14. 5α-24-Norcholestan-3β-ol and (24*Z*)-Stigmasta-5,7,24(28)-trien-3β-ol, Two New Marine Sterols from the Pacific Sponges *Terpios zeteki* and *Dysidea herbacea*[1])

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Summary

The steroidal components of 2 marine sponges, *Terpios zeteki* (from Hawaii) and *Dysidea herbacea* (from Australia) were fractionated through a combination of chromatographic methods, including reversed phase HPLC., and were analyzed by a combination of physical methods, including high resolution GC.-MS. and 360 MHz ^{1}H-NMR. *T. zeteki* contains 6 conventional 5α-stanols which comprise 91% of the sterol mixture, and traces (0.5%) of a new C_{26} sterol, 5α-24-norcholestan-3β-ol. Minor amounts of conventional Δ^5-sterols (6.5%) and of a single Δ^4-3-keto-steroid (1.5%) were also present. In contrast, the Australian sponge *(D. herbacea)* contains 3 $\Delta^{5,7}$-sterols which comprise 1.5% of the sterol mixture, and one new C_{29} sterol, (24*Z*)-stigmasta-5,7,24(28)-trien-3β-ol, as the major component (75%). In addition, minor amounts of conventional 5α-stanols (0.5%), Δ^5-sterols (5%) and 5α-Δ^7-sterols (18%) were present in this complex sterol mixture. The possible dietary or endosymbiotic origins of these sterols are discussed.

Introduction. – Marine organisms contain very complex sterol mixtures which often include sterols with unusual structures compared to those from terrestrial organisms [2–5]. These sterols, including sterols with unprecedented side chain alkylation patterns or unconventional steroid ring systems, seem to be particularly prevalent in primitive animals, notably the sponges (Porifera). Therefore, in our continuing search for novel sterols [1] and key marine sterol biosynthetic intermediates [6] among the minor and trace components of marine sterol mixtures, we have paid particular attention to the sponges. We report the analysis of the sterol mixtures of 2 Pacific sponges, *Terpios zeteki,* from Hawaii, and *Dysidea herbacea,* from Australia which included respectively a novel saturated C_{26} sterol and a novel triunsaturated C_{29} sterol.

[1]) Part XI in the Stanford series 'Minor and Trace Sterols in Marine Invertebrates'. For X: see [1].

Results. – The gas-liquid chromatographic (GLC.) and combined gas chromatographic mass spectrometric (GC.-MS.) analyses of the free sterol mixtures of both sponges revealed their complex composition. Separations by high pressure liquid chromatography (HPLC.) on a reversed phase (C_{18}) column and subsequent GLC. and GC.-MS. analysis of the fractions made possible the identification of the sterols listed in *Table 1* for *T. zeteki* and in *Table 2* for *D. herbacea*, with the specific structures reproduced in the *Figure*. The identification of known sterols was based on direct comparison with authentic samples (GLC. retention times and coinjection, GC.-MS.). The novel sterols, 5α-24-norcholestan-3β-ol (**1b**) and (24*Z*)-stigmasta-5,7,24(28)-trien-3β-ol (**4j**) were isolated from the crude mixture and purified by

Table 1. *Sterols present in* T.zeteki

Relative retention time[a])	Structure	Percent in the sterol mixture[b])		Enriched HPLC. fraction nb.
0.76	**1b**	0.5		1
0.95	**1c** or **1d**	~0.3	}	1
1.00	**1e**	~59	} mixt. 60.3	2 and 3
1.00	**2e**	~ 1	}	2
1.14	**1f**	~ 1	}	1
1.14	**2f**	~ 0.5	} mixt. 3.1	2
1.15	**5e**	~ 1.5	}	1
1.29	**1h**	~15.0	}	4
1.29	**2h**	~ 1.0	}	2
1.34	**1g**	~ 5.0	} mixt. 22.1	1
1.34	**2g**	~ 1	}	2
1.42	**2i**	1.8		2
1.61	**1l**	~10.5	}	4
1.61	**2l**	~ 1	} mixt. 11.8	3
1.93	**2** (see text)	0.2		4

[a]) Retention time (relative to cholesterol = 1.00) measured using OV 25–3% on Gas Chrom Q at 265°.
[b]) Estimated from the GLC. peaks of the sterol mixture before and after HPLC. fractionation.

