208. Isolation and Structure of Eucosterol and 16β-Hydroxy-eucosterol, Two Novel Spirocyclic Nortriterpenes, and of a New 24-Nor-5α-chola-8,16-diene-23-oic Acid from Bulbs of Several *Eucomis* Species

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Summary. Eucosterol and 16β-hydroxy-eucosterol which have been isolated from several *Eucomis* species have been shown to be (23*S*)-17,23-epoxy-3β,31-dihydroxy-27-nor-5α-lanost-8-ene-15,24-dione (**1**) and (23*S*)-17,23-epoxy-3β,16β,31-trihydroxy-27-nor-5α-lanost-8-ene-15,24-dione (**2**) by chemical transformations and spectral data. The spiro-fused furanoic ether linkage of both metabolites represents a *novel structural element* for natural nortriterpenes. The structure of another metabolite (**16**), 3β-hydroxy-4β-hydroxymethyl-4,14α-dimethyl-15-oxo-24-nor-5α-chola-8,16-diene-23-oic acid, from *Eucomis autumnalis* (*Mill.*) *Chitt.* was elucidated by chemical correlation of its methyl ester **17** with a degradation product of eucosterol (**1**).

1. Introduction. – During the past twelve years investigation of the bulbs of *Eucomis L'Hérit.* (*Liliaceae*) has led to identification of the homoisoflavanones (3-benzyl- and 3-benzylidene-chroman-4-ones) [1], the dibenzo-α-pyrones autumnariol and autumnariniol [2] and (*R*)-(−)-eucomic acid [3]. Recently we have isolated three hitherto unknown steroidal substances, eucosterol, 16β-hydroxy-eucosterol and a 24-nor-5α-chola-8,16-diene-oic acid. Hydroxy-eucosterol occurs in *Eucomis bicolor Bak.* whereas the acid was found in *Eucomis autumnalis* (*Mill.*) *Chitt.*[1]). Eucosterol is a metabolite of these two species as well as of *Eucomis punctata L'Hérit.*[2]), *Eucomis Pole-Evansii N. E. Brown* and a botanically undefined *Eucomis* species. The structure and absolute configuration of eucosterol was deduced from chemical and spectroscopic data and from the X-ray diffraction of its *p*-bromo-benzenesulfonyl derivative to be (23*S*)-17,23-epoxy-3β,31-dihydroxy-27-nor-5α-lanost-8-ene-15,24-dione (**1**) as reported in a preliminary communication [4]. We present here the details of these chemical and physical investigations as well as the determination of the structure of 16β-hydroxy-eucosterol (**2**) and evidence for the structure of 3β-hydroxy-4β-hydroxymethyl-4,14α-dimethyl-15-oxo-24-nor-5α-chola-8,16-diene-23-oic acid (**16**).

2. Isolation. – Morphological studies with *Eucomis* indicated that the homoisoflavanones and waxy compounds are concentrated on the surface of the bulb layers [5]. The intact layers were therefore extracted with ether in a *Soxhlet* apparatus to eliminate these substances prior to homogenization in a mechanical mixer. The

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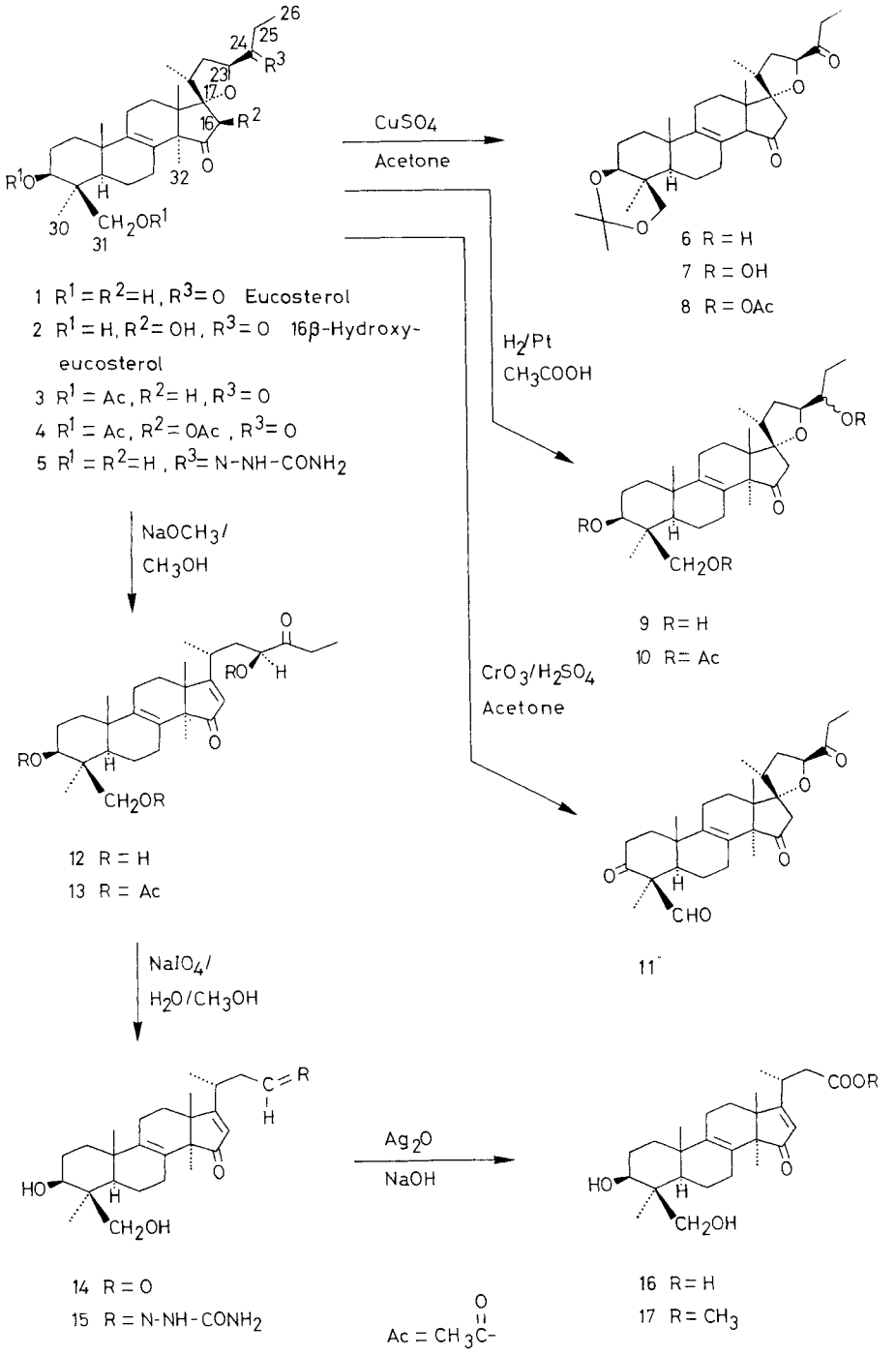
[1]) Syn. *Eucomis undulata Ait.*
[2]) Syn. *Eucomis comosa Hort.*

Scheme



1 $R^1 = R^2 = H$, $R^3 = O$ Eucosterol
2 $R^1 = H$, $R^2 = OH$, $R^3 = O$ 16β-Hydroxy-eucosterol
3 $R^1 = Ac$, $R^2 = H$, $R^3 = O$
4 $R^1 = Ac$, $R^2 = OAc$, $R^3 = O$
5 $R^1 = R^2 = H$, $R^3 = N\text{-}NH\text{-}CONH_2$

6 R = H
7 R = OH
8 R = OAc

9 R = H
10 R = Ac

11

12 R = H
13 R = Ac

14 R = O
15 R = N-NH-CONH2

16 R = H
17 R = CH3

homogenate was extracted with acidic (pH = 4) ethanol/water mixtures (95:5 up to 60:40) at 40°. Finally, the desired compounds were separated by column chromatography on *Florisil®* and recrystallized.

