46. On the First Marine Stigmastane Sterols and Sterones Having a 24,25-Double Bond. Isolation from the Sponge *Stelletta* sp. of Deep Coral Sea

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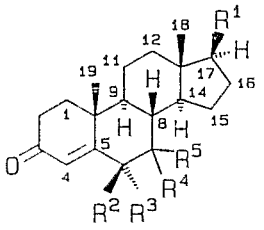
The sponge *Stelletta* sp. (Astrophorida, Stellettidae), collected at −700 m in the Coral Sea, is shown to contain sterones and sterols of the stigmastane type with a C(24)=C(25) bond for which there is no precedent in the sea. Structure elucidation of the second abundant of these steroids, stigmasta-4,24(25)-dien-3-one ((+)-**1**), is based on 1D and 2D NMR spectra and chemical transformation to acetate (−)-**5**. Stigmasta-4,24(25)-diene-3,6-dione ((−)-**3**), present in trace amounts in the sponge, was obtained in sufficient quantity for NMR study by oxidation of the also present, inseparable, and abundant 4:1 mixture of stigmasta-5,24(25)-dien-3β-ol (**6**) and its 5,6-dihydro derivative **7** (*Scheme 1*). This oxidation also afforded the ketone analogues (+)-**8** and (+)-**9**, which could be separated, thus making structure elucidation possible. The 6β-hydroxystigmasta-4,24(25)-dien-3-one ((+)-**4**), also present in trace amounts in the sponge, was obtained in sufficient amount for NMR study, together with its C(6) epimer (+)-**11**, by hydroperoxidation of (+)-**8** followed by deoxygenation (*Scheme 2*). The last trace steroid of the sponge, stigmasta-4,6,24(25)-trien-3-one ((−)-**2**), was structurally elucidated using limited NMR data and comparison with the other stigmastanes. These stigmastanes, as the only steroids of this sponge, are likely to function as stabilizers of its cell walls; their phytosteroid structure, for a sponge which lives in the dark of deep waters, suggest origin through a complex food chain, possibly followed by bioelaboration in the sponge.

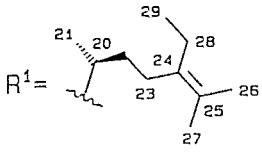
1. Introduction. – Demosponges belonging to the order Astrophorida (= Choristida) are known to contain terpenoids (malabaricane triterpenes [1] and enzyme-inhibiting aromatic sesquiterpenes [2]), terpenoids of mixed biogenesis (amino-sugar-containing triterpenoidic saponins [3], triterpene galactosides [4], and indolizidine-type alkaloidal terpenes [5]), other alkaloids (of the acridine [6] and bromoindole-diketopiperazine [7] classes), anthelmintic aminoacid derivatives [8], peptides (cyclodepsipeptides [9] and insecticidal and antifungal modified peptides [10]), proteins (cytotoxic chromoproteins [11a], other than cytotoxic and hemolytic proteins [11b]), and unusual nucleosides [12]. Steroids have only been reported from members of the family Stellettidae and comprise steroid glycosides and free steroids. Thus, antifugal and cytotoxic sterol glycosides based on 4α-methylcholestanes or polyhydroxylated ergostanes have been isolated from *Erylus lendenfeldi* (Geodiidae) of the Red Sea [13] or *Pachastrella* sp. (Geodiidae) of Kamagi Bay, Japan [14], respectively. Free steroids are represented by ergosta-4,24(28)-dien-3-one and (*E*)-stigmasta-4,24(28)-dien-3-one and similar, minor ergostanes and stigmastanes, isolated from *Stelletta clarella,* collected intertidally in the Monterey, Pacific Grove, area [15], and Δ^5-sterols, accompanied by 5α-stanols, isolated from *Stryphnus mucronatus* of the Bay of Naples [16].

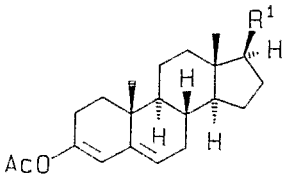
We report here on the first marine stigmast-24(25)-ene sterols and sterones; they are the only free steroids of a sponge of the genus *Stelletta* of deep Coral Sea.

2. Results and Discussion. – The first compound which was isolated, after extensive flash chromatography (FC) and HPLC, in sufficient amount for structural study from

the non-polar extracts of our freeze-dried *Stelletta* sp. is optically active and possesses an enone chromophore. The structure of this novel phytosterone is (+)-stigmasta-4,24(25)-dien-3-one ((+)-**1**), as established by spectroscopic means and acetylation to (−)-**5**.