Table 2. *Sterols present in* D.herbacea

Relative retention time[a])	Structure	Percent in the sterol mixture[b])		Enriched HPLC. fraction nb.
0.95	**2c** or **2d**	~ 0.5	}	2
1.00	**1e**	~ 0.5	} mixt. 3.1	4
1.00	**2e**	~ 2	}	4
1.10	**3c** or **3d**	~ 1	}	1
1.10	**4c** or **4d**	~ 0.1	} mixt. 1.0	1
1.14	**2f**	1.5		2
1.34	**2g**	1.3		2
1.40	**4f**	0.8		2
1.59	**3g**	~ 7	}	2 and *3*
1.59	**4g**	~ 0.5	} mixt. 7.7	*2* and 3
2.18	**3j**	~10	}	3 and *4*
2.18	**4j**	~75	} mixt. 84.5	*3* and *4*

[a,b]) Same remarks as in *Table 1*.

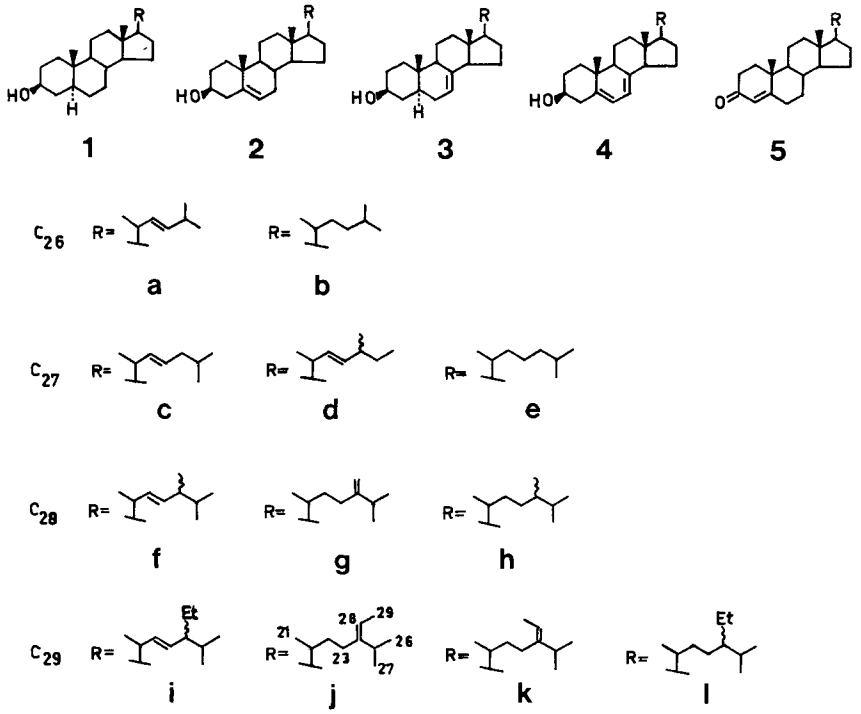

Figure. *Structure of the sterols listed in* Tables 1 *and* 2

repeated HPLC. and argentic preparative thin layer chromatography (TLC.) of the acetates. Their structural assignments were made with the aid of high resolution mass spectrometry, 360 MHz proton nuclear magnetic resonance (NMR.) spectroscopy and ultraviolet (UV.) spectroscopy.