3. Structure of Eucosterol (1). – Eucosterol (**1**) crystallized from acetone as colourless needles, m.p. 235–236° (dec.); $[\alpha]_D^{25} = +20.4 \pm 2°$ ($c = 1.00$, chloroform). The molecular formula, $C_{29}H_{44}O_5$, was deduced by high resolution mass spectrometry (*m/e* calc. 472.3189, found 472.3175) and by elemental analysis. The positive *Liebermann-Burchard* reaction [6] suggested the presence of a nortriterpene with a double bond and a hydroxyl group in the ring system. Eucosterol (**1**) gave an orange colour-reaction with vanillin/H_2SO_4 [7] on silica gel at room temperature which turned to blue violet at 120°.

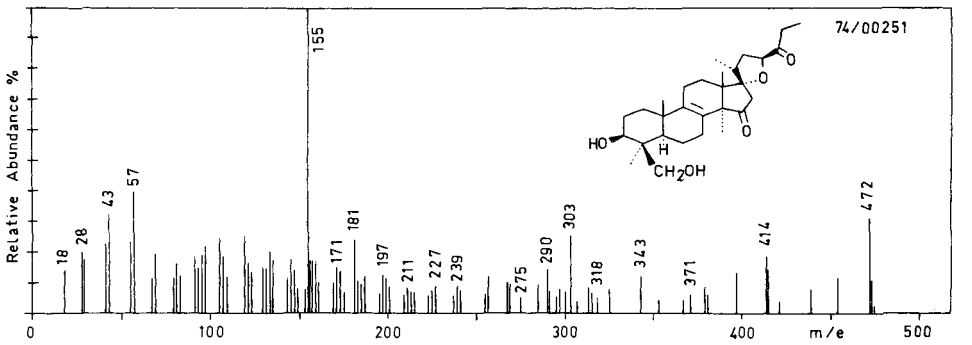


Fig. 1. *Mass spectrum of eucosterol* (**1**)

The presence of a propionyl group, instead of the terminal isobutyl group normally found in the lanostanes, was evident from the mass spectrum showing the fragment ion $[C_{26}H_{39}O_4]^+$ (*m/e* calc. 415.2848, found 415.2856) resulting by loss of a propionyl radical. Confirmation of this was obtained from the ^{1}H-NMR. spectrum (Fig. 2; Table 1) where the presence of an ethyl group adjacent to a carbonyl group appeared as a two proton quartet at 2.51 ppm and a three proton triplet at 1.09 ppm. The mutual coupling of the two signals was demonstrated by double resonance experiments. Finally, the presence of a propionyl group in the side chain was shown by the degradation of eucosterol (**1**) to the aldehyde **14** (see below).

The four tertiary methyl groups of eucosterol (**1**) appeared as four singlets in the ^{1}H-NMR. spectrum. Exact assignments were achieved by the paramagnetic shift of these signals upon addition of increasing amounts of Eu(fod)$_3$[3]. The singlet corresponding to C(30) exhibited the most powerful downfield shift due to complexation of lanthanide ion at the two hydroxyl functions. The protons at C(19) were also shifted appreciably whereas the protons at C(18) and C(32) showed small paramagnetic shifts. The assignment of the latter signals was aided by the presence of a carbonyl group at C(15) which caused a downfield shift of the protons at C(32) to 1.41 ppm.

[3] Tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium(III).

Table 1. *^1H-NMR. Chemical shifts (selected data) of the eucosterols* **1** *and* **2** *and derivatives in $CDCl_3$.* All chemical shift values are given in ϑ (ppm) relative to TMS; 90 MHz spectra unless otherwise stated. – Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad. J_{vic}/J_{gem} = vicinal or geminal spin-spin coupling constant in Hz. $J' = J_{AX} + J_{BX}$ of the X-part of an ABX-system in Hz

	H at C(3)	H at C(16)	H at C(18) and C(19)	H at C(21)	H at C(23) [a]	H at C(25) and C(26)	H at C(30) and C(32)	H at C(31) [b]	other signals
1 [e]	3.47 m $J' = 15$	2.78, 2.23 AB, $J_{gem} = 20$	0.96 s 0.98 s	1.15 d $J_{vic} = 7$	4.67 $J' = 18$	2.51 q 1.09 t $J_{vic} = 7$	1.28 s 1.41 s	4.23, 3.38 AB, $J_{gem} = 11$	3.43 br. s (3-OH) [d] 3.02 br. s (31-OH) [d]
1 [e]	3.20 m		0.87 s 0.93 s		4.73 $J' = 18$		1.10 s 1.28 s	3.89, 3.26 [f]	4.98 d, $J_{vic} = 5$ (3-OH) [d] 4.08 m, $J' = 10$ (31-OH) [d]
2	3.44 m $J' = 16$	4.38 d $J_{vic} = 3$ [g]	0.87 s 0.96 s	1.13 d $J_{vic} = 7$	4.81 m $J' = 18$	2.48 q 1.07 t $J_{vic} = 7$	1.28 s 1.51 s	4.25, 3.38 AB, $J_{gem} = 11$	3.21 d, $J_{vic} = 3$ (16-OH) [d]
3 [e]	4.54 m $J' = 16$	2.74, 2.19 AB, $J_{gem} = 20$	0.93 s 1.01 s	1.10 d $J_{vic} = 7$	4.64 $J' = 18$	2.47 q 1.05 t $J_{vic} = 7$	1.03 s 1.36 s	4.35, 4.14 AB, $J_{gem} = 11$	2.02 s, 2.00 s (CH_3COO)
4	4.63 m $J' = 16$	5.69 s	0.97 s 1.03 s		4.74 m $J' = 18$	2.53 q $J_{vic} = 7$	1.07 s 1.52 s	4.40, 4.20 AB, $J_{gem} = 11$	2.15 s, 2.07 s, 2.04 s (CH_3COO)
6	3.47 m $J' = 15$	2.78, 2.21 AB, $J_{gem} = 20$	0.97 s 1.18 s	1.16 d $J_{vic} = 7$	4.67 $J' = 18$	2.50 q 1.08 t $J_{vic} = 7$	1.27 s 1.40 s	4.08, 3.29 AB, $J_{gem} = 11$	1.46 s, 1.40 s (acetonide)
7	3.50 m $J' = 15$	4.42 d $J_{vic} = 2$ [g]	0.88 s 1.18 s	1.14 d $J_{vic} = 7$	4.83 m $J' = 18$	2.47 q 1.10 t $J_{vic} = 7$	1.30 s 1.53 s	4.13, 3.34 AB, $J_{gem} = 11$	1.48 s, 1.41 s (acetonide)
8	3.50 m $J' = 15$	5.72 s	0.97 s 1.19 s		4.76 m $J' = 18$	2.56 q 1.12 t $J_{vic} = 7$	1.30 s 1.53 s	4.13, 3.34 AB, $J_{gem} = 11$	1.49 s, 1.42 s (acetonide) 2.18 s (CH_3COO)

