all-cis substituted cyclopropanes (23*S*,24*R*,28*R*)-**14** and (23*R*,24*S*,28*S*)-**17** would in each case be expected to be the kinetically controlled major component. If true, the assignment of the absolute configurations of the missing centers in calysterol (**1**) and 23*H*-isocalysterol (**2**) should easily be possible by determination of both the ratio and the absolute configurations of their primary reduction products. The results of our hydrogenolytic investigations using palladium as the catalyst and a hydrogen pressure of 2 atm at room temperature are summarized in Table II.

Considering the ^{1}H NMR spectroscopic analysis of the major hydrogenation products **14** and **17** of calysterol (**1**) and 23*H*-isocalysterol (**2**), we may presume similar shift behavior among the cyclopropane protons compared to that of our recently reported[14] 23,24-methylenecholesterols **24** and **25** (Table III). In fact, the protons at C-28 of **14** and **17**, which were assigned by decoupling experiments, show a similar downfield shift (0.711 → 0.782 ppm) as is documented for H_b (0.55 → 0.61 ppm) in **24** and **25**, respectively. Furthermore, the easily detectable C-24 protons show for both pairs of diastereoisomers (**14** and **17** vs. **24** and **25**) a reversed shift mode, namely 0.392 and 0.43 ppm for **14** and **24** vs. 0.346 and 0.36 ppm for **17** and **25**. Additionally, the 9-Hz coupling constants of the d × d × d of H-C(24) in **17** indicate an all-cis substitution pattern[13] of the cyclopropane ring. These findings clearly point to a configurational similarity between **14** and **24** as well as between **17** and **25**. Consequently, using the Cahn–Ingold–Prelog rules,[22] we can assign the steroidal cyclopropane **14** the 23*S*,24*R*,28*R* configuration and **17** the 23*R*,24*S*,28*S* configuration.

The minor hydrogenation product (Table II) of calysterol (**1**), the steroidal cyclopropane **15**—in contrast to the major component **14**—shows (Table III) a high-field shifted H-C(28) signal (0.102 ppm), which is quite typical for a trans-oriented three-membered ring.[13] A similar shift behavior is observed (Table III) for H-C(23) of the minor reduction product **18** of 23*H*-isocalysterol (**2**). From all these considerations we are now able to assign the 28*R* configuration to the naturally occurring calysterol (**1**) and the 23*R* configuration to its naturally occurring isomer (**2**) as indicated in Chart I.

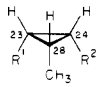
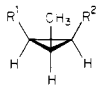
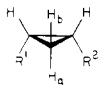
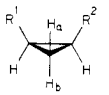
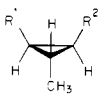
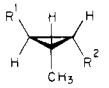
Turning now to all hydrogenolysis products of calysterol (**1**) and 23*H*-isocalysterol (**2**) (cf. Table II), we were able to detect a greater variety of saturated sterols from **1** than from the isomeric

(20) Lukina, M. Yu.; Nesmeyanova, O. A.; Rudashevskaya, T. Yu. *Dokl. Akad. Nauk SSSR* **1967**, *174*, 1101–1104.

(21) Some preliminary results on the catalytic hydrogenation of calysterol (**1**) have been reported by the Italian group (see ref 5), but the absence of spectroscopic data and isolation of all products precluded any stereochemical conclusions.

(22) Cahn, R. S.; Ingold, Ch.; Prelog, V. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385–415.

Table III. ^{1}H NMR (360 MHz, $CDCl_3$) Chemical Shifts of the Cyclopropane Protons of Comparable Sterols[a]

	H_a, C-28	H_b, C-28	H, C-24	H, C-23
		0.734	0.392 (ddd, 9, 9, 9)	0.734
14 (23*S*,24*R*,28*R*)				
		0.782	0.346 (ddd, 9, 9, 9)	0.578
17 (23*R*,24*S*,28*S*)				
	−0.33 (ddd, 5, 5, 5)	0.55 (ddd, 5, 8.5, 8.5)	0.43 (dddd, 5.5, 8.5, 8.5, 8.5)	ca. 0.79 (m)
24 (23*R*,24*S*)				
	−0.26 (ddd, 4.5, 4.5, 4.5)	ca. 0.61 (ddd)	0.36 (dddd, 5.5, 8.5, 8.5, 8.5)	ca. 0.79 (m)
25 (23*S*,24*R*)				
	0.102		0.313 (ddd, 4.5, 9, 9)	0.635
15 (23*R*,24*S*,28*R*)				
		0.473	0.053	0.053
18 (23*R*,24*R*,28*R*)				

[a] Given as σ values; multiplicity and J values in hertz are given in parentheses.

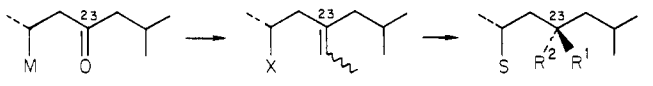
cyclopropene **2**. Such a behavior can only be explained if some double bond migration occurs during the hydrogenation of calysterol (**1**). Indeed, the occurrence of 7% of the "forbidden" hydrogenation product **17** from **1** points to such an isomerization process.