(+)-**1** $R^2, R^3 = 2H$, $R^4, R^5 = 2H$
(−)-**2** $R^2, R^3 = H$, $R^4, R^5 = H$, *i.e.* Δ^6
(−)-**3** $R^2, R^3 = O$, $R^4, R^5 = 2H$
(+)-**4** $R^2 = OH$, $R^3 = H$, $R^4, R^5 = 2H$

(−)-**5**

Table. *^{13}C-NMR Data for the Natural Novel Steroids (+)-**1**, (−)-**2**, (−)-**3**, (+)-**4**, and **7** in $CDCl_3$*

	(+)-1	(−)-2	(−)-3	(+)-4	7[c])
C(1)	35.70(*t*)	33.96(*t*)	35.54(*t*)	37.09(*t*)	37.02(*t*)
C(2)	34.00(*t*)	33.90(*t*)	33.98(*t*)	34.28(*t*)	31.51(*t*)
C(3)	199.70(*s*)	[b])	199.51(*s*)	200.44(*s*)	71.35(*d*)
C(4)	123.76(*d*)	123.50(*d*)	125.46(*d*)	126.36(*d*)	38.19(*t*)
C(5)	171.72(*s*)	[b])	161.07(*s*)	168.42(*s*)	44.87(*d*)
C(6)	32.96(*t*)	127.76(*d*)	202.36(*s*)	73.30(*d*)	28.76(*t*)
C(7)	32.04(*t*)	141.62(*d*)	46.82(*t*)	38.52(*t*)	32.10(*t*)
C(8)	35.62(*d*)	37.74(*d*)	34.22(*d*)	29.72(*d*)	35.47(*d*)
C(9)	53.80(*d*)	50.66(*d*)	50.97(*d*)	53.59(*d*)	54.36(*d*)
C(10)	38.61(*s*)	[b])	39.82(*s*)	37.98(*s*)	35.52(*s*)
C(11)	21.03(*t*)	20.66(*t*)	20.88(*t*)	20.97(*t*)	21.27(*t*)
C(12)	39.60(*t*)	39.52(*t*)	39.11(*t*)	39.56(*t*)	40.02(*t*)
C(13)	42.42(*s*)	[b])	42.56(*s*)	42.52(*s*)	42.63(*s*)
C(14)	55.80(*d*)[a])	53.40(*d*)	55.65(*d*)	55.84(*d*)	56.48(*d*)
C(15)	24.22(*t*)	23.75(*t*)	24.00(*t*)	24.18(*t*)	24.27(*t*)
C(16)	28.15(*t*)	28.13(*t*)	27.98(*t*)	28.14(*t*)	[d])
C(17)	55.85(*d*)[a])	55.71(*d*)	56.52(*d*)	55.84(*d*)	56.00(*d*)
C(18)	11.96(*d*)	11.88(*q*)	11.89(*q*)	12.02(*q*)	12.09(*q*)
C(19)	17.39(*q*)	16.28(*q*)	17.52(*q*)	19.52(*q*)	12.34(*q*)
C(20)	36.06(*d*)	36.07(*d*)	35.97(*d*)	36.06(*d*)	[d])
C(21)	18.74(*q*)	18.73(*q*)	18.74(*q*)	18.76(*q*)	18.78(*q*)
C(22)	34.94(*t*)	34.93(*t*)	34.88(*t*)	34.95(*t*)	[d])
C(23)	30.70(*t*)	30.71(*t*)	30.67(*t*)	30.71(*t*)	[d])
C(24)	129.44(*s*)	[b])	129.56(*s*)	129.45(*s*)	129.34(*s*)
C(25)	127.99(*s*)	[b])	127.85(*s*)	127.99(*s*)	128.08(*s*)
C(26)	17.84(*q*)	17.83(*q*)	17.84(*q*)	17.84(*q*)	[d])
C(27)	18.59(*q*)	18.57(*q*)	18.58(*q*)	18.59(*q*)	[d])
C(28)	27.00(*t*)	27.05(*t*)	27.00(*t*)	26.99(*t*)	[d])
C(29)	13.31(*q*)	13.30(*q*)	13.31(*q*)	13.31(*q*)	[d])

[a]) These values can be interchanged. [b]) Not detected. [c]) Data from a 4:1 mixture **6**/**7**. [d]) Superimposed with signals of the major component **6**.