Sterols of T. zeteki. The presence of cholestanol and lesser amounts of other saturated compounds in this sponge, as well as a sterol believed to possess unsaturation only in the side chain, was reported by *Bergmann et al.* [7] almost 30 years ago. Our reexamination confirms these findings in that 7 5α-stanols comprise more than 90% of the sterol mixture, 5α-cholestanol (**1e**) being the predominant one (59%). Five other 5α-stanols, previously described as constituents of marine organisms [5, 8–11], are also present: 24ξ-methylcholest-22-en-3β-ol (**1f**), 24ξ-methylcholestan-3β-ol (**1h**), 24-methylenecholestan-3β-ol (**1g**), 24ξ-ethylcholestan-3β-ol (**1l**) and cholest-22-en-3β-ol (**1c**) or 27-nor-24ξ-methylcholest-22-en-3β-ol (**1d**)[2]).

A low-molecular weight stanol with shorter GLC. retention time than cholestanol was also present. A complete high resolution mass spectrum yielded the empirical formula $C_{26}H_{46}O$ and also demonstrated the presence of ions characteristic of the sterol nucleus [12] and diagnostic for the fully reduced nucleus: $C_{22}H_{38}^+$ (loss of A ring by cleavage of C(1)–C(10) and C(4)–C(5) bonds); $C_{19}H_{29}^+$ (loss of side

[2]) Low concentrations and difficulties in separation prevented the isolation of sufficient material for securing NMR. data necessary to distinguish between side chain structures **c** and **d**.

chain and water); $C_{18}H_{32}^+$ (loss of A ring by cleavage of C(6)–C(7) and C(9)–C(10) bonds); $C_{16}H_{26}O^+$ (loss of D ring by cleavage of C(14)–C(15) and C(13)–C(17) bonds); $C_{16}H_{25}O^+$ (loss of D ring with H transfer) and $C_{16}H_{23}^+$ (loss of water and D ring with H transfer). The above data support the assignment of the structure of this sterol as 5α-24-norcholestan-3β-ol (**1b**), a sterol previously not encountered in nature. Several ions in the mass spectrum (*m/e* 248, 234, 233 and 215) establish the difference between the 24-norcholestanol (C_{19} nucleus) and the 19-norcholestanol (C_{18} nucleus) isolated previously from *Axinella polypoides* [11] [13].

The 360 MHz proton NMR. spectrum of this C_{26} sterol also supports the proposed structure. There are no olefinic protons and all the other protons characteristic of sterols are observed: singlets at 0.631 ppm ($H_3C(18)$) and 0.787 ppm ($H_3C(19)$); doublets centered at 0.875 ppm ($J = 6.8$ Hz, $H_3C(21)$), 0.851 ppm and 0.834 ppm ($J = 6.5$ Hz, $H_3C(25)$, $H_3C(26)$); multiplet at 3.580 ppm (HC(3)). Two doublets are observed for the methyls of the isopropyl group. Splitting due to the long-range induction of nonequivalence in prochiral side chain groups is also reported in the NMR. spectrum of 5α-24-norcholestane [14] and can be used as a model for the assignment of the peaks in the natural compound. Coinjection of **1b** with an authentic sample of 24-norcholest-5-en-3β-ol (**2b**) confirmed the presence of a 5α-stanol nucleus in this new C_{26} sterol since the 2 sterols coelute [15].

In addition to the 5α-stanols, the sterol mixture of *T. zeteki* contains a smaller amount of 6 Δ^5-sterols widely distributed among marine organisms [5]: cholesterol (**2e**), 24ξ-methylcholesta-5,22-dien-3β-ol (**2f**), 24ξ-methylcholest-5-en-3β-ol (**2h**), ergosta-5,24(28)-dien-3β-ol (**2g**), 24ξ-ethylcholesta-5,22-dien-3β-ol (**2i**) and 24ξ-ethylcholest-5-en-3β-ol (**2l**). Another sterol with a relative GLC. retention time of 1.93 was detected in trace amounts. After HPLC., this component remained at trace levels in fraction 4 *(Table 1)* with **1l** and **1h** as the major components. A weak mass spectrum (GC.-MS.) of this compound showed a molecular ion of mass 428 with typical fragmentations (M^+-85, M^+-111) of a Δ^5-3β-hydroxysterol [12]. The low concentration of this sterol and the difficulty of its isolation prevented the attainment of good quality NMR. and mass spectral data necessary for a secure structural assignment. Therefore identification of the structure of the saturated side chain of this Δ^5-C_{30} sterol must await further studies.