It is known[23] that in cyclopropenes, it is the single rather than the double bond that is opened preferentially. The behavior (Table II) of 23*H*-isocalysterol (**2**) is consistent with this generalization, since only the 23–28 and 23–24 bonds are ruptured to yield **21** and **23**, respectively. On the other hand, calysterol (**1**)— in addition to the expected hydrogenolysis products **21** and **22**—also yields (Table II) some 24-homosterol **23** (formally resulting from fission of the 23–24 olefinic bond), which suggests that some isomerization of calysterol (**1**) to 23*H*-isocalysterol (**2**) occurs on the catalyst surface.

Among the ring-opening products **21–23** (cf. Table II), which correspond to all possible bond cleavages of the three-membered ring, (24*R*)-stigmastanol (**21a**) and its C-24 epimer (24*S*) 24-ethyl-5α-cholestan-3β-ol (clionastanol, **21b**) were readily identified by comparison with literature data.[24,25] Particularly noteworthy is the observation that while *both* (24*R*)- and (24*S*)-ethyl isomers **21a** and **21b** arose from hydrogenolysis of the cyclopropenes **1** and **2**, only the (24*S*) isomer **21b** was produced by hydrogenolytic opening of naturally occurring 23,24-dihydrocalysterol (**3**). Therefore, we conclude that this sterol possesses the 23*S*,24*S*,28*R* configuration (**3b** in Chart I) rather than the 23*R*,24*R*,28*S* alternative (**3a**).

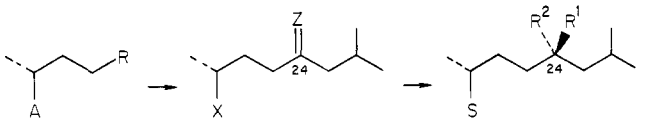
In order to substantiate the constitutional assignments of the hitherto unknown side-chain substitution patterns of the remaining cyclopropene hydrogenolysis products **22** and **23**, we undertook their independent synthesis.

Scheme IV



26 (23*Z*/*E*)-**27**, X = M; (23*Z*/*E*)-**28**, X = N; $R^1 = C_2H_5$; $R^2 = H$; $R^1 = H$; $R^2 = C_2H_5$

Scheme V



29, R = COOH; **30**, R = COCl; **31**, X = A; Z = O; **32**, X = N; Z = O; **33**, X = N; Z = CH_2; **23**, $R^1 = CH_3$; $R^2 = H$; $R^1 = H$; $R^2 = CH_3$

Synthesis of 23-Ethylcholestanol (22). The starting material for the synthesis of both C-23 epimers of **22** was 6β-methoxy-3α,5-cyclo-5α-cholest-23-one (**26**)[26] (cf. Scheme IV). Wittig olefination with the appropriate phosphorane gave an easily separable (HPLC) mixture (1:1 ratio) of (23*Z*/*E*)-**27**. After acid-induced recovery of the 3β-hydroxy-Δ^5 system, one $\Delta^{23(28)}$ stereoisomer of **28** was hydrogenated to yield both anticipated C-23 epimers (23*R*)-**22** and (23*S*)-**22**. HPLC separation of the (1:2) mixture of these C-23 epimers and spectral analysis of each pure isomer (whose absolute configurations have note yet been elucidated) showed them to be identical with the reduction products **22a** and **22b** (cf. Table II) of calysterol (**1**) (see Experimental Section).

Synthesis of 24-Methyl-24-homocholestanol (23). 3β-Acetoxy-5-cholenic acid (**29**), which was first transformed into its acid

(23) Kasai, P. H.; Myers, R. J.; Eggers, D. F., *Jr.*, Wiberg, K. B. *J. Chem. Phys.* **1959**, *30*, 512–516.
(24) Rubenstein, I.; Goad, L. J.; Claque, A. D. J.; Mulheirn, L. J. *Phytochemistry* **1976**, *15*, 195–200.
(25) Bohlin, L.; Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Phytochemistry* **1981**, *20*, 2397–2401.
(26) Li, H-T.; Duax, W. L., Djerassi, C., to be published in connection with the synthesis and absolute configurational assignment of the epimeric 23-methylcholesterols.

chloride **30**,[27] was treated with diisobutylcadmium to give 24-oxo-24-homocholesteryl acetate (**31**) (Scheme V). After saponification, the free sterol **32** was subjected to a Wittig olefination, yielding the 24-methylene compound **33**. Hydrogenation of the free sterol **33** furnished an easily separable (HPLC) mixture of the two anticipated C-24 epimers of **23**, whose configuration has not yet been determined. Their spectroscopic properties were in all aspects (see Experimental Section) identical with those of the two stereoisomeric hydrogenolysis products (cf. Table II) of naturally occurring calysterol (**1**) and 23*H*-isocalysterol (**2**), respectively.