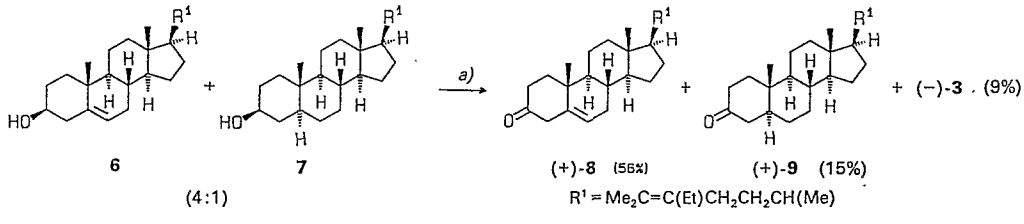
The ^{13}C-NMR spectrum of (+)-**1** (*Table*) shows 2 quaternary C-atoms and 6 *q* for Me groups, and the ^{1}H-NMR spectrum (*Exper. Part*) a *s* for a Me group at $\delta < 1$, besides a *s*, a *d*, and a *t* at δ *ca.* 1 for 3 Me groups and two br. *s* at δ 1.60 for 2 other Me groups. This points to a sterone with an Et group in the side chain, which ends with an isopropylidene group. Support to these deductions is given by the MS which indicates a M^+ in the C_{29} steroidal range and fragmentations at the bonds C(17)–C(20), C(20)–C(22), and C(22)–C(23) (see *Exper. Part*). NOE experiments and comparison with NMR data of cholest-4-en-3-one [17], obtained by oxidation and double-bond migration from cholesterol, are of help in assigning the ^{13}C-NMR data.

Sterone (+)-**1** was accompanied by three other sterones, (–)-**2**, (–)-**3**, and (+)-**4**, which were isolated in such small amounts as to make their structural elucidation uncertain. However, (–)-**3** and (+)-**4** could also be obtained by chemical transformation of the more abundant steroids of this sponge, which allowed their identification (see below).

The abundant sterol **6** and the accompanying inseparable dihydro derivative **7** (see *Scheme 1*) were isolated from slightly more polar fractions of the above FC. NMR data (*Exper. Part*) allowed us to identify **6** as identical to a sterol of our diet. In fact, **6** is also present both in the common bean (seeds of *Phaseolus vulgaris*) [18a] and in maize (*Zea mais*) [18b], but not in the unrelated plant *Withania somnifera* [18c] (only ^{1}H-NMR of Me groups given in [18]). The NMR data of **7** (*Exper. Part*) suggest the structure of the 5,6-dihydro derivative of sterol **6**.

The 4:1 mixture **6**/**7** was treated with pyridinium chlorochromate (PCC) [19], to give a mixture of sterones (+)-**8**, (+)-**9**, and (–)-**3** which were separated (*Scheme 1*)[1]. The minor sterone (–)-**3** proved to be identical with natural (–)-**3** and was combined with further (–)-**3**, obtained from (+)-**8** (see below) for the collection of detailed spectral data (*Table* and *Exper. Part*). Compared with those of (+)-**1**, they suggest the presence of an additional carbonyl group at C(6) which is confirmed by a bathochromic shift in the UV spectrum of (–)-**3**.

Scheme 1



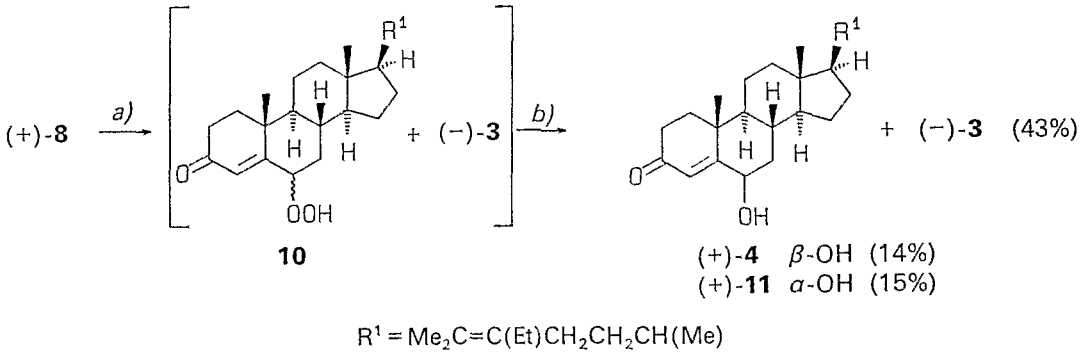
a) 1) PCC/AcONa CH_2Cl_2, r.t., 2 h; 2) filtration on *Celite*; 3) reversed-phase HPLC.

Moreover, the spectral data of (+)-**8** and of its dihydro derivative (+)-**9** allow a more detailed structural assignment for **6** and **7**: in particular, the ^{13}C-NMR spectra (*Exper. Part*) indicate a *trans* ring A/B junction for (+)-**9** [20], and thus also for **7**.

The nonconjugated-enone structure of (+)-**8** suggests to try its oxidation by O_2 in acidic medium [21]. Thus, (+)-**4** and its 6-epimer (+)-**11** were obtained, besides (–)-**3**

[1]) The choice of PCC was based on extensive investigations. *E.g.* working with cholesterol as a substitute, either CrO_3/pyridine or pyridinium dichromate led to the undesired 4-en-3-one derivative. The latter was also obtained from the oxidation of cholesterol with PCC if the reaction mixture was chromatographed on silica gel, which is, therefore, the isomerization agent. In accordance, also (+)-**8** in contact with silica gel gave the conjugated enone.