The last steroidal component identified in *T. zeteki* was cholest-4-en-3-one (**5e**). Its presence is not surprising since such 3-oxosteroids occur in other marine organisms [5] [16] [17] and are intermediates [18] in the conversion of Δ^5-sterols (*e.g.*, cholesterol **2e**) to 5α-stanols (*e.g.*, cholestanol **1e**) both of which are present in the *T. zeteki* sterol mixture.

Sterols of D. herbacea. The sterols of this sponge contain a diversity of nuclei, ranging from 5α-stanols to Δ^5-, Δ^7- and $\Delta^{5,7}$-sterols. The following 4 Δ^5-3β-hydroxysterols were detected in small amounts (less than 6% of the mixture) but are widely distributed in the marine environment [5]: cholesterol (**2e**), 24ξ-methylcholesta-5,22-dien-3β-ol (**2f**), ergosta-5,24(28)-dien-3β-ol (**2g**) and cholesta-5,22-dien-3β-ol (**2c**) or 27-nor-24ξ-methylcholesta-5,22-dien-3β-ol (**2d**)[2]).

Three Δ^7-5α-sterols account for about 18% of the sterol mixture. These 3 sterols, 5α-cholesta-7,22-dien-3β-ol (**3c**) or 5α-27-nor-24ξ-methylcholesta-7,22-dien-3β-ol (**3d**)[2]), 5α-ergosta-7,24(28)-dien-3β-ol (**3g**) and (24*Z*)-5α-stigmasta-7,24(28)-dien-

3β-ol (**3j**) have been encountered as constituents of other marine organisms, notably the echinoderms [5].

UV. analysis of the total sterol mixture revealed maxima at 262, 270, 281, 293 nm typical of $\Delta^{5,7}$-sterols; their extinction coefficients indicated that they amount to more than 75% of the total sterols present. Four separate $\Delta^{5,7}$-sterols were identified, the first one being either cholesta-5,7,22-trien-3β-ol (**4c**) or 27-nor-24ξ-methyl-cholesta-5,7,22-trien-3β-ol (**4d**)[2]). The former has been identified in other sponges [11] [19] [20] but to our knowledge **4d** has not been encountered previously in nature. Another component (0.8%) of *D. herbacea* was 24ξ-methylcholesta-5,7,22-trien-3β-ol (**4f**), one of the most widely distributed $\Delta^{5,7}$-sterols in marine organisms [5]. The third tri-unsaturated component (0.5%) was ergosta-5,7,24(28)-trien-3β-ol (**4g**). Its occurrence in the terrestrial fungus *Phycomyces blakesleeanus* together with its spectroscopic properties have been recorded [21], but to our knowledge this is the first identification of **4g** in the marine environment.

The last $\Delta^{5,7}$-sterol of *D. herbacea*, the major one ($\sim$75%) in the mixture, is an hitherto undescribed natural product identified on the basis of its spectroscopic data as (24*Z*)-stigmasta-5,7,24(28)-trien-3β-ol (**4j**). The high resolution mass spectrum ($M^+ = C_{29}H_{46}O$) exhibited peaks diagnostic for the $\Delta^{5,7}$-diunsaturated nucleus: $C_{26}H_{39}^+$ (loss of part of the A ring by cleavage of the C(1)-C(10) and C(3)-C(4) bonds plus H transfer [21] [22]), $C_{12}H_{14}^+$, $C_{11}H_{11}^+$ and $C_{10}H_8^+$, the origins of these last 3 ions being described by *Zaretskii* [12]. Additional ions characteristic of a sterol with a doubly unsaturated nucleus and an unsaturated side chain [12] [23] are those corresponding to $C_{19}H_{25}O^+$ (loss of side chain plus 2 H transfer), $C_{19}H_{25}^+$ (loss of side chain and water) and $C_{16}H_{19}^+$ (loss of D ring and water). Finally, the presence of the 24(28) side chain double bond can be deduced from the presence of the ions of *m/e* 312.24604 ($C_{22}H_{32}O^+$, *McLafferty* rearrangement by cleavage of the 22–23 bond together with one H transfer from C(20)), 294.23600 ($C_{22}H_{30}^+$, loss of water from *m/e* 312) and 279.21075 ($C_{21}H_{27}^+$, loss of methyl and water from *m/e* 312) [12] [23] [24].