Experimental Section

General Procedures. Conditions and equipment used in the synthetic section were those described in ref 14. Optical rotations $[\alpha]^{20}_{589}$ of the synthetic compounds were measured on a Perkin-Elmer 141 polarimeter in chloroform. For the HPLC separation of the hydrogenation products of **1–3**, we used two Altex Ultrasphere ODS columns in series and methanol/water (97.5:2.5) as the mobile phase.

(28*R*)-Calysterol (1): mp 111–112 °C (Et_2O/MeOH); $[\alpha]^{20}_{589}$ −30° (*c* 6.0 mg/mL, $CHCl_3$) [mp 114–116 °C; $[\alpha]^{20}_{589}$ −29.3°; ref 5]; GLC t_R (OV-17) 1.20 (cholesterol); ^{1}H NMR (360 MHz) δ 2.694 (septet, *J* = 6.8 *hz*, 1 H, C-25), 1.330 (q, *J* = 4.1 Hz, 1 H, C-28), 1.105 (d, *J* = 6.8 Hz, 3 H) and 1.097 (d, *J* = 6.85 Hz, 3 H, C-26 and C-27), 1.010 (s, 3 H, C-19), 0.999 (d, *J* = 4.55 Hz, 3 H, C-29), 0.965 (d, *J* = 6.6 Hz, 3 H, C-21), 0.700 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 410.3550 (M^+, 15, calcd for $C_{29}H_{46}O$ 410.3549), 395 (10), 392 (1), 377 (2), 367 (33), 349 (6), 300 (10), 271 (87), 253 (20), 231 (8), 213 (18), 110 (100).

(23*R*)-23*H*-Isocalysterol (2): mp 115–116 °C (Et_2O/MeOH); $[\alpha]^{20}_{589}$ −47.3° (*c* 4.0 mg/mL, $CHCl_3$); HPLC (ODS-2) t_R 1.31 (calysterol); GLC t_R (OV-17) 1.52 (cholesterol); ^{1}H NMR (360 MHz) δ 2.687 (septet × d, *J* = 6.4, 1.4 Hz, 1 H, C-25), 1.994 (d, *J* = 1.42 Hz, 3 H, C-29), 1.151 (d, *J* = 6.8 Hz, 3 H) and 1.099 (d, *J* = 6.8 Hz, 3 H, C-26 and C-27), 1.010 (s, 3 H, C-19), 1.006 (d, *J* = 6 Hz, 3 H, C-21), 0.692 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 410.3537 (M^+, 8, calcd for $C_{29}H_{46}O$ 410.3549), 395 (2), 314 (4), 300 (11), 271 (82), 253 (7), 231 (3), 213 (6), 96 (65), 95 (100).

(23*S*,24*S*,28*R*)-23,24-Dihydrocalysterol (3b): HPLC (ODS-2) t_R 1.68 (calysterol); GLC t_R (OV-17) 1.42 (cholesterol); ^{1}H NMR (360 MHz) δ 1.019 (d, *J* = 6.9 Hz, 3 H, C-21) 1.009 (s, 3 H, C-19), 0.990 (d, *J* = 6.8 Hz, 3 H, C-29), 0.930 (d, *J* = 6.0 Hz, 3 H) and 0.929 (d, *J* = 6.0 Hz, 3 H, C-26 and C-27), 0.688 (s, 3 H, C-18), 0.439 (m, 2 H, C-23, C28), −0.126 (ddd, *J* = 4.5, 8.0, 4.5 Hz, 1 H, C-24); mass spectrum, *m/z* (relative intensity) 412.3708 (M^+, 33, calcd for $C_{29}H_{48}O$ 412.3705), 397 (6), 394 (9), 379 (6), 356 (4), 328 (7), 314 (41), 300 (25), 273 (10), 271 (100), 255 (13), 231 (7), 213 (15).

Catalytic Hydrogenation of (28*R*)-Calysterol (1). A solution of **1** (15 mg, 0.037 mmol) in 8 mL of ethanol was hydrogenated over Pd/C (2%; 15 mg) at 2 atm of H_2 (room temperature) for 24 h. Usual workup and separation by using HPLC (Altex) gave eight fractions, A–H, in the ratio 17:58:2:7:4:3:1:8.

Fraction A. (23*R*,24*S*,28*R*)-23,24-Dihydrocalystanol (**15**): HPLC (Altex) t_R 1.85 (calysterol); GLC t_R (OV-17) 1.27 (cholesterol); ^{1}H NMR (360 MHz) δ 1.018 (d, *J* = 6.6 Hz, 3 H, C-29), 0.999 (d, *J* = 6.82 Hz, 3 H, C-21), 0.970 (d, *J* = 6.5 Hz, 3 H) and 0.940 (d, *J* = 6.44 Hz, 3 H, C-26 and C-27), 0.803 (s, 3 H, C-19), 0.663 (s, 3 H, C-18), 0.633 (m, 1 H, C-23), 0.313 (m, 1 H, C-24), 0.102 (m, 1 H, C-28); mass spectrum, *m/z* (relative intensity) 414.3863 (M^+, 11, calcd for $C_{29}H_{50}O$ 414.3862), 399 (3), 381 (2), 358 (1), 330 (1), 316 (26), 302 (20), 273 (100), 257 (15), 255 (8), 233 (6), 215 (9).