Scheme 2



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(+)-**4** β-OH (14%)
(+)-**11** α-OH (15%)

$R^1 = Me_2C{=}C(Et)CH_2CH_2CH(Me)$

a) O_2/AcOH, r.t., dark, 5 h. *b*) 1) PPh_3/Et_2O; 2) FC; 3) *CN*HPLC.

(*Scheme 2*), allowing to assign to natural (+)-**4** the structure of a (−)-**3** reduction product obtained *via* a formal equatorial attack by H^-. The ^{1}H-NMR spectra of (+)-**4** indicate indeed that H–C(6) is equatorial, while CH_3(19) is deshielded by the axial OH group.

By comparison with all above spectral data, also the structure of sterone (−)-**2**, available only from the sponge[2]), could be elucidated from its incomplete NMR data (*Table* and *Exper. Part*). The presence of an additional C=C bond extending the chromophore of (+)-**1** and of a δ(H) at 6.09 (*dd*) indicating coupling to both H–C(8) and H–C(6) places the extra olefinic bond at C(6)=C(7).

C=C bonds in the steroid side chain are suitable positions for biomethylation by *S*-adenosylmethionine according to biosynthetic experiments which have been carried out in the last decade with steroids possessing various unsaturated side chains [22]. Sterols having a C(24)=C(25) bond have been postulated as intermediates in these biomethylations, but only recently, it has been demonstrated that both desmosterol and 24-methyldesmosterol are methylated at C(24) by cell-free extracts of the sponge *Aplysina fistularis* [23]. However, to the best of our knowledge, no products of methylation of stigmast-24(25)-enes have ever been reported. Whether there are products of this type in our *Stelletta* sp. is uncertain: chromatographic fractions from this sponge revealed ^{1}H-NMR signals for a side-chain $=CH_2$ group, although this material was present in only trace amounts so that we could not establish if this methylidene group belongs to a C_{30} steroid, as expected for the methylation of a C_{29} stigmast-24(25)-ene, or to a C_{28} steroid.

The fact that stigmastanes are the only steroids of our *Stelletta* sp. suggests that they take part in the cell-wall organization of this sponge. Such a vital role is amazing in view of the fact that stigmastanes are typical phytosteroids (actually **6** was isolated from terrestrial plants too [18]), while any symbiontic origin of these steroids in the sponge can be ruled out: photosynthesis is not allowed in the dark of the sponge habitat (−700 m). Although certain sponges are capable of *de novo* synthesis of sterols [22] [24], it is likely that our *Stelletta* sp. obtains the stigmastanes described here – or close precursors of them, which are then elaborated in the sponge – through a complex food chain initiated in photic waters with phytoplankton. It could also be that the end source of the stigmastanes

[2]) All attempts to dehydrate (+)-**4** or (+)-**11** in either aq. H_3PO_4 solution or $POCl_3$/pyridine failed.

is merely debris deposited from surface waters and filter-fed by our sponge. As a matter of fact, mysterious food chains with sponges exist, such as in obtaining macrophyte products [25].

This work has been carried out within the collaborative program *ORSTOM-CNRS* on marine substances of biological interest. The work in Trento has been supported by both *MPI* (Progetti di Interesse Nazionale) and *CNR*, Roma.

Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. Yields of extracted products are given on dry animal weight, those for chemical reactions on reacted substrate. Flash chromatography (FC): *Merck* silica gel *Si60*, 20–50 μm. HPLC: either *Merck-LiChrosorb Si-60* (7 μm) or *Merck-LiChrosorb CN* (7 μm). Reversed-phase HPLC: *Merck-LiChrosorb RP18* (7 μm); UV monitoring (254 nm if not otherwise stated). All HPLC columns were 25 × 1 cm. Polarimetric data: *JASCO-DP-181* polarimeter. UV (λ_{max} in nm, ε in $mol^{-1}cm^{-1}$): *Perkin-Elmer-Lambda-3* spectrophotometer. NMR: *Varian-XL-300* (^{13}C-NMR at 75.43 MHz, ^1H-NMR at 299.94 MHz); δ's (ppm) relative to internal Me_4Si (= 0 ppm) and J's in Hz; δ values derived from either HETCOR traces or COSY maps are rounded off to 0.05 ppm. MS: home-built quadrupole mass spectrometer based on the *ELFS-4-162-8* extranuclear quadrupole [26].