The 360 MHz proton NMR. spectrum of this new sterol exhibits 3 olefinic signals. The ring olefinic protons (2 H) are deshielded to 5.581 and 5.390 ppm, indicating a conjugated system. The olefinic signal at 5.112 ppm has an intensity corresponding to one proton and is split into a quartet (J = 6.5 Hz) which supports the coupling to a methyl group (*i.e.*, >C=C*H*–CH_3). The multiplet due to the C(3) proton occurs at 3.636 ppm. Methyl signals are observed at 0.623 ppm (*s*, $H_3C(18)$), 0.945 ppm (*s*, $H_3C(19)$), 0.992 ppm (*d*, J = 7 Hz, $H_3C(21)$), 0.998 ppm (*d*, J = 7 Hz, $H_3C(26)$ and $H_3C(27)$) and 1.591 ppm (*d*, J = 6.5 Hz, $H_3C(29)$). The above spectral data require the assignment of structure **j** or **k** for the side chain of this new sterol. The deshielded doublet confirms the presence of an ethylidene group and the chemical shift, identical to that of $H_3C(29)$ of isofucosteryl acetate (acetate of **2j** [25]), suggests that the new sterol is the 24(28)*Z* isomer **4j**. Unambiguous assignment for the geometry of the 24(28) double bond is based on the chemical shift of the allylic proton at C(25), which occurs as a septet at 2.8 ppm in isofucosterol (**2j**) but at 2.2 ppm in fucosterol (**2k**) [26]. In our case, the septet (J = 7 Hz, 1H) is at 2.851 ppm confirming the *Z* configuration of **4j**. The other allylic protons at C(23) yield a triplet at 2.286 ppm (J = 12 Hz, 2 H).

Discussion. – The sponge *Terpios zeteki* (de Laubenfels, 1936) is classified in the order *Hadromerida* and in the family *Suberitidae* of the class *Desmospongia.* Little work has been reported on the sterols of sponges belonging to this family since the pioneering studies of *Bergmann* [7]. He found that cholestanol and other nuclear saturated sterols predominate in 5 other species of this family. Recently *Sica et al.* [27] reported exclusively Δ^5-sterols in another species *(Cliona viridis)* within the order *Hadromerida.* A reexamination of additional members in this family may help to clarify the relationship between sterol content and taxonomy.

In *T. zeteki* the presence of stanols as major components as well as Δ^5-sterols as minor ones might indicate that this organism is able to reduce the 5,6-double bond, which is the position of nuclear unsaturation in the most common and widespread marine sterols. This conversion usually proceeds through the intermediate production of steroidal 3-oxo-4-enes [18]. The detection of cholest-4-en-3-one in *T. zeteki* tends to confirm the above hypothesis. The occurrence in the sponge of sterols with saturated side chains such as **1h**, **2h**, **1l**, **2l** may reflect the ability of this sponge to reduce side chain double bonds, although it is also possible that they are of dietary origin.

If the above suggestion is correct, the new C_{26} sterol, 5α-24-nor-cholestan-3β-ol (**1b**), could originate from the biological reduction of 24-norcholesta-5,22-dien-3β-ol (**2a**) by the sponge. The C_{26} sterol **2a** has a very wide distribution in the marine environment, occurring in the sterol mixtures of nearly every marine animal phylum [3] [5] [28] and even sea water [29]. Therefore, **2a** is very likely a component of the diet of *T. zeteki.* Other C_{26} steroids – 24-nor-5α-cholest-22-en-3β-ol (**1a**), 24-norcholest-5-en-3β-ol (**2b**) and 24-norcholesta-4,22-dien-3-one (**5a**) – which could be intermediates in the production of **1b**, have been isolated from various marine invertebrates [3] [5] [28 and ref. therein]. However, the very limited distribution of these sterols suggests that the widespread C_{26} sterol **2a** is consumed and reduced by the sponge to the completely saturated 5α-stanol **1b**. A C_{26} 5α-stanol with a C_7 side chain has been found in lacustrine sediments but its structure was not elucidated [30].