Fraction B. (23*S*,24*R*,28*R*)-23,24-Dihydrocalystanol (**14**): HPLC (Altex) t_R 1.91 (calysterol); GLC t_R (OV-17) 1.91 (cholesterol); ^{1}H NMR (360 MHz) δ 0.978 (d, *J* = 6.48 Hz, 3 H, C-21), 0.952 (d, *J* = 6.3 Hz, 3 H, C-29), 0.922 (d, *J* = 6.48 Hz, 3 H) and 0.914 (d, *J* = 6.49 Hz, 3 H, C-26 and C-27), 0.798 (s, 3 H, C-19), 0.734 (m, 2 H, C-23, C-28), 0.656 (s, 3 H, C-18), 0.392 (ddd, *J* = 9, 9, 9 Hz, 1 H, C-24); mass spectrum, *m/z* (relative intensity) 414.3863 (M^+, 21, calcd for $C_{29}H_{50}O$ 414.3862), 399 (5), 381 (2), 358 (2), 330 (5), 316 (47), 302 (26), 273 (100), 257 (27), 255 (8), 233 (14), 215 (18).

Fraction C. (23ξ)-23-Ethylcholestanol (**22a,b**): HPLC (Altex) t_R 1.94 (calysterol); GLC t_R (OV-17) 1.28 (cholesterol); ^{1}H NMR (360 MHz) δ 0.876 (d, *J* = 6.51 Hz, 3 H, C-21), 0.871 (d, *J* = 6.4 Hz, 3 H) and 0.833 (d, *J* = 6.89 Hz, 3 H) C-26 and C-27, 0.823 (t, *J* = 6.89 Hz, 3 H, C-29), 0.805 (s, 3 H, C-19), 0.668 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 416.3986 (M^+, 100, calcd for $C_{29}H_{52}O$ 416.4018), 401 (18), 398 (4), 290 (8), 234 (56), 233 (46), 215 (35).

Fraction D. (23*R*,24*S*,28*S*)-23,24-Dihydrocalystanol (**17**): HPLC (Altex) t_R 2.02 (calysterol); GLC t_R (OV-17) 1.91 (cholesterol); ^{1}H NMR (360 MHz) δ 0.980 (d, *J* = 6.5 Hz, 3 H, C-21), 0.955 (d, *J* = 6.5 Hz, 3 H) and 0.912 (d, *J* = 6.5 Hz, 3 H) C-26 and C-27, 0.914 (d, *J* = 6.5 Hz, 3 H, C-29), 0.803 (s, 3 H, C-19), 0.782 (m, 1 H, C-28), 0.665 (s, 3 H, C-18), 0.578 (m, 1 H, C-23), 0.346 (ddd, *J* = 9, 9, 9 Hz, 1 H, C-24); mass spectrum, *m/z* (relative intensity) 414.3846 (M^+, 11, calcd for $C_{29}H_{50}O$ 414.3862), 399 (5), 381 (2), 330 (2), 316 (28), 302 (22), 273 (100), 257 (24), 233 (7), 215 (24).

Fraction E. (23ξ)-23-Ethylcholestanol (**22b,a**): HPLC (Altex) t_R 2.07 (calysterol); GLC t_R (OV-17) 1.33 (cholesterol); ^{1}H NMR (360 MHz) δ 0.874 (d, *J* = 6.35 Hz, 3 H, C-21), 0.864 (d, *J* = 6.52 Hz, 3 H) and 0.837 (d, *J* = 6.58 Hz, 3 H) C-26 and C-27, 0.802 (s, 3 H, C-19), 0.795 (t, *J* = 7.26 Hz, 3 H, C-29), 0.661 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 416.4041 (M^+, 100 calcd for $C_{29}H_{52}O$ 416.4018), 401 (17), 398 (4), 383 (6), 359 (2), 273 (7), 257 (6), 233 (44), 215 (38).

Fraction F. (24ξ)-24-Methyl-24-homocholestanol (**23a,b**): HPLC (Altex) t_R 2.09 (calysterol); GLC t_R (OV-17) 1.44 (cholesterol); ^{1}H NMR (360 MHz) δ 0.886 (d, *J* = 6.43 Hz, 3 H), 0.855 (d, *J* = 6.74 Hz, 3 H), 0.832 (d, *J* = 6.46 Hz, 3 H) and 0.803 (d, *J* = 6.2 Hz, 3 H, C-21, C-26, C-27, and C-28), 0.800 (s, 3 H, C-19), 0.645 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 416.4110 (M^+, 100, calcd for $C_{29}H_{52}O$ 416.4018), 401 (19), 398 (4), 383 (6), 359 (2), 316 (3), 273 (5), 233 (59), 215 (45).