2. *Collection and Isolation.* The sponge was collected in September 1988 in the Coral Sea southeast of Nouméa by dredging at a depth of 700 m and was identified by Professor *Claude Levi*, from the Musée National d'Histoire Naturelle, Paris. The sponge was immediately lyophilized at *ORSTOM* in Nouméa to get a powder whose CH_2Cl_2 extract proved active against Kb and P388 cells. This lyophilized powder (350 g) was then shipped to Trento where it was first extracted with petroleum ether and then with EtOH. The residue from evaporation of the petroleum-ether extract (0.47 g) was subjected to gradient FC (petroleum ether to Et_2O). The residue from evaporation of the fraction eluted with petroleum ether/Et_2O 3:2 (0.052 g) was subjected to *CN* HPLC with hexane/EtOH 97:3 (10 ml/min) to get a head fraction, besides (–)-**3** (t_R 3 min; 1.2 mg, 0.00034%) and (–)-**2** (t_R 4.5 min; 2.7 mg, 0.00077%). The head fraction was evaporated and the residue subjected to *Si60* HPLC with hexane/AcOEt 9:1 to give (+)-**1** (t_R 9 min; 12 mg, 0.0034%). The combined residues from evaporation of the fractions eluted with petroleum ether/Et_2O 1:1 and 2:3 were subjected to *CN* HPLC with hexane/EtOH 95:5 (λ 196 nm) to get a 4:1 mixture **6**/**7** (52 mg, 0.015%). The residue from evaporation of the fraction eluted with petroleum ether/Et_2O 1:4 was subjected to *CN* HPLC with hexane/EtOH 49:1 to get (+)-**4** (t_R 11 min; 0.9 mg, 0.00026%).

3. *Stigmasta-4,24(25)-dien-3-one* ((+)-**1**). $[\alpha]^{20} = +54.0$ (589), +101.7 (435; $c = 0.66$, EtOH). UV (EtOH): 240 (16500). ^1H-NMR ($CDCl_3$; values in brackets for C_6D_6 soln.): 5.72 (br. *s*, H–C(4)); 0.71 (*s*, 3 H–C(18)); 1.18 (*s*, 3 H–C(19)); 0.96 (*d*, $J = 6.8$, 3 H–C(21)); 1.61 [1.66, 1.72] (br. *s*, 3 H–C(26), 3 H–C(27)); 2.01 (*q*, $J = 7.4$, 2 H–C(28)); 0.94 (*t*, $J = 7.4$, 3 H–C(29)); from HETCOR traces: 1.50, 2.00 (H_α–C(1), H_β–C(1)); 2.40 (2 H–C(2)); 2.35 (2 H–C(6)); 1.00, 1.90 (H_α–C(7), H_β–C(7)); 1.50 (H–C(8)); 0.90 (H–C(9)); 1.45 (2 H–C(11)); 1.15, 2.05 (H_α–C(12), H_β–C(12)); 1.05 (H–C(14)); 1.10, 1.60 (H_α–C(15), H_β–C(15)); 1.25, 1.90 (H_α–C(16), H_β–C(16)); 1.05 (H–C(17)); 1.40 (H–C(20)); 1.10, 1.40 (2 H–C(22)); 1.25 (2 H–C(23)). MS: 410 (15, M^+), 395 (3, $[M - CH_3]^+$), 327 (4), 313 (22, C(22)–C(23) break), 312 (31), 299 (25, C(20)–C(22) break), 297 (37), 271 (23, C(17)–C(20) break), 269 (28), 245 (18), 243 (13), 231 (24), 229 (23), 227 (23), 97 (57), 55 (100).

4. *Stigmasta-4,6,24(25)-trien-3-one* ((–)-**2**). $[\alpha]^{20} = -6.0$ (589), +12.0 (546), +114.0 (435; $c = 0.05$, EtOH). UV (EtOH): 283 (17000). ^1H-NMR ($CDCl_3$): 2.44 (*dddd*, $J_{gem} = 18.0$, $J(2\alpha,1\beta) = 5.3$, $J(2\alpha,1\alpha) = 2.4$, $J(2\alpha,4) = 0.7$, H_α–C(2)); 2.57 (*ddd*, $J_{gem} = 18.0$, $J(2\beta,1\alpha) = 14.1$, $J(2\beta,1\beta) = 5.1$, H_β–C(2)); 5.67 (br. *s*, H–C(4)); 6.14 (br. *d*, $J(6,7) = 9.9$, H–C(6)); 6.09 (*dd*, $J(7,6) = 9.9$, $J(7,8) = 2.4$, H–C(7)); 0.76 (*s*, 3 H–C(18)); 1.01 (*s*, 3 H–C(19)); 0.97 (*d*, $J = 6.6$, 3 H–C(21)); 1.61 (br. *s*, 3 H–C(26), 3 H–C(27)); 2.09 (*q*, $J = 7.5$, 2 H–C(28)); 0.95 (*t*, $J = 7.5$, 3 H–C(29)); from COSY maps: 1.70, 2.00 (H_α–C(1), H_β–C(1)); 2.20 (H–C(8)). MS: 408 (37, M^+), 393 (7, $[M - CH_3]^+$), 379 (3), 311 (7), 310 (24), 297 (14), 295 (27), 281 (8), 269 (21), 267 (32), 242 (10), 241 (11), 55 (100).