9	3.44 *m*	2.82, 2.36 *AB*, J_{gem} = 20	0.93 *s* 0.96 *s*	1.13 *d* J_{vic} = 7	4.06 *m*	0.97 *t*, J_{vic} = 7	1.27 *s* 1.27 *s*	4.21, 3.35 *AB*, J_{gem} = 11	3.53 *m* (H at C(24))[h]
10	4.56 *m*	2.71, 2.36 *AB*, J_{gem} = 20	0.95 *s* 1.03 *s*	1.13 *d* J_{vic} = 7	4.27 *m*	0.93 *t* J_{vic} = 7	1.07 *s* 1.23 *s*	4.38, 4.18 *AB*, J_{gem} = 11	2.04 *s*, 2.07 *s* (CH_3COO) 4.54–5.18 *m* (H at C(24))[h]
11[c]		2.76, 2.19 *AB*, J_{gem} = 20	0.95 *s* 1.13 *s*	1.11 *d* J_{vic} = 7	4.63 J' = 18	2.44 *q* 1.04 *t* J_{vic} = 7	1.27 *s* 1.37 *s*		9.65 *s* (CHO)
12	3.47 *m* J' = 15	5.58 br. *s*	0.93 *s* 0.93 *s*	1.11 *d* J_{vic} = 7	3.95 *m*	2.44 *J* 1.09 *t* J_{vic} = 7	1.24 *s* 1.20 *s*	4.20, 3.39 *AB*, J_{gem} = 11	3.41 *d*, J_{vic} = 5 (23-OH)[d]
13	4.61 *m* J' = 16	5.62 br. *s*	0.87 *s* 1.05 *s*		4.91 *m*	2.55 *q*, J_{vic} = 7	1.08 *s* 1.23 *s*	4.40, 4.18 *AB*, J_{gem} = 11	2.04 *s*, 2.07 *s*, 2.13 *s* (CH_3COO)
14	3.47 *m* J' = 16	5.53 br. *s*	0.96 *s* 0.98 *s*				1.27 *s* 1.20 *s*	4.22, 3.38 *AB*, J_{gem} = 11	9.76 br. *s* (CHO) 2.48 *m* (H at C(22))
16[e]	3.20 *m*	5.53 br. *s*	0.87 *s* 0.95 *s*	1.02 *d* J_{vic} = 7			1.11 *s* 1.11 *s*	3.89, 3.27 *AB*, J_{gem} = 11	2.86 *m* (H at C(22))
17	3.46 *m* J' = 16	5.56 br. *s*	0.93 *s* 0.98 *s*	1.16 *d* J_{vic} = 7			1.29 *s* 1.20 *s*	4.22, 3.38 *AB*, J_{gem} = 11	3.67 *s* ($COOCH_3$) 2.56 *m* (H at C(22))

a) Unless otherwise stated the proton appears as a pseudotriplet.
b) The signal at lower field was assigned to H_R, the one at higher field to H_S by Eu(fod)$_3$ induced paramagnetic shift and by stereochemical considerations; for H_R and H_S see [8].
c) 100 MHz spectrum.
d) Exchangeable with D_2O.
e) In DMSO-d_6.
f) Multiplet of 8 lines; *AB*-spectrum with J_{gem} = 11 after addition of D_2O.
g) Singlet after addition of D_2O.
h) Complicated multiplet due to the presence of a mixture of C(23) epimers.

The doublet at 1.15 ppm was assigned to the protons of the secondary methyl group at C(20), in accordance with the chemical shifts normally observed in a steroid side chain.

The presence of a tetra-substituted double bond was detected only by the ^{13}C-NMR. spectrum (Table 2) which showed two signals at 133.1 and 136.6 ppm re-

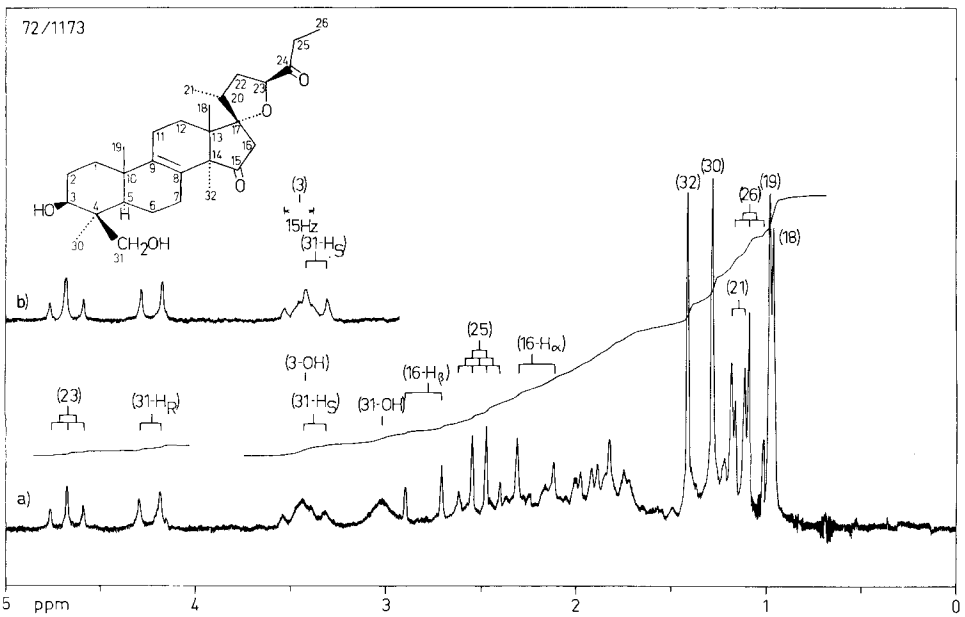


Fig. 2. *100 MHz ^1H-NMR. spectrum of eucosterol* (**1**) *in $CDCl_3$: a) before, b) after D_2O exchange*

Table 2. *^{13}C-NMR. chemical shifts of the eucosterols* **1** *and* **2** *in pyridine-d_5 and tentative assignments of some signals*[a])

1		**2**		**1**		**2**	
7.6 *q*	C(26)	7.7	C(26)	43.4 *d*	C(20)		
17.2		17.0				47.1	
18.8 *t*	C(11)	19.0	C(11)	47.7 *s*	C(13)		
19.9				51.1	C(5)	51.1	C(5)
20.4	C(19) [b])	20.2	C(19) [e])	51.9	C(16)		
20.8	C(6) [b])	20.7	C(6) [e])			55.2	
23.3	C(30) [d])	23.2	C(30) [e])	57.9 *s*	C(14)		
23.4	C(12) [d])	23.4	C(12) [e])	64.3 *t*	C(31)	64.3	C(31)
24.1		24.4		79.9 *d*	C(3)	80.1	C(3) [f])
27.2		26.7				80.4	C(16) [f])
28.9 *t*	C(2)	29.0	C(2)	81.8 *d*	C(23)	82.1	C(23)
32.3 *t*	C(7)	32.2	C(7)	91.2 *s*	C(17)	93.6	C(17)
35.8	C(1)	35.9	C(1)	133.1 *s*	C(8)	132.7	C(8)
36.9	C(25)			136.6 *s*	C(9)	136.6	C(9)
37.6	C(10)	37.6	C(10) + C(25)	211.7 *s*	C(24)	211.5	C(24)
43.1 *s*	C(4)	43.2	C(4)	215.1 *s*	C(15)	217.7	C(15)

[a]) 22.63 MHz spectra; chemical shifts are given in ppm relative to TMS; *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet; the assignments are based on data from [9], [10] and [12].

[b–f]) These assignments could be reversed.

spectively. Their positions are in good agreement with those found for the 8,9-double bond in lanosterol [9] and isovirescenol B [10].

In the IR. spectrum (Fig. 3) eucosterol (**1**) exhibited two carbonyl bands. The absorption at 1735 cm^{-1} is similar to that recorded for a steroid with carbonyl function at C(15) [11]. The presence of a cyclopentanone system was further supported by the signal at 215.1 ppm in the ^{13}C-NMR. spectrum [12]. The carbonyl group of the side chain showed the normal band at 1715 cm^{-1} and a signal at 211.7 ppm. Catalytic hydrogenation of eucosterol (**1**) with H_2/Pt in glacial acetic acid yielded

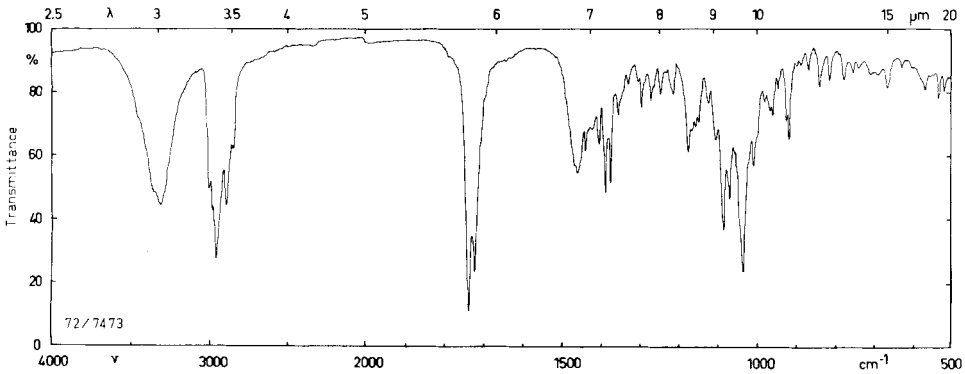


Fig. 3. *IR. spectrum of eucosterol* (**1**) *in KBr*

a mixture of the two epimers of dihydro-eucosterol (**9**) ($C_{29}H_{46}O_5$, *m/e* calc. 474.3345, found 474.3343) which were transformed to their tri-O-acetyl derivatives **10** by treatment with acetic anhydride/pyridine. The same reduction products were obtained with $LiBH_4$ in tetrahydrofuran at room temperature. Attempts to reduce both ketone functions with $LiAlH_4$ or $LiBH_4$ in refluxing ether or tetrahydrofuran failed, probably because of base-catalysed opening of the ether linkage. The steric hindrance at the C(15)-ketone was also demonstrated by the formation of only the mono-semicarbazone **5**.