Fraction G. (24ξ)-24-Methyl-24-homocholestanol (**23b,a**): HPLC (Altex) t_R 2.11 (calysterol); GLC t_R (OV-17) 1.44 (cholesterol); ^{1}H NMR (360 MHz) δ 0.890 (d, *J* = 6.4 Hz, 3 H), 0.868 (d, *J* = 6.55 Hz, 3 H), 0.828 (d, *J* = 6.5 Hz, 3 H) and 0.816 (d, *J* = 6.48 Hz, 3 H) C-21, C-26, C-27, and C-28, 0.800 (s, 3 H, C-19), 0.641 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 416.4110 (M^+, 100, calcd for $C_{29}H_{52}O$ 416.4018), 401 (19), 398 (4), 383 (6), 359 (2), 316 (3), 273 (5), 257 (5), 233 (59) 215 (45).

Fraction H. (24*R*)-Stigmastanol (**21a**) and (24*S*)-ethyl-5α-cholestan-3β-ol (clionastanol, **21b**) in a ratio 5:3 (^{1}H NMR evidence: HPLC (Altex) t_R 2.16 (calysterol); GLC t_R (OV-17) 1.59 (cholesterol); ^{1}H NMR (360 MHz) δ 0.901 (d, *J* = 6.3 Hz, 3 H, C-21 of **21a**), 0.903 (d, *J* = 6.3 Hz, 3 H, C-21 of **21b**), 0.838 (t, *J* = 7.6 Hz, 3 H, C-29 of **21a**), 0.847 (t, *J* = 7.4 Hz, 3 H, C-29 of **21b**), 0.827 (d, *J* = 6.6 Hz, 3 H) and 0.807 (d, *J* = 6.7 Hz, 3 H, C-26 and C-27), 0.799 (s, 3 H C-19), 0.644 (s, 3 H, C-18); mass spectrum, *m/z* (relative intensity) 416.3979 (M^+, 100, calcd for $C_{29}H_{52}O$ 416.4018), 401 (9), 398 (8), 383 (7), 359 (2), 273 (3), 257 (4), 233 (50), 215 (36).

Catalytic Hydrogenation of (23*R*)-23*H*-Isocalysterol (2). A solution of **2** (4 mg, 0.01 mmol) in 5 mL of ethanol was hydrogenated over Pd/C (2%, 4 mg) at 2 atm of H_2 (room temperature) for 36 h. Usual workup and separation by using HPLC (Altex) gave five fractions, A–E, in a ratio of 17:58:6:7:12.

Fraction A. (23*R*,24*R*,28*R*)-23,24-Dihydrocalystanol (**18**): HPLC (Altex) t_R 1.89 (calysterol); GLC t_R (OV-17) 1.38 (cholesterol); ^{1}H NMR (360 MHz) δ 0.982 (d, *J* = 6.5 Hz, 3 H, C-21), 1.033 (d, *J* = 6.3 Hz, 3 H, C-29), 0.994 (d, *J* = 6.4 Hz, 3 H) and 0.935 (d, *J* = 6.4 Hz, 3 H, C-26 and C-27), 0.802 (s, 3 H, C-19), 0.650 (s, 3 H, C-18), 0.63 (m, 1 H, C-24), 0.473 (m, 1 H, C-28), 0.053 (m, 2 H, C-24); mass spectrum, *m/z* (relative intensity) 414.3864 (M^+, 11, calcd for $C_{29}H_{50}O$ 414.3862), 399 (3), 381 (2), 316 (31), 302 (24), 273 (100), 257 (10), 233 (7), 215 (9).

Fraction B. (23*R*,24*S*,28*S*)-23,24-Dihydrocalystanol (**17**).

Fraction C. (24ξ)-24-Methyl-24-homocholestanol (**23a,b**).

Fraction D. (24ξ)-24-Methyl-24-homocholestanol (**23b,a**).

Fraction E. (24*R*)-Stigmastanol (**21a**) and (24*S*)-ethyl-5α-cholestan-3β-ol (clionastanol, **21b**) in the ratio 8:4 (^{1}H NMR evidence).

Catalytic Hydrogenation of (23*S*,24*S*,28*R*)-23,24-Dihydrocalysterol (3b). A solution of **3b** (2 mg, 0.005 mmol) in 5 mL of ethanol was hydrogenated over Pd/C (10%, 5 mg) at 3 atm of H_2 (65 °C) for 100 h. Usual workup and separation by using HPLC (Altex) gave two fractions, A and B, in a ratio of 86:14.