5. *Stigmasta-4,24(25)-diene-3,6-dione* ((–)-**3**). $[\alpha]^{20} = -18.8$ (589), –28.4 (546; $c = 0.37$, $CHCl_3$). UV ($CHCl_3$): 250 (19400). ^1H-NMR ($CDCl_3$): 2.46 (*dddd*, $J_{gem} = 17.5$, $J(2\alpha,1\beta) = 5.5$, $J(2\alpha,1\alpha) = 2.9$, $J(2\alpha,4) = 0.9$, H_α–C(2)); 2.58 (*ddd*, $J_{gem} = 17.5$, $J(2\beta,1\alpha) = 13.8$, $J(2\beta,1\beta) = 5.0$, H_β–C(2)); 6.17 (*d*, $J(4,2\alpha) = 0.9$, H–C(4)); 2.68 (*dd*, $J_{gem} = 15.6$, $J(7\beta,8) = 3.8$, H_β–C(7)); 0.72 (*s*, 3 H–C(18)); 1.17 (*s*, 3 H–C(19)); 0.99 (*d*, $J = 6.7$, 3 H–C(21)); 1.61 (br. *s*, 3 H–C(26), 3 H–C(27)); 2.01 (*q*, $J = 7.5$, 2 H–C(28)); 0.95 (*t*, $J = 7.5$, 3 H–C(29)); from

HETCOR traces: 1.90, 2.15 (H_α–C(1), H_β–C(1)); 2.05 (H_α–C(7)); 1.90 (H–C(8)); 1.35 (H–C(9)); 1.25, 2.10 (H_α–C(12), H_β–C(12)); 1.15 (H–C(14)); 1.15, 1.65 (H_α–C(15), H_β–C(15)); 1.35, 1.95 (H_α–C(16), H_β–C(16)); 1.15 (H–C(17)); 1.40 (H–C(20)); 1.10, 1.40 (2 H–C(22)); 2.05 (2 H–C(23)). MS: 424 (28, M^+), 341 (6), 327 (40, C(22)–C(23) break), 313 (27), 311 (45), 285 (22), 283 (24), 270 (25), 259 (33), 257 (60), 137 (59, C(9)–C(10) and C(6)–C(7) break), 55 (100).

6. *6β-Hydroxystigmasta-4,24(25)-dien-3-one* ((+)-4). $[\alpha]^{20} = +17.1$ (589), +20.6 (546), +25.1 (435; $c = 0.26$, $CHCl_3$). UV ($CHCl_3$): 242 (12200). ^{1}H-NMR ($CDCl_3$): 2.35 (*dddd*, $J_{gem} = 17.2$, $J(2\alpha,1\beta) = 4.9$, $J(2\alpha,1\alpha) = 3.0$, $J(2\alpha,4) = 0.9$, H_α–C(2)); 2.52 (*ddd*, $J_{gem} = 17.2$, $J(2\beta,1\alpha) = 14.5$, $J(2\beta,1\beta) = 4.9$, H_β–C(2)); 5.82 (*d*, $J(4,2\alpha) = 0.9$, H–C(4)); 4.35 (*dd*, $J = 2.7$, 2.7, H–C(6)); 0.74 (*s*, 3 H–C(18)); 1.38 (*s*, 3 H–C(19)); 0.96 (*d*, $J = 6.7$, 3 H–C(21)); 1.61 (br. *s*, 3 H–C(26), 3 H–C(27)); 2.03 (*q*, $J = 7.4$, 2 H–C(28)); 0.95 (*t*, $J = 7.4$, 3 H–C(29)); from COSY maps: 1.70, 2.05 (H_α–C(1), H_β–C(1)); 1.25, 2.05 (H_α–C(7), H_β–C(7)); 1.40 (H–C(20)). MS: 426 (20, M^+), 411 (5, $[M - CH_3]^+$), 408 (3, $[M - H_2O]^+$), 400 (7), 393 (2), 343 (7), 329 (32), 328 (60), 313 (81), 310 (9), 285 (29), 259 (58), 55 (100).