The presence of two hydroxyl groups in eucosterol (**1**) was established by the formation of the di-O-acetyl derivative **3** upon treatment with acetic anhydride/pyridine at 35° and by two broad signals in the ^1H-NMR. spectrum at 3.02 and 3.34 ppm which disappeared after addition of D_2O. Formation of the keto-aldehyde **11** ($C_{29}H_{40}O_5$, *m/e* calc. 468.2876, found 468.2874), using CrO_3/H_2SO_4 in acetone, revealed the presence of a sterically hindered primary and a secondary hydroxyl function. The ^1H-NMR. spectrum in DMSO-d_6 (Table 1) showed a doublet at 4.98 ppm due to the proton of the secondary hydroxyl group. Because of its location adjacent to two magnetically unequivalent protons, the primary hydroxyl group appeared as the *X*-part of an *ABX*-system at 4.08 ppm. Both signals disappeared after addition of D_2O.

The 1,3-relationship of the two hydroxyl groups was established by the formation of the acetonide **6** with $CuSO_4$/acetone and by the fact that eucosterol (**1**) showed no reaction with $NaIO_4$ in aqueous methanol. Ring A stereochemistry was further deduced from the ^1H-NMR. spectra. The proton at C(3) formed a four line signal at

3.47 ppm as the *X*-part of an *ABX*-system with a vicinal spin-spin coupling constant sum of 15 Hz. The latter value is typical for an axial proton at C(3) [13]. In the di-O-acetyl derivative **3** this signal was shifted, as expected, to 4.54 ppm [14]. The methylene protons of the primary hydroxyl group appeared as an *AB*-quartet at 3.38 and 4.23 ppm with a geminal spin-spin coupling constant of 11 Hz. The large chemical shift difference is probably due to steric interaction with the methyl group at C(10) and formation of a hydrogen bond between the two hydroxyl groups. The latter effect can be seen in the IR. spectrum in chloroform at various concentrations. Downfield shift of the protons at C(31) to 4.14 and 4.35 ppm on acetylation is typical for the acetate of an axial hydroxymethylene group at C(4) [15]. The ^{1}H-NMR. data of the acetonide **6** are in good agreement with data published by Japanese workers [16], who have studied ring A acetonides of triterpene alcohols, and support the postulated stereochemistry of the substituents in ring A of eucosterol (**1**).

The signals at 64.3 and 81.8 ppm in the ^{13}C-NMR. spectrum of eucosterol (**1**) were assigned to the carbon atoms C(31) and C(3), respectively, by comparison with the published values for isovirescenol B [10] which possesses the same substitution pattern in ring A.

The presence of an ether linkage in eucosterol (**1**) was deduced from its ^{13}C-NMR. spectrum which showed two further signals for carbon atoms adjacent to oxygen at 79.9 and 91.2 ppm. Its position between C(17) and C(23) was established by the *AB*-spectrum of the protons at C(16) which had no further coupling and by base-catalyzed formation of the α,β-unsaturated ketone **12** (λ_{max} = 239 nm, log ε = 4.06, ethanol) which yielded the tri-O-acetyl derivative **13** on treatment with acetic anhydride/pyridine. Compound **12** was cleaved by $NaIO_4$ in aqueous methanol to give aldehyde **14** (M^+ at *m/e* 414) from which the semicarbazone **15** was obtained. Oxidation of **14** with Ag_2O in ethanolic NaOH [17] and subsequent methylation of the product with CH_2N_2 in ether yielded the carboxylic ester **17** (M^+ at *m/e* 444), identical with the methyl ester of the acid **16** described below.

4. Structure of 16β-Hydroxy-eucosterol (2). – Crystallization from chloroform/methanol gave 16β-hydroxy-eucosterol (**2**) as colourless needles, m.p. 264–267° (dec.); $[\alpha]_D^{25} = +4.7 \pm 2°$ (c = 1.04, chloroform/methanol 2:1). The molecular formula, $C_{29}H_{44}O_6$, which was deduced by high resolution mass spectrometry (*m/e* calc. 488.3138, found 488.3124) and by elemental analysis indicated the presence of an additional hydroxy group as compared with eucosterol (**1**). Substance **2** showed a positive *Liebermann-Burchard* reaction [6]. On silica gel it was slightly more polar than eucosterol (**1**) and gave a red colour with vanillin/H_2SO_4 [7] at room temperature.

The elimination of a propionyl radical in the mass spectrum (Fig. 4) and the signals of an ethyl ketone in the ^{1}H-NMR. spectrum (Fig. 5 and Table 1) indicated the presence of the same propionyl side chain as in eucosterol (**1**). The ^{1}H-NMR. spectrum of **2** showed four tertiary methyl singlets and one secondary methyl doublet, similar to the corresponding signals in **1**. The presence of a cyclopentanone system and of a normal ketone was confirmed by the IR. absorptions at 1745 and 1718 cm^{-1} and by the ^{13}C-NMR. signals (Table 2) at 217.7 and 211.5 ppm, respectively. The tetra-substituted double bond displayed peaks at 132.7 and 136.6 ppm in the ^{13}C-NMR. spectrum.

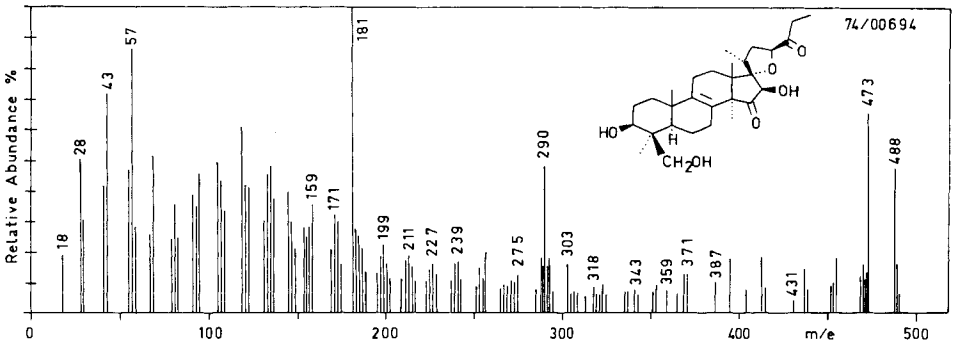


Fig. 4. *Mass spectrum of 16β-hydroxy-eucosterol* (**2**)