Fraction A. (23*S*,24*S*,28*R*)-23,24-Dihydrocalystanol (**20b**): HPLC (Altex) t_R 1.93 (calysterol); GLC t_R (OV-17) 1.54 (cholesterol); ^{1}H NMR (360 MHz) δ 1.003 (d, *J* = 6.5 Hz, 3 H, C-21), 0.986 (d, *J* = 6.5 Hz, 3 H, C-29), 0.930 (d, *J* = 6.0 Hz, 3 H) and 0.927 (d, *J* = 6.0 Hz, 3 H, C-26 and C-27), 0.802 (s, 3 H, C-19), 0.656 (s, 3 H, C-18), 0.63 (m, 1 H, C-23), 0.451 (m, 2 H, C-23,C-28), −0.125 (ddd, *J* = 4.5, 8.0, 4.5 Hz, 1 H, C-24); mass spectrum, *m/z* (relative intensity) 414.3865 (M^+, 15, calcd for $C_{29}H_{50}O$ 414.3862), 399 (3), 381 (2), 316 (26), 302 (17), 273 (100), 257 (12), 233 (6), 215 (10).

Fraction B. (24*S*)-Ethyl-5α-cholestan-3β-ol (**21b**): HPLC (Altex) t_R 2.16 (calysterol); GLC t_R (OV-17) 1.59 (cholesterol); ^{1}H NMR (360 MHZ) δ 0.906 (d, *J* = 6.5 Hz, 3 H, C-21), 0.849 (t, *J* = 7.4 Hz, 3 H, C-29), 0.825 (d, *J* = 6.6 Hz, 3 H) and 0.806 (d, *J* = 6.7 Hz, 3 H, C-26

(27) Kurath, P.; Capezzuto, M. *J. Am. Chem. Soc.* **1956**, *78*, 3527–2529.

and C-27), 0.800 (s, 3 H, C-19), 0.645 (s, 3 H, C-18); mass spectrum, m/z (relative intensity) 416.3979 (M^+, 100, calcd for $C_{29}H_{52}O$ 416.4018), 401 (9), 398 (8), 383 (7), 359 (2), 273 (3), 257 (4), 233 (50), 215 (36).

Dibromocarbene Addition to (23*Z*)-9.[14] To a vigorously stirred solution of the *i*-methyl ether **9** (1.00 g, 2.51 mmol) and benzyltriethylammonium chloride (BTEAC; 20 mg, 0.09 mmol) in bromoform (2 mL) was added slowly an aqueous solution of sodium hydroxide (50%, 3.0 mL) and a trace of ethanol (12 μL). The mixture was stirred for 6 days at room temperature, diluted with water, and extracted with methylene chloride. The extract, after being washed with water and brine, was dried over potassium carbonate and evaporated in a RV at reduced pressure. After purification of the crude material by column chromatography, the product separation was realized by HPLC (ODS-2). Two fractions (32% yield; A and B, 4:1) were collected, and the analytical data are listed below.

Fraction A. HPLC, t_R 50 min. According to its mass and ^1H NMR spectra, this fraction represents a mixture of unreacted starting material **9** and (23*R*,24*S*)-, 23,24-(dibromomethylene)-6β-methoxy-3α,5-cyclo-5α-cholestane (**10**). No further attempt was made to resolve this fraction.

Fraction B. (23*S*,24*R*)-23,24-(Dibromomethylene)-6β-methoxy-3α,5-cyclo-5α-cholestane (**11**): HPLC t_R 57 min; ^1H NMR (360 MHz) δ 3.327 (s, 3 H, OCH_3), 2.78 (m, 1 H, C-6), 1.126 (d, J = 5.47 Hz, 3 H) and 1.114 (d, J = 6.36 Hz, 3 H, C-26 and C-27), 1.026 (s, 3 H, C-19), 0.984 (d, J = 5.98 Hz, 3 H, C-21), 0.741 (s, 3 H, C-18), 0.65 (t, J = 5 Hz, 1 βH, C-4), 0.43 (dd, J = 5, 9 Hz, 1 αH, C-4); mass spectrum, m/z (relative intensity) 568 (M^+, 30), 553 (10, M − CH_3), 536 (51, M − CH_3OH), 513 (28, M − A ring), 512 (9, M − C_4H_8!), 457 (10), 415 (5), 337 (4), 315 (5), 310 (9), 283 (30), 267 (9), 255 (45).

23,24-(Bromomethylene)-6β-methoxy-3α,5-cyclo-5α-cholestane (12). Reaction of 85 mg (0.15 mmol) of **11** with tributyltin hydride (0.15 mmol) in 1 mL of dry benzene for 2 days at room temperature[15] yielded almost quantitatively the desired monobromo compound **12** after column chromatography (hexane/ethyl acetate 9:1): mass spectrum, m/z (relative intensity) 490 (M^+, 10), 458 (12, M − CH_3OH), 411 (15), 379 (40), 315 (20), 310 (18), 295 (17), 285 (95), 255 (85), 241 (50), 227 (55), 213 (100).