7. *Stigmasta-5,24(25)-dien-3β-ol* (6). ^{1}H-NMR ($CDCl_3$): 3.51 (*m*, H–C(3)); 5.35 (*m*, H–C(6)); 0.67 (*s*, 3 H–C(18)); 1.00 (*s*, 3 H–C(19)); 0.96 (*d*, $J = 6.8$, 3 H–C(21)); 1.60 (br. *s*, 3 H–C(26), 3 H–C(27)); 2.01 (*q*, $J = 7.5$, 2 H–C(28)); 0.94 (*t*, $J = 7.5$, 3 H–C(29)); from HETCOR traces: 1.05, 1.85 (H_α–C(1), H_β–C(1)); 1.50, 1.80 (H_α–C(2), H_β–C(2)); 2.25 (2 H–C(4)); 2.05–1.80 (2 H–C(7)); 1.45 (H–C(8)); 0.90 (H–C(9)); 1.50 (2 H–C(11)); 1.15, 2.00 (H_α–C(12), H_β–C(12)); 1.10 (H–C(14)); 1.05, 1.55 (H_α–C(15), H_β–C(15)); 1.25, 1.85 (H_α–C(16), H_β–C(16)); 1.00 (H–C(17)); 1.40 (H–C(20)); 1.05, 1.40 (2 H–C(22)); 1.70–2.10 (2 H–C(23)). ^{1}H-NMR (C_6D_6): 3.45 (*m*, H–C(3)); 5.37 (*m*, H–C(6)); 0.66 (*s*, 3 H–C(18)); 0.97 (*s*, 3 H–C(19)); 1.04 (*d*, $J = 6.7$, 3 H–C(21)); 1.65 (br. *s*, 3 H–C(26)); 1.70 (br. *s*, 3 H–C(27)); 2.12 (*q*, $J = 7.4$, 2 H–C(28)); 1.03 (*t*, $J = 7.4$, 3 H–C(29)). ^{13}C-NMR ($CDCl_3$): 37.27 (*t*, C(1)); 31.63 (*t*, C(2)); 71.75 (*d*, C(3)); 42.28 (*t*, C(4)); 140.77 (*s*, C(5)); 121.69 (*d*, C(6)); 31.91 (*t*, C(7)); 31.91 (*d*, C(8)); 50.13 (*d*, C(9)); 36.51 (*s*, C(10)); 21.10 (*t*, C(11)); 39.76 (*t*, C(12)); 42.35 (*s*, C(13)); 55.85 (*d*, C(14)); 24.34 (*t*, C(15)); 28.21 (*t*, C(16)); 56.74 (*d*, C(17)); 11.88 (*q*, C(18)); 19.42 (*q*, C(19)); 36.09 (*d*, C(20)); 18.84 (*q*, C(21)); 35.03 (*t*, C(22)); 30.73 (*t*, C(23)); 129.36 (*s*, C(24)); 128.06 (*s*, C(25)); 17.85 (*q*, C(26)); 18.60 (*q*, C(27)); 27.00 (*t*, C(28)); 13.33 (*q*, C(29)). ^{13}C-NMR (C_6D_6): 37.71 (*t*, C(1)); 32.14 (*t*, C(2)); 71.68 (*d*, C(3)); 42.92 (*t*, C(4)); 141.30 (*s*, C(5)); 121.60 (*d*, C(6)); 32.31 (*t*, C(7)); 32.24 (*d*, C(8)); 50.54 (*d*, C(9)); 36.81 (*s*, C(10)); 21.44 (*t*, C(11)); 40.15 (*t*, C(12)); 42.62 (*s*, C(13)); 56.27 (*d*, C(14)); 24.63 (*t*, C(15)); 28.61 (*t*, C(16)); 56.99 (*d*, C(17)); 12.07 (*q*, C(18)); 19.55 (*q*, C(19)); 36.48 (*d*, C(20)); 19.14 (*q*, C(21)); 35.52 (*t*, C(22)); 31.24 (*t*, C(23)); 129.60 (*s*, C(24)); 128.24 (*s*, C(25)); 18.04 (*q*, C(26)); 18.82 (*q*, C(27)); 27.47 (*t*, C(28)); 13.58 (*q*, C(29)). MS: 412 (24, M^+), 410 (2, $[M - 2]^+$), 397 (6, $[M - CH_3]^+$), 394 (13, $[M - H_2O]^+$), 379 (11, $[M - (CH_3 + H_2O)]^+$), 314 (100, C(22)–C(23) break), 301 (10), 299 (25), 296 (36), 281 (54), 253 (21), 231 (12), 229 (33), 213 (30), 145 (37).