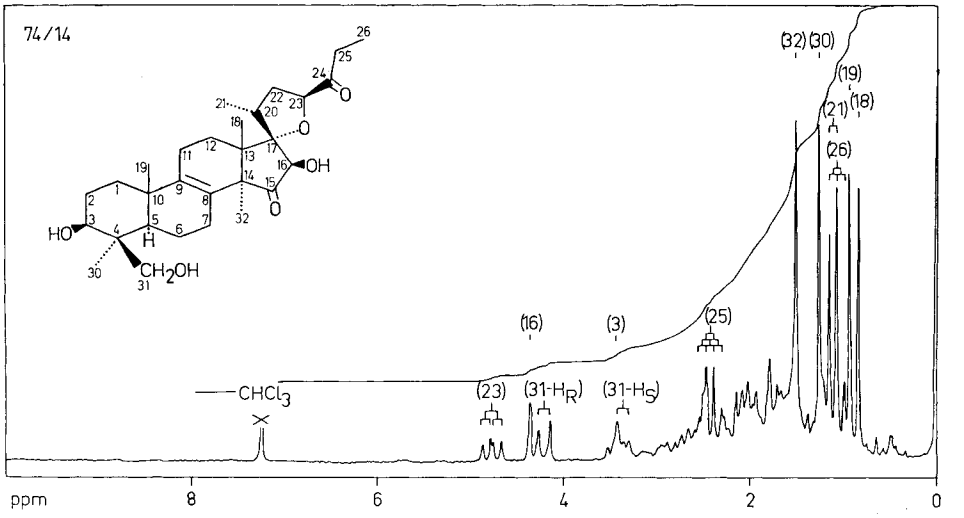


Fig. 5. *90 MHz ^{1}H-NMR. spectrum of 16β-hydroxy-eucosterol* (**2**) *in $CDCl_3$*

The formation of the tri-O-acetyl derivative **4** on treatment with acetic anhydride/pyridine revealed the presence of three hydroxyl groups in the molecule. The position and configuration of those in ring A are the same as in eucosterol (**1**), based on the ^{1}H-NMR. spectra of the metabolites, their O-acetyl derivatives (**3** and **4**) and their acetonides (**6** and **7**). Instead of the C(16) *AB*-quartet of eucosterol (**1**), a one proton doublet (J_{vic} = 3 Hz) appeared at 4.38 ppm due to the additional hydroxyl group. On treatment with D_2O this signal collapsed to a singlet. The configuration of the hydroxyl group was established on the basis of the following observation. Addition of increasing amounts of $Eu(fod)_3$ to a $CDCl_3$ solution of the acetonide **7** caused a more pronounced paramagnetic shift of the protons at C(18) than of those at C(32), in agreement with an equatorial hydroxyl group at C(16). As expected the proton at C(16) showed the most powerful paramagnetic shift.

Like eucosterol (**1**), 16β-hydroxy-eucosterol (**2**) exhibited two further carbon atoms adjacent to oxygen in the ^{13}C-NMR. spectrum. The presence of the novel

spiro-ether was also confirmed by the absence of a proton at C(17) in the ^{1}H-NMR. and by elimination of propionyl radical in the mass spectrum.

5. Structure of the Carboxylic Acid 16. – The carboxylic acid **16** crystallized from ethanol/*n*-hexane as colourless microcrystals, m.p. 246–250°. Its molecular formula, $C_{26}H_{38}O_5$, was deduced from high resolution mass spectrometry (*m/e* calc. 430.2719, found 430.2742). The broad absorption in the IR. spectrum (KBr) between 3500 and 2500 cm^{-1} and the solubility in 10 per cent $NaHCO_3$ suggested the presence of a carboxylic group. This was supported by the formation of the methyl ester **17** with CH_2N_2 in ether ($C_{27}H_{40}O_5$, *m/e* calc. 444.2876, found 444.2877). The base peaks in the mass spectra of **16** (Fig. 6) and **17** at *m/e* 371 ($[C_{24}H_{35}O_3]^+$, *m/e* calc. 371.2586, found 371.2584 and 371.2580 respectively) and the peaks at *m/e* 343 ($[C_{22}H_{31}O_3]^+$, *m/e* calc. 343.2273, found 343.2281 and 343.2239 respectively) are due to the fragment ions shown in Fig. 7. Both peaks also occurred in the high resolution mass spectra of

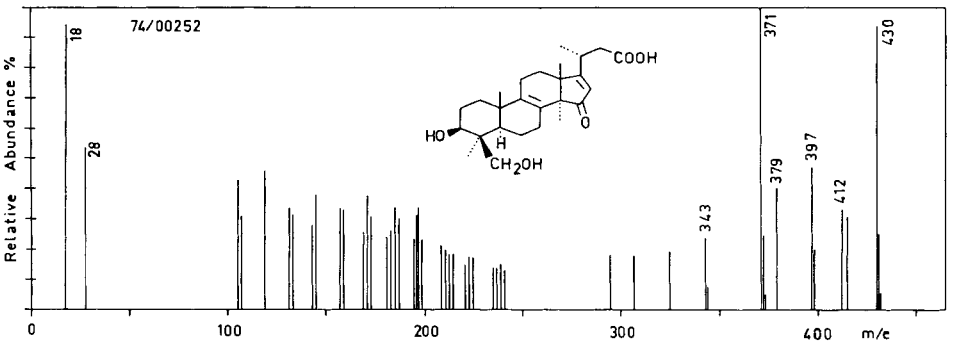
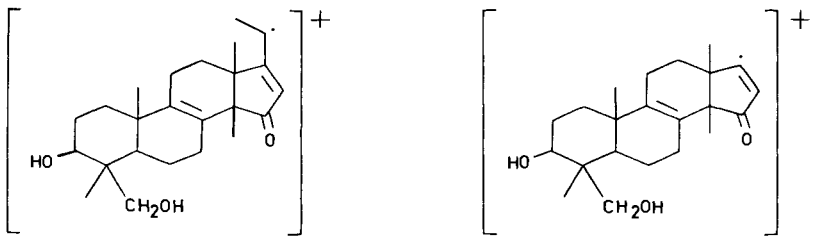


Fig. 6. *Mass spectrum of the carboxylic acid* **16**



m/e 371 $C_{24}H_{35}O_3$ m/e 343 $C_{22}H_{31}O_3$

Fig. 7. *Fragment ions in the mass spectra of the acid* **16** *and the ester* **17**

eucosterol (**1**) and 16β-hydroxyl-eucosterol (**2**). The easier formation, relative to **1** and **2**, of these fragments in the mass spectra of **16** and **17** is possibly due to the open chain structure of these compounds[4]).

In the UV. (ethanol) the acid **16** exhibited a maximum at 239 nm (log ε = 3.92); the same maximum was observed in the UV. spectra of the degradation products,

[4]) For further details see [18].

12 and **14**, of eucosterol (**1**). The bathochromic shift of 13 nm with respect to the calculated value of 226 nm indicated the presence of a strained cyclopentenone [19].

The IR. spectrum (KBr) showed the expected bands at 1690 and 1585 cm^{-1} for an α,β-unsaturated ketone, and at 1710 cm^{-1} for the carbonyl group of the carboxylic acid. The vinyl proton at C(16) was a broad singlet at 5.53 ppm due to allylic coupling with the proton at C(20). In the ^{1}H-NMR. spectrum (DMSO-d_6) (Table 1) the *AB*-quartet of the protons at C(31) appeared at 3.27 and 3.89 ppm with a geminal spin-spin coupling constant of 11 Hz. A multiplet at 3.20 ppm was attributed to the axial proton at C(3). These assignments are in good agreement with those found for eucosterol (**1**) in the same solvent.

The methyl ester **17** obtained from naturally occurring **16** showed ^{1}H-NMR. and mass spectra identical with those of a sample of **17** obtained by degradation of eucosterol (**1**) (see *Scheme*). Behaviour of the two samples on silica gel in different solvent systems was also identical. The novel nor-cholanoic acid **16** is therefore interrelated chemically to eucosterol (**1**) whose absolute configuration was established by X-ray analysis [4].

6. Conclusions. – The spiro-fused furanoic system of the eucosterols (**1** and **2**) is a *novel structural element* in the field of natural triterpenoid compounds. It is present in the diterpene lasiocoryn [20] and in ophiobolin A [21], a sesterterpene. The biosynthesis of the ether linkage has been established for the latter compound. If the biosynthesis of eucosterol (**1**) is similar, hydroxylation at C(17) and ring closure at C(23) would precede elimination of a carbon atom at the terminus of the side chain. A reversed process by which C(23) is hydroxylated first, followed by the attack of this hydroxyl group at the Δ^{16} double bond cannot be excluded.