The sponge *Dysidea herbacea* is a member of the order *Dictyoceratida* and of the family *Dysideidae* within the class *Desmospongia.* From the 4 other species of this family studied [5] [11], 3 were reported to contain conventional C_{26}, C_{27}, C_{28}, C_{29}-Δ^5 sterols whereas one, *Dysidea avara,* contained Δ^5-sterols together with more than 40% of $\Delta^{5,7}$-sterols. Several sponges within that order but belonging to another family *(Spongidae)* contain almost exclusively $\Delta^{5,7}$-sterols [11]. These findings support the view that sterol analyses may be an aid in some aspects of sponge taxonomy. However, one of these sponges, *Spongia officinalis,* has recently been reported [10] to contain exclusively Δ^5-sterols, a finding which is in contradiction to the earlier report [11] which states that $\Delta^{5,7}$-sterols predominate in that sponge. The contradictory nature of the above findings may reflect the difficulty in determining the origins of sterols contained in a sample of sponge because the sterols may arise from the sponge tissues, from dietary sources or from endosymbionts inhabiting the tissues of sponges [31]. Conventional methods of lipid extraction and natural products analyses do not allow the differentiation of the origins of the various sterols contained within a sample of sponge. Sponges often house large numbers of symbiotic

organisms in their tissues and cavities and can be considered as true 'living hotels' [31]. In this regard, since species within the family *Spongidae* are intermittently infected by marine fungi [32], and since marine fungi contain $\Delta^{5,7}$-sterols [5], the seemingly contradictory finding reported for *Spongia officinalis* may actually reflect the presence of fungi in the sample [11] containing $\Delta^{5,7}$-sterols. In this respect, the sponges of the family *Dysideidae* are known to contain a large amount of extraneous particles [31] and the microscopic examination of the particulate materials contained within *D. herbacea* (the subject of this report) indicates that blue-green algae predominate within the spongal matrix. Blue-green algae contain Δ^{7}-, $\Delta^{5,7}$- and Δ^{5}-C_{29} sterols [28] [33] as well as sterols with 24-methylene and 24 *Z*-ethylidene side chains **g** and **j** [5] [28]. Conversely, reports of Δ^{7}-sterols (usually typical of asteroids and holothurians [5]) in sponges are rare [10] [11]. Therefore, the sterol mixture of the present collection of *D. herbacea* seems more typical of an alga. Indeed, the presence of cholestanol along with $\Delta^{5,7}$-, Δ^{7}- and Δ^{5}-sterols in the sterol mixture reinforces the view that the individual components do not have a single origin.

Several $\Delta^{5,7}$-sterols occur in sponges [11] [19] [20]. The identification of **4j** and **4g** in *D. herbacea* reported here completes the list of hypothetical intermediates in sterol biosynthetic schemes. Interestingly, **4j** has never been encountered in green plants and **4g** has only been encountered once in a fungus [21], although compounds with a 24(28)-double bond are considered essential intermediates in the biosynthesis of the alkylated side chains of plant sterols [34–36].

Although (24*Z*)-stigmasta-5,7,24(28)-trien-3β-ol (**4j**) has not previously been identified as a natural product, *Nes et al.* [37] have demonstrated that the cultured ciliated protozoan *Tetrahymena pyriformis* can biosynthesize this compound from isofucosterol (**2j**) when this was added to the culture medium.

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Experimental Part

Terpios zeteki was collected on reef flats in Kaneohe Bay, Oahu, Hawaii. The collection of *Dysidea herbacea* was made from the Great Barrier Reef of Australia.