(23*S*,24*R*,28*S*)-23,24-(Bromomethylene)-cholest-5-en-3β-ol (13). The 3β-hydroxy-Δ^5 system was regenerated from **12** in the usual manner to give the crystalline sterol **13**: mp 115–116 °C (MeOH); HPLC (two Altex in series) t_R 56 min; ^1H NMR (360 MHz) δ 5.36 (m, 1 H, C-6), 3.53 (m, 1 αH, C-3), 3.25 (dd, J = 7.9, 7.9 Hz, 1 H, C-28), 1.052 (d, J = 6.56 Hz, 3 H) and 1.020 (d, J = 6.5 Hz, 3 H, C-26 and C-27), 1.011 (s, 3 H, C-19), 1.005 (d, J = 6.5 Hz, 3 H, C-21), 0.700 (s, 3 H, C-18); high-resolution mass spectrum, m/z (relative intensity) 476.2648 (M^+, 94, calcd for $C_{28}H_{45}OBr$ 476.2654), 461.2425 ($C_{27}H_{42}OBr$, 18, M − CH_3), 458.2520 ($C_{28}H_{43}Br$, 15, M − H_2O), 443.2299 ($C_{27}H_{40}Br$, 10, M − CH_3 − H_2O), 397.3434 ($C_{28}H_{45}O$, 34), 391.2005 ($C_{23}H_{36}Br$, 14), 379.3357 ($C_{28}H_{43}$, 40), 365.1801 ($C_{21}H_{34}Br$, 11), 328.2817 ($C_{23}H_{36}O$, 19), 301.2526 ($C_{21}H_{33}O$, 14), 283.2431 ($C_{21}H_{31}$, 20), 273.2211 ($C_{19}H_{29}O$, 16), 271.2035 ($C_{19}H_{27}O$, 42), 255.2118 ($C_{19}H_{27}$, 20).

(23*Z*/*E*)-23-Ethylidene-6β-methoxy-3α,5-cyclo-5α-cholestane (27). A suspension of ethyltriphenylphosphonium bromide (1.56 g, 4.2 mmol) in benzene (31 mL) was treated with *n*-butyllithium (1.8 mL, 2.4 M in hexane). A solution of the *i*-methyl ether (**26**, 350 mg, 0.84 mmol) in benzene was then added and the reaction mixture stirred under reflux for 40 h. Workup as usual gave the anticipated product **27** as a 1:1 Z/E mixture (34%). HPLC separation (ODS-2) furnished the two pure stereoisomers whose spectroscopic data are listed herewith.

27a: ^1H NMR (360 MHz) δ 5.250 (q, J = 6.64 Hz, 1 H, C-28), 1.566 (m, 3 H, C-29), 1.026 (s, 3 H, C-19), 0.877 (d, J = 6.28 Hz, 3 H, C-21), 0.816 (d, J = 6.44 Hz, 3 H) and 0.786 (d, J = 6.35 Hz, 3 H, C-26 and C-27), 0.733 (s, 3 H, C-18); high-resolution mass spectrum, m/z (relative intensity) 426.3869 (M^+, 3, $C_{30}H_{50}O$), 411.3591 (10, $C_{29}H_{47}O$), 394.3593 (9, $C_{29}H_{46}$), 371.3302 (12, $C_{26}H_{43}O$), 314.2603 (100, $C_{22}H_{34}O$), 299.2373 (20, $C_{21}H_{31}O$), 282.2351 (91, $C_{21}H_{31}O$).

27b: ^1H NMR (360 MHz) δ 5.212 (q, J = 6.64 Hz, 1 H, C-28), 1.576 (m, 3 H, C-29), 1.023 (s, 3 H, C-19), 0.923 (d, J = 6.33 Hz, 3 H, C-21), 0.819 (d, J = 6.11 Hz, 3 H) and 0.803 (d, J = 5.79 Hz, 3 H, C-26 and C-27), 0.734 (s, 3 H, C-18); for mass spectrum see above.

(23*Z*/*E*)-23-Ethylidenecholesterol (28). The *i*-methyl ether protecting group in both **27a** and **27b** was removed in the usual manner to give **28a** and **28b**, respectively.

28a: mp 136–137 °C (MeOH); $[\alpha]^{20}_{589}$ −66° (*c* 7.3 mg/mL, $CHCl_3$); HPLC (ODS-2) t_R 0.82 (cholesterol); GLC t_R (OV-17) 1.49 (cholesterol); ^1H NMR (360 MHz) δ 5.250 (q, J = 6.55 Hz, 1 H, C-28), 1.565 (m, 3 H, C-29), 1.013 (s, 3 H, C-19), 0.874 (d, J = 6.35 Hz, 3 H, C-21), 0.824 (d, J = 6.48 Hz, 3 H) and 0.785 (d, J = 6.33 Hz, 3 H, C-26 and C-27), 0.699 (s, 3 H, C-18); high-resolution mass spectrum, m/z (relative intensity) 412.3708 (M^+, 1, $C_{29}H_{48}O$), 300.2444 (100, $C_{21}H_{32}O$), 285.2219 (18, $C_{20}H_{29}O$), 283.2427 (15, $C_{21}H_{31}$), 282.2361 (15, $C_{21}H_{30}$), 267.2094 (17, $C_{20}H_{27}$).