Oxidation at C(31) is also unusual for tetracyclic triterpenes. The normal biosynthetic pathway includes elimination of the equatorial C(30) by stepwise oxidation, prior to isomerization of axial C(31) into the equatorial position [22]. The eucosterols are therefore interesting exceptions to this rule, and with their missing side chain carbon atom represent biosynthetically uncommon nortriterpenes.

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Experimental Part

1. *General.* Melting points were determined on a *Kofler* block and are corrected. Anhydrous sodium sulfate was employed as drying agent in reaction work-ups. Progress of most reactions and purity of all products were checked by thin layer chromatography (TLC.) on silica gel F 254 (*E. Merck*, Darmstadt, W. Germany) with visualization by UV. light or vanillin/H_2SO_4 spray reagent followed by heating to 120° [7]. Column chromatography with *Florisil*® (60–100 mesh, *Sigma Chemical Company*, St. Louis, U.S.A.) was used for the separation of the crude extracts. Reaction products were purified by preparative TLC. with precoated silica gel plates 60 F 254 (*E. Merck*, Darmstadt) and the TLC. zones were extracted with dichloromethane/methanol 9:1. All substances were dried at least 4 h at 0.01 Torr and 25° before spectral measurements.

Microanalyses were performed in the microanalytical laboratory of the Institute (*E. Thommen*). IR. (in cm^{-1}), UV. (λ_{max} nm, ($\log \varepsilon$)) and 90 MHz-^{1}H-NMR. spectra (Table 1) were measured on a *Perkin Elmer* Model 125 grating spectrometer, a *Beckman* D.K.2 spectrophotometer and a

Bruker WH90 spectrometer with *Fourier* transform, resp., in the spectral laboratories of the Institute (*K. Aegerter*). The ^{13}C-NMR. spectra (Table 2) were recorded on a *Bruker* WH90 (22.63 MHz) spectrometer with *Fourier* transform at the Institute laboratories (*K. Aegerter*), and the 100 MHz-^{1}H-NMR. spectra (Table 1) were measured on a *Varian* HA-100D spectrometer at the Physikalisch-chemisches Institut, Basel (*E. Wullschleger*). Rotations and additional IR. spectra were determined on a *Perkin Elmer* Model 141 polarimeter and a *Perkin Elmer* Model 177 grating spectrometer, resp. We thank *Dr. H. Lichti, Sandoz AG.*, Basel, for measurements of all mass spectra (data given in *m/e*) which were carried out on a *CEC* 21-110B instrument at 70 eV (high resolution) and on a *AEI* MS-30 instrument (normal resolution).

2. *Isolation.* To eliminate homoisoflavanones and waxes the layers of 3.47 kg fresh bulbs of *Eucomis punctata* L'*Herit.*[5]) were extracted in a *Soxhlet* apparatus with ether and worked up by the method described in [5] prior to homogenization in a mechanical mixer. The homogenate was extracted with acidic (pH 4) ethanol/water mixtures (3 l, 95:5, 80:20, 70:30 and 60:40, resp.) at 40° overnight and filtered over Celite. The combined alcoholic phases (12 l) were concentrated *in vacuo* to 800 ml, adjusted to pH 4 with solid K_2CO_3 and back-extracted with petroleum ether (3 × 500 ml) to eliminate remaining fats and waxes. Further extraction with ether (3 × 500 ml) and evaporation of the solvent yielded 2.1 g of crude extract. One per cent (v/v) of methanol was added during all these separations to avoid formation of emulsions.

The crude extract (2.1 g) was chromatographed on 250 g of *Florisil*® (250 ml fraction volume) using benzene, benzene/ether mixtures, ether and mixtures of ether and chloroform/ethyl acetate/methanol 1:1:1, successively as solvents. The 100 per cent ether fractions gave 190 mg eucosterol (**1**) after crystallization and purification of the mother liquor by preparative TLC. with chloroform/methanol 9:1.

Extractions of the other *Eucomis* species were performed in an analogous manner. *Eucomis bicolor Bak.* yielded a 2:1 mixture of eucosterol (**1**) and 16β-hydroxy-eucosterol (**2**), separated by chromatography on *Florisil*® using dichloromethane/methanol 96:4 as solvents. The nor-cholanoic acid **16** resulted from polar fractions of *Eucomis autumnalis* (*Mill.*) *Chitt.*

Eucosterol (*(23S)-17,23-epoxy-3β,31-dihydroxy-27-nor-5α-lanost-8-ene-15,24-dione*) (**1**), colourless needles from acetone, m.p. 235–236° (dec.); $[\alpha]_D^{25} = +20.4 \pm 2°$ (*c* = 1.00, chloroform). – UV. (ethanol): 196.5 (4.03), 280 (2.10), 293 (2.20). – IR. (KBr): 3300 (br., assoc. OH); 2940, 2875 (CH); 1735 (five ring C=O), 1715 (C=O); 1382, 1369, 1080, 1030. – ($CHCl_3$): 3620 (free OH), 3450 (br., assoc. OH); 1730, 1722 (C=O); 1025. – MS.: 472.3175 (M^+, calc. for $C_{29}H_{44}O_5$ 472.3189); 454 (M^+-18, H_2O); 439 (M^+-33, H_2O+CH_3); 415 (M^+-57, CH_3CH_2CO); 414 ($C_{26}H_{38}O_4$); 371 ($C_{24}H_{35}O_3$); 343 ($C_{22}H_{31}O_3$); 303 ($C_{19}H_{27}O_3$); 290 ($C_{19}H_{30}O_2$); 181 ($C_{11}H_{17}O_2$); 155 ($C_9H_{15}O_2$, base peak).

$C_{29}H_{44}O_5$ (472) Calc. C 73.69 H 9.38% Found C 73.93 H 9.49%

16β-Hydroxy-eucosterol (*(23S)-17,23-epoxy-3β,16β,31-trihydroxy-27-nor-5α-lanost-8-ene-15,24-dione*) (**2**), colourless needles from chloroform/methanol, m.p. 264–267° (dec.); $[\alpha]_D^{25} = +4.7 \pm 2°$ (*c* = 1.04, chloroform/methanol 2:1). – UV. (ethanol): 197 (4.04), 288 (2.16). – IR. (KBr): 3500 (br., assoc. OH); 2980, 2940, 2880 (CH); 1745 (five ring C=O), 1718 (C=O); 1085, 1040, 1030, 990, 965, 930, 920. – MS.: 488.3124 (M^+, calc. for $C_{29}H_{44}O_6$ 488.3138); 473 (M^+-15, CH_3); 455 (M^+-33, CH_3+H_2O); 431 (M^+-57, CH_3CH_2CO); 387 ($C_{24}H_{35}O_4$); 371 ($C_{24}H_{35}O_3$); 343 ($C_{22}H_{31}O_3$); 303 ($C_{19}H_{27}O_3$); 290 ($C_{19}H_{30}O_2$); 181 ($C_{11}H_{17}O_2$, base peak).

$C_{29}H_{44}O_6$ (488) Calc. C 71.28 H 9.08% Found C 70.13 H 9.09%

3β-Hydroxy-4β-hydroxymethyl-4,14α-dimethyl-15-oxo-24-nor-5α-chola-8,16-diene-23-oic acid (**16**), colourless microcrystals from ethanol/*n*-hexane, m.p. 246–250°. – UV. (ethanol): 237 (3.92). – IR. (KBr): 3380, 3270 (br., OH, COOH); 2960, 2940, 2870 (CH); 1710 (acid C=O), 1690 (α,β-unsat. C=O); 1585 (C=C); 1180, 1035. – MS.: 430.2742 (M^+, calc. for $C_{26}H_{38}O_5$ 430.2719); 415 (M^+-15, CH_3); 412 (M^+-18, H_2O); 397 (M^+-33, H_2O+CH_3); 379 (M^+-51, $2H_2O+CH_3$); 371 ($C_{24}H_{35}O_3$, base peak); 343 ($C_{22}H_{31}O_3$).