The isolation of the sterol mixture from both sponges was achieved by chromatography on silica gel using earlier described [38] [39] methods.

Sterol characterization. GLC. was performed using a *Hewlett Packard* 402A chromatograph equipped with a flame ionization detector; 1.80 m × 4 mm ID. 'U'-shaped glass column containing 3% OV-25 on gas Chrom Q (*Applied Sci.* Inc.); temperature: 265°; carrier gas: He (100 ml/min). Combined GC.-MS. analysis was performed on a *Varian* MAT 44 quadrupole spectrometer system using a coiled GLC. column (1.80 m × 2 mm ID.) containing 3% SP-2250 on Supelcoport 100/120 (*Supelco* Inc.); temperature: 270°; electron ionization energy: 70 eV. The high resolution mass spectra were recorded on a *Varian* MAT 711 system using the conditions reported [17]. The UV. spectra (MeOH) were recorded on a *Cary* 14 spectrophotometer. 360 MHz ^{1}H-NMR. spectra ($CDCl_3$, TMS as internal reference, *J* Hz) were

measured on a *Bruker* HXS 360 spectrometer at the Stanford Magnetic Resonance Laboratory. Melting points (m.p.) are uncorrected.

Sterol fractionation. Reversed phase HPLC. [17] on a Partisil M9 10/50 ODS-2 column (*Whatman* Inc.), 50 cm × 8 mm OD., was used at a pressure of 1200 psi. with absolute methanol as the mobile phase and sample loading of 15 mg sterol mixture in 5 ml of methanol. Three separations were made for *T. zeteki* and 2 for *D. herbacea.* Four fractions were collected each time and evaporated under vacuum at RT. before further analysis.

5α-24-Norcholestan-3β-ol (**1b**). The steryl acetate mixture of *T. zeteki* was obtained by treating the total sterols with acetic anhydride/pyridine (1:1) for 84 h at RT. The excess acetic anhydride was destroyed with water. The steryl acetates were extracted 4 times with CH_2Cl_2, and chromatographed over a $AgNO_3$ (20%) impregnated silica gel (*Merck* 60, 0.063–0.2 mm diam. particles) column, using hexane, hexane/benzene (5:2, 3:2) and hexane/ether (1:1) as eluent. Four fractions were collected and after regeneration to the free sterols (treatment with $LiAlH_4$ in ether) were analyzed by GC.-MS.

The sterol component with $M^+ = 374$ was present in the 3 earliest eluted fractions. Further purification of this compound was carried out by reversed phase HPLC. Three fractions were collected containing the $M^+ = 374$ compound at concentrations of 88, 71 and 55%. Recycling of the combined fractions resulted in a better separation (98% purity). GLC. coinjection of this sterol with authentic 24-norcholest-5-en-3β-ol gave a single peak (relative retention time 0.76, OV 25–3%, 265°, see *Table 1*). – ^{1}H-NMR.: 3.851 (*m*, 1 H, C*H*OH); 0.875 (*d*, $J = 6.8$, 3 H, $H_3C(21)$); 0.851 (*d*, $J = 6.5$, 3 H, $H_3C(26)$ or $H_3C(27)$); 0.834 (*d*, $J = 6.5$, 3 H, $H_3C(26)$ or $H_3C(27)$); 0.787 (*s*, 3 H, $H_3C(19)$); 0.631 (*s*, 3 H, $H_3C(18)$). – MS. characteristic fragmentations: 374.35305 (100, $C_{26}H_{46}O = M^+$); 359.33543 (40, $C_{25}H_{43}O^+$); 356.34580 (14, $C_{26}H_{44}^+$); 341.31740 (17, $C_{25}H_{41}^+$); 302.29454 (7, $C_{22}H_{38}^+$); 257.22776 (9, $C_{19}H_{29}^+$); 248.25298 (14, $C_{18}H_{32}^+$); 248.21348 (10, $C_{17}H_{28}O^+$); 234.19903 (68, $C_{16}H_{26}O^+$); 233.19367 (92, $C_{16}H_{25}O^+$); 215.17829 (86, $C_{16}H_{23}^+$); 201.16182 (14, $C_{15}H_{21}^+$); 165.12602 (31, $C_{11}H_{17}O^+$); 149.13260 (20, $C_{11}H_{17}^+$); 147.11663 (23, $C_{11}H_{15}^+$); 121.10201 (27, $C_9H_{13}^+$); 108.09322 (46, $C_8H_{12}^+$); 107.08560 (38, $C_8H_{11}^+$); 95.08497 (38, $C_7H_{11}^+$); 81.07087 (40, $C_6H_9^+$); 69.07057 (30, $C_5H_9^+$); 57.07067 (34, $C_4H_9^+$).