28b: mp 146–147 °C (MeOH); $[\alpha]^{20}_{589}$ −40° (*c* 4.2 mg/mL, $CHCl_3$); HPLC (ODS-2) t_R 0.92 (cholesterol); GLC t_R (OV-17) 1.53 (cholesterol); ^1H NMR (360 MHz) δ 5.213 (q, J = 6.48 Hz, 1 H, C-28), 1.576 (m, 3 H, C-29), 1.010 (s, 3 H, C-19), 0.919 (d, J = 6.27 Hz, 3 H, C-21), 0.818 (d, J = 6.37 Hz, 3 H) and 0.810 (d, J = 6.17 Hz, 3 H, C-26 and C-27), 0.699 (s, 3 H, C-18); for mass spectrum see above.

(23ξ)-23-Ethylcholestanol (22a,b). A solution of **28a** (7 mg, 0.018 mmol) in methanol (4 mL) was hydrogenated with 5% Pd/C (2 mg) for 16 h at room temperature. The anticipated C-23 epimers **22a** and **22b** were obtained in a ratio of 1:2 after HPLC (Altex) separation. **22a**: mp 155–157 °C (MeOH); $[\alpha]^{20}_{589}$ +43° (*c* 6.7 mg/mL, $CHCl_3$). **22b**: mp 134–135 °C (MeOH); $[\alpha]^{20}_{589}$ +36° (*c* 4.5 mg/mL, $CHCl_3$); for spectroscopic properties see above.

24-Oxo-24-homocholesterol (32). 3β-Acetoxy-5-cholenyl chloride (**30**)[27] (prepared from 2 g of **29**) was dissolved in dry benzene (20 mL) and added dropwise to a cold diisobutylcadmium solution (prepared from 8.8 g of anhydrous cadmium bromide according to ref 28). The reaction mixture was stirred at room temperature for 20 h and was directly transformed after acidic workup into the free sterol **32** (35% yield after recrystallization from aqueous methanol): mp 139–140 °C; $[\alpha]^{20}_{589}$ −35° (C 11.8 mg/mL, $CHCl_3$); ^1H NMR (360 MHz) δ 1.005 (s, 3 H, C-19), 0.909 (d, J = 6.55 Hz, 9 H, C-21, C-26, and C-27), 0.673 (s, 3 H, C-18); high-resolution mass spectrum, m/z (relative intensity) 414.3475 (M^+, 83, $C_{28}H_{46}O_2$), 399.3241 (13, $C_{27}H_{43}O_2$), 396.3398 (56, $C_{28}H_{44}O$), 381.3183 (18, $C_{27}H_{41}O$), 357.2814 (11, $C_{24}H_{37}O_2$), 329.2852 (13, $C_{23}H_{37}O$), 314.2610 (45, $C_{22}H_{34}O$), 303.2681 (21, $C_{21}H_{35}O$), 271.2068 (25, $C_{19}H_{27}O$), 57.0709 (100, C_4H_9).

24-Methylene-24-homocholesterol (33). The desired sterol **33** was obtained from **32** by Wittig olefination[29] in 45% yield: mp 125–126 °C (MeOH); $[\alpha]^{20}_{589}$ −35° (*c* 4.5 mg/mL, $CHCl_3$); ^1H NMR (360 MHz) δ 4.713 and 4.654 (2 m, 2 H, H_2C=), 1.009 (s, 3 H, C-19), 0.935 (d, J = 6.46 Hz, 3 H, C-21), 0.875 (d, J = 6.36 Hz, 3 H) and 0.860 (d, J = 6.24 Hz, 3 H) C-26 and C-27, 0.680 (s, 3 H, C-18); high-resolution mass spectrum, m/z (relative intensity) 412.3711 (M^+, 28, $C_{29}H_{48}O$), 397.3467 (13, $C_{28}H_{45}O$), 314.2615 (100, $C_{22}H_{34}O$), 299.2369 (24, $C_{21}H_{31}O$), 271.2058 (45, $C_{19}H_{27}O$).

(24ξ)-24-Methyl-24-homocholestanol (23a,b). A solution of **33** (45 mg, 0.11 mmol) in methanol (10 mL) was hydrogenated with 5% Pd/C (5 mg) for 16 h at room temperature. The anticipated C-24 epimers **23a** and **23b** were obtained after HPLC (Altex) separation. **23a**: mp 125–126 °C (MeOH); $[\alpha]^{20}_{589}$ +16 (C 1.5 mg/mL, $CHCl_3$). **23b**: mp 126–127 °C (MeOH); $[\alpha]^{20}_{589}$ +18° (*c* 1.6 mg/mL, $CHCl_3$); for spectroscopic properties see above.

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