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[5]) The bulbs of all *Eucomis* species were purchased from *C. G. van Tubergen, Bloembollen- en Zaadhandel N.V.*, Haarlem, Holland.

3. *(23S)-3β,31-Diacetoxy-17,23-epoxy-27-nor-5α-lanost-8-ene-15,24-dione* (**3**). A solution of 40 mg of eucosterol (**1**) (0.085 mmol) in 1 ml of abs. pyridine and 2 ml of acetic anhydride was kept at 35° for 16 h. Evaporation of the solvent *in vacuo* with benzene and dichloromethane successively, followed by crystallization from ether yielded 44 mg of the acetate **3** as colourless needles, m.p. 168–170° (dec.); $[\alpha]_D^{25} = +17.4 \pm 2°$ ($c = 0.213$, chloroform). – IR. (KBr): 2970, 2950, 2880 (CH); 1740, 1730 (C=O); 1250, 1235 (acetate); 1090, 1065, 1030. – MS.: 556 (M^+); 498 ($M^+ - 58$, CH_3CH_2CHO, base peak); 481, 455, 427, 397, 387, 374, 181, 155.

4. *(23S)-3β,16β,31-Triacetoxy-17,23-epoxy-27-nor-5α-lanost-8-ene-15,24-dione* (**4**). A solution of 6 mg of **2** (0.012 mmol) in 0.4 ml of abs. pyridine and 0.2 ml of acetic anhydride was kept at 35° for 16 h. Evaporation of the solvent *in vacuo* with benzene and dichloromethane successively gave a brown oil. Purification by TLC., using chloroform/methanol 96:4, yielded 5.6 mg of the acetate **4** as a colourless oil, $[\alpha]_D^{25} = +31.5 \pm 2°$ ($c = 0.499$, chloroform). – IR. (CH_2Cl_2): 2960, 2930, 2880, 2860 (CH); 1760, 1740, 1730 (C=O); 1340, 1220 (acetate). – MS.: 614 (M^+); 599 ($M^+ - 15$, CH_3), 572, 554 ($M^+ - 60$, CH_3COOH); 498, 387, 181 (base peak).

5. *Semicarbazone* **5** *of eucosterol* (**1**). A solution of 6.3 mg of eucosterol (**1**) (0.013 mmol) in 1.6 ml of abs. methanol was treated with 0.4 ml of reagent (see below) and kept at 35° for 16 h. Addition of 0.1 ml of H_2O afforded **5** as colourless needles (5.1 mg), m.p. 240–242° (dec.); $[\alpha]_D^{25} = -12.4 \pm 2°$ ($c = 0.081$, cthanol). The reagent was prepared by mixing 200 mg of semicarbazide hydrochloride with 300 mg of anhydrous sodium acetate in a mortar. The mixture was triturated with 2 ml of abs. methanol and filtered. – IR. (KBr): 3480 (NH); 3300 (br., assoc. OH); 2930, 2880 (CH); 1740 (five ring C=O); 1695 (br., C=O and C=N); 1570 (C—N); 1460 1065, 1040, 1030. – MS.: 529 (M^+); 371 (base peak).

6. *(23S)-17,23-Epoxy-3β,31-di-O-isopropylidene-27-nor-5α-lanost-8-ene-15,24-dione* (**6**). A solution of 23 mg of eucosterol (**1**) (0.049 mmol) in 23 ml of acetone was shaken with 200 mg of anhydrous $CuSO_4$ at room temp. for 18 h. Filtration and evaporation of the solvent *in vacuo* yielded a white crystalline solid (18 mg) which was purified by TLC. with chloroform/methanol 97:3. The acetonide **6** crystallized from benzene/*n*-hexane as colourless needles, m.p. 203–204°; $[\alpha]_D^{25} = +17.4 \pm 2°$ ($c = 0.098$, chloroform). – IR. (KBr): 2980, 2950, 2890 (CH); 1730 (C=O); 1380, 1370, 1090, 1075. – MS.: 512 (M^+); 497 ($M^+ - 15$, CH_3); 454 ($M^+ - 58$, CH_3CH_2CHO); 439, 397, 343, 155 (base peak).

7. *(23S)-17,23-Epoxy-16β-hydroxy-3β,31-di-O-isopropylidene-27-nor-5α-lanost-8-ene-15,24-dione* (**7**). A solution of 16 mg of **2** (0.033 mmol) in 16 ml of acetone was shaken with 200 mg of anhydrous $CuSO_4$ at 25° for 18 h. Filtration and evaporation of the solvent *in vacuo* gave 12 mg of a colourless solid. Purification by TLC. using chloroform/methanol 98:2 as solvent, followed by crystallization from benzene/*n*-hexane afforded the acetonide **7** as colourless needles, m.p. 228–230°; $[\alpha]_D^{25} = -2.6 \pm 2°$ ($c = 0.230$, chloroform). – IR. (KBr): 3440 (br., assoc. OH); 2980, 2940, 2890 (CH); 1740, 1730 (C=O); 1170, 1120, 1020, 980. – MS.: 528 (M^+); 513 ($M^+ - 15$, CH_3); 470 ($M^+ - 58$, CH_3CH_2CHO); 444, 333, 181 (base peak).

8. *(23S)-16β-Acetoxy-17,23-epoxy-3β,31-di-O-isopropylidene-27-nor-5α-lanost-8-ene-15,24-dione* (**8**). The acetonide **8** (2.6 mg; 0.005 mmol) was acetylated with 0.3 ml of acetic anhydride in 0.3 ml of abs. pyridine at 35° for 16 h. The solvent was removed *in vacuo* by addition of benzene and the oily residue (2.1 mg) was purified by filtration in dichloromethane over *Florisil®*, finally affording **8** as a colourless oil. – MS.: 570 (M^+); 555 ($M^+ - 15$, CH_3); 512 ($M^+ - 58$, CH_3CH_2CHO, base peak); 396, 343, 181.

9. *(23S)-17,23-Epoxy-3β,24ξ,31-trihydroxy-27-nor-5α-lanost-8-ene-15,24-dione* (**9**). Eucosterol (**1**) (11.64 mg, 0.025 mmol) was hydrogenated with a trace of PtO_2 in 2 ml of glacial acetic acid. After 30 min the solution was filtered and the solvent evaporated *in vacuo*. The colourless solid (13 mg) was recrystallized twice from acetone and methanol successively, yielding an epimeric mixture of **9** as colourless needles, m.p. 235–237°; $[\alpha]_D^{25} = +37.1 \pm 2°$ ($c = 0.280$, chloroform). – IR. (KBr): 3300 (br., assoc. OH); 2960, 2930 (CH); 1731 (five ring C=O); 1080, 1030. – MS.: 474.3343 (M^+, calc. for $C_{29}H_{46}O_5$ 474.3345); 456 ($M^+ - 18$, H_2O); 441 ($M^+ - 33$, $H_2O + CH_3$); 416 ($M^+ - 58$, CH_3CH_2CHO); 371 ($C_{24}H_{35}O_3$); 343 ($C_{22}H_{31}O_3$); 303 ($C_{19}H_{27}O_3$); 290 ($C_{19}H_{30}O_2$, base peak); 183, 157.

10. *(23S)-3β,24ξ,31-Triacetoxy-17,23-epoxy-27-nor-5α-lanost-8-ene-15,24-dione* (**10**). A solution of 2.3 mg of **9** (0.0049 mmol) in 0.2 ml of abs. pyridine and 0.2 ml of acetic anhydride was kept at 35° for 16 h. Evaporation of the solvent *in vacuo* by addition of benzene and dichloromethane, successively, yielded a yellow oil which was purified by filtration in dichloromethane over *Florisil*®. The acetate **10** finally obtained was a colourless oil (3.42 mg), $[\alpha]_D^{25} = +35.4 \pm 2°$ ($c = 0.342$, chloroform). – IR. (CH_2Cl_2): 2940, 2880 (CH); 1730 (br., C=O); 1540, 1230 (acetate); 1070, 1030. – MS.: 600 (M^+); 542 ($M^+ - 58$, CH_3CH_2CHO); 522, 427, 387, 374 (base peak); 359, 239, 225, 199.