(24Z)-Stigmasta-5,7,24(28)-trien-3β-ol (**4j**). The fourth HPLC. fraction in the separation of the crude sterol mixture of *D. herbacea* exhibited a single GLC. peak but was a mixture of **3j** and **4j** in the approximate ratio 1:8 (GC.-MS.). Further purification of **4j** was achieved by acetylation of this HPLC. fraction (14 mg, pyridine acetic anhydride at RT.) with subsequent separation of the sterol acetates by preparative TLC. on a $AgNO_3$ (20%) impregnated silica gel (*Merck*, HF-254+366, type 60), glass plate (20 × 20 cm, thickness 0.5 mm) with hexane/benzene 1:1 (2 developments). The UV. active band was recovered and regenerated to the free sterol ($LiAlH_4$ in ether), affording **4j** (7 mg of white crystals of 98% purity, m.p. (MeOH) 119–121°). Like all the $\Delta^{5,7}$-sterols, **4j** was sensitive to air and light exposure. – UV.: λ_{max} nm (ε): 262 (8200), 270 (11300), 281 (11900) and 293 (6900). ^{1}H-NMR.: 5.581 (*m*, 1 H, olefinic ring H); 5.390 (*m*, 1 H, olefinic ring H); 5.112 (*qa*, $J = 6.5$, 1 H, HC(28)); 3.636 (*m*, 1 H, HC(3)); 2.851 (*septet*, $J = 7$, 1 H, HC(25)); 2.286 (*t*, $J = 12$, 2 H, $H_2C(23)$); 1.591 (*d*, $J = 6.5$, 3 H, $H_3C(29)$); 0.998 (*d*, $J = 7$, 6 H, $H_3C(26)$ and $H_3C(27)$); 0.992 (*d*, $J = 7$, 3 H, $H_3C(21)$); 0.945 (*s*, 3 H, $H_3C(19)$); 0.623 (*s*, 3 H, $H_3C(18)$). – MS. characteristic fragmentations: 410.35548 (100, $C_{29}H_{46}O = M^+$); 395.33176 (5, $C_{28}H_{43}O^+$); 392.34459 (30, $C_{29}H_{44}^+$); 377.32111 (63, $C_{28}H_{41}^+$); 351.30405 (15, $C_{26}H_{29}^+$); 312.24604 (3, $C_{22}H_{32}O^+$); 294.23600 (13, $C_{22}H_{30}^+$); 279.21075 (4, $C_{21}H_{27}^+$); 271.20245 (3, $C_{19}H_{27}O^+$); 269.19061 (4, $C_{19}H_{25}O^+$); 253.19639 (33, $C_{19}H_{25}^+$); 251.17978 (15, $C_{19}H_{23}^+$); 211.14801 (20, $C_{16}H_{19}^+$); 159.11655 (18, $C_{12}H_{15}^+$); 158.10954 (18, $C_{12}H_{14}^+$); 157.10134 (17, $C_{12}H_{13}^+$); 143.08631 (26, $C_{11}H_{11}^+$); 128.06383 (6, $C_{10}H_8^+$); 97.10199 (30, $C_7H_{13}^+$); 95.08671 (31, $C_7H_{11}^+$); 69.07042 (60, $C_5H_9^+$); 55.05469 (75, $C_4H_7^+$).

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