11. *(23S)-17,23-Epoxy-3,15,24-trioxo-27-nor-5α-lanost-8-ene-31-al* (**11**). A solution of 62 mg of eucosterol (**1**) (0.13 mmol) in 25 ml of acetone was treated with *Jones'* reagent (2.67 g CrO_3, 2.3 ml of conc. H_2SO_4, water *ad* 10 ml) until the brown colour persisted. After addition of 0.05 ml of methanol the solution was concentrated to a volume of 5 ml, diluted with 25 ml of water and extracted three times with 10 ml of dichloromethane. Evaporation of the solvent *in vacuo* yielded 67 mg of a yellow solid which on crystallization from methanol gave **11** as colourless plates, m.p. 205–211° (dec.); $[\alpha]_D^{25} = +1.8 \pm 2°$ ($c = 1.08$, chloroform). – IR. (KBr): 2970, 2950, 2880 (CH); 2820 (CH of the aldehyde); 1735, 1730, 1725, 1700 (C=O); 1380, 1370, 1090, 1070. – MS.: 468.2874 (M^+, calc. for $C_{29}H_{40}O_5$ 468.2876); 411 ($M^+ - 57$, CH_3CH_2CO); 410 ($M^+ - 58$, CH_3CH_2CHO); 367, 339, 299, 286, 271, 149.

12. *(23S)-3β,23,31-Trihydroxy-27-nor-5α-lanosta-8,16-diene-15,24-dione* (**12**). Eucosterol (**1**) (13 mg, 0.028 mmol), dissolved in 2.5 ml of abs. methanol, was treated with 0.35 mg of $NaOCH_3$ and the solution kept at 25° for 24 h. The reaction was stopped by addition of 0.1 ml of glacial acetic acid, the solvent evaporated *in vacuo* and the oily residue purified in chloroform/methanol 9:1 by TLC. Crystallization from benzene/*n*-hexane afforded **12** (7 mg) as colourless needles, m.p. 166–169°; $[\alpha]_D^{25} = +47.4 \pm 2°$ ($c = 0.118$, ethanol). – UV. (ethanol): 239 (4.06). – IR. (KBr): 3400 (br., assoc. OH); 2970, 2940, 2880 (CH); 1700 (br., C=O); 1595 (C=C); 1100, 1085, 1035. – MS.: 472 (M^+, base peak); 457 ($M^+ - 15$, CH_3); 454 ($M^+ - 18$, H_2O); 439 ($M^+ - 33$, $H_2O + CH_3$); 414 ($M^+ - 58$, CH_3CH_2CHO); 371, 343, 313, 303, 290, 155, 149.

13. *(23S)-3β,23,31-Triacetoxy-27-nor-5α-lanosta-8,16-diene-15,24-dione* (**13**). The α,β-unsaturated ketone **12** (4 mg, 0.0085 mmol) was acetylated with 0.2 ml of acetic anhydride in 0.4 ml of abs. pyridine at 35° for 16 h. Evaporation of the solvent *in vacuo* by addition of benzene and dichloromethane, successively, yielded a brown oil, which was purified in chloroform/methanol 94:6 by TLC. The acetate **13** resulted as a colourless oil (4.79 mg), $[\alpha]_D^{25} = +32.1 \pm 2°$ ($c = 0.249$, ethanol). – UV. (ethanol): 229 (3.69). – IR. (CCl_4): 2970, 2940, 2880 (CH); 1735, 1715, 1700 (C=O); 1600 (C=C); 1345, 1230 (acetate); 1130, 1025. – MS.: 598 (M^+); 583 ($M^+ - 15$, CH_3); 538 ($M^+ - 60$, CH_3COOH); 525, 480, 465, 455, 427, 307, 133 (base peak).

14. *3β-Hydroxy-4β-hydroxymethyl-4,14α-dimethyl-15-oxo-24-nor-5α-chola-8,16-diene-23-al* (**14**). A stirred solution of 16 mg (0.034 mmol) of **12** in 2 ml of methanol was treated with a solution of 20 mg (0.093 mmol) of $NaIO_4$ in 2 ml of water at 25°. After 2 h the mixture was diluted with 6 ml of water and methanol evaporated *in vacuo*. The remaining aqueous solution was extracted three times with 5 ml of chloroform/ether 1:3 and the organic phase was washed with 2N HCl, water, 2N Na_2CO_3 and water, successively. Evaporation of the solvent *in vacuo* yielded 10 mg of a yellow crystalline solid which was purified in chloroform/methanol 94:6 by TLC. Crystallization from acetone afforded the aldehyde **14** as colourless needles, m.p. 147–149°; $[\alpha]_D^{25} = +44.7 \pm 2°$ ($c = 0.338$, ethanol). – UV. (ethanol): 237 (3.90). – IR. ($CHCl_3$): 3680 (free OH); 3440 (br., assoc. OH); 2940 (CH); 2720 (CH of the aldehyde); 1730 (aldehyde C=O); 1700 (α,β-unsat. C=O); 1600 (C=C); 1375, 1365, 1100, 1085, 1030. – MS.: 414 (M^+); 399 ($M^+ - 15$, CH_3); 381 ($M^+ - 33$, $H_2O + CH_3$); 371, 343, 303, 290, 181, 155, 119, 69 (base peak).

15. *Semicarbazone* **15** *of the aldehyde* **14**. A solution of 7 mg (0.017 mmol) of the aldehyde **14** in 0.2 ml of abs. methanol was treated with 0.4 ml of the reagent, described in section 5, and kept at 35° for 16 h. Addition of 0.2 ml of water afforded **15** as colourless needles, m.p. 252–254°; $[\alpha]_D^{25} = +10.6 \pm 2°$ ($c = 0.161$, ethanol). – IR. (KBr): 3470 (NH); 3200 (br., assoc. OH); 2930, 2860 (CH); 1690 (br., C=O and C=N); 1580 (C—N); 1435, 1370, 1125, 1040. – MS.: 471 (M^+); 371 (base peak).

16. *Methyl 3β-hydroxy-4β-hydroxymethyl-4,14α-dimethyl-15-oxo-24-nor-5α-chola-8,16-diene-23-oate* (**17**). A suspension of freshly prepared Ag_2O (4 mg $AgNO_3$ dissolved in 0.1 ml of water and

0.3 ml of 10 per cent NaOH added) was shaken for 90 min at 25° with a solution of 3 mg of **14** in 0.3 ml of ethanol, diluted with 2 ml of water and extracted with 1 ml of dichloromethane to eliminate unreacted educt. The aqueous phase was acidified with 2N HCl, extracted five times with 2 ml of dichloromethane and the solvent evaporated *in vacuo*. The crude acid **16** (1.9 mg) was directly esterified in 1 ml of methanol/water 9:1 with an etheral solution of CH_2N_2. Evaporation of the solvent *in vacuo* gave a brown oil which was purified by filtration in dichloromethane over *Florisil®*, affording the methyl ester **17** as a colourless, microcrystalline solid. – MS.: 444.2877 (M^+, calc. for $C_{27}H_{40}O_5$ 444.2876); 429 (M^+-15, CH_3); 411 (M^+-33, CH_3+H_2O); 393 (M^+-51, CH_3+2H_2O); 371 ($C_{24}H_{35}O_3$, base peak); 343 ($C_{22}H_{31}O_3$); 220, 197, 195, 181, 169, 167, 155, 119.

A sample of the methyl ester **17**, obtained from natural acid **16** by an analogous procedure to that described above, had the same spectral properties and Rf-values as the methyl ester which resulted from the degradation of eucosterol (**1**) (see sections 12–16).

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