8. *Stigmasta-3,5,24(25)-trien-3-yl Acetate* ((−)-5). A mixture of Ac_2O (1 ml), Et_3N (0.5 ml), and 4-dimethylamino)pyridine (*ca.* 1 mg) was added to (+)-1 and heated at 80° for 18 h. The mixture was evaporated and the residue subjected to *CN* HPLC with hexane (λ 254 nm) to get (−)-5 (6 mg, 73%). $[\alpha]_D^{20} = -48.5$ ($c = 0.39$, EtOH). UV (EtOH): 235 (22900). ^{1}H-NMR ($CDCl_3$): 2.13 (*s*, Ac); 5.68 (*d*, $J = 2.4$, H–C(4)); 5.39 (*m*, H–C(6)); 0.70 (*s*, 3 H–C(18)); 1.00 (*s*, 3 H–C(19)); 0.96 (*d*, $J = 6.6$, 3 H–C(21)); 1.61 (br. *s*, 3 H–C(26), 3 H C(27)); 2.02 (*q*, $J = 7.5$, 2 H–C(28)); 0.95 (*t*, $J = 7.5$, 3 H–C(29)); from HETCOR traces: 1.30, 1.85 (H_α–C(1), H_β–C(1)); 2.20, 2.40 (H_α–C(2), H_β–C(2)); 1.70, 2.15 (H_α–C(7), H_β–C(7)); 1.65 (H_β–C(8)); 1.00 (H–C(9)); 1.50 (2 H–C(11)); 1.20, 2.05 (H_α–C(12), H_β–C(12)); 1.10 (H–C(14)); 1.10, 1.60 (H_α–C(15), H_β–C(15)); 1.25, 1.85 (H_α–C(16), H_β–C(16)); 1.05 (H–C(17)); 1.40 (H–C(20)); 1.10, 1.40 (2 H–C(22)); 2.00 (2 H–C(23)). ^{13}C-NMR ($CDCl_3$): 33.78 (*t*, C(1)); 24.82 (*t*, C(2)); 146.97 (*s*, C(3)); 21.11 (*q*), 169.41 (*s*, Ac); 117.02 (*d*, C(4)); 139.37 (*s*, C(5)); 124,11 (*d*, C(6)); 31.87 (*t*, C(7)); 31.75 (*d*, C(8)); 47.96 (*d*, C(9)); 35.03 (*s*, C(10)); 21.21 (*t*, C(11)); 39.71 (*t*, C(12)); 42.47 (*s*, C(13)); 55.83 (*d*, C(14)); 24.23 (*t*, C(15)); 28.21 (*t*, C(16)); 56.82 (*d*, C(17)); 11.98 (*q*, C(18)); 18.86 (*q*, C(19) or C(21)); 36.09 (*d*, C(20)); 18.81 (*q*, C(21) or C(19)), 34.90 (*t*, C(22)); 30.72 (*t*, C(23)); 129.34 (*s*, C(24)); 128.07 (*s*, C(25)); 17.83 (*q*, C(26)); 18.56 (*q*, C(27)); 26.99 (*t*, C(28)); 13.90 (*q*, C(29)).

9. *Oxidation of* 6/7. To a soln. of PPC [19] (0.195 mmol) and AcONa (0.043 mmol) in dry CH_2Cl_2 (0.5 ml) was added a 4:1 mixture 6/7 (52 mg, 0.13 mmol) in dry CH_2Cl_2 (0.5 ml). The mixture was stirred at r.t. for 2 h. After addition of Et_2O (5 ml) and precipitation of a solid, the supernatant soln. was decanted, filtered on *Celite*, and concentrated by evaporation. Nine tenths of this soln. were subjected to reversed-phase HPLC (MeCN, flux 10 ml/min. λ = 205 nm): (−)-3 (5 mg, 9%; t_R = 8.5 min), (+)-8 (29 mg, 56%, t_R = 24 min), and (+)-9 (8 mg, 15%; t_R = 31 min). The remaining one tenth of the above soln. was subjected to FC (hexane/Et_2O 1:1): (+)-1 as main product.

Stigmasta-5,24(25)-dien-3-one ((+)-8). $[\alpha]_D^{20} = +6.9$ ($c = 1.1$, abs. EtOH). UV (EtOH): < 210, weak absorption at 241. ^{1}H-NMR (C_6D_6): 2.97, 2.83 (br *AB*, $J_{gem} = 15.8$, H_α–C(4), H_β–C(4)); 5.13 (*m*, H–C(6)); 0.64 (*s*, 3 H–C(18)); 0.85 (*s*, 3 H–C(19)); 1.05 (*d*, $J = 6.7$, 3 H–C(21)); 1.66 (br. *s*, 3 H–C(26)), 1.71 (br. *s*, 3 H–C(27));

2.14 (*q*, $J = 7.5$, 2 H–C(28)); 1.04 (*t*, $J = 7.5$, 3 H–C(29)); from COSY maps: 2.15 (2 H–C(2)); 1.40, 1.80 (H_α–C(7), H_β–C(7)). ^{13}C-NMR (C_6D_6): 37.04 (*t*, C(1)); 37.67 (*t*, C(2)); 207.15 (*s*, C(3)); 48.58 (*t*, C(4)); 139.12 (*s*, C(5)); 122.49 (*d*, C(6)); 32.07 (*t*, C(7)); 32.10 (*d*, C(8)); 49.39 (*d*, C(9)); 37.02 (*s*, C(10)); 21.52 (*t*, C(11)); 39.95 (*t*, C(12)); 42.59 (*s*, C(13)); 56.18 (*d*, C(14)); 24.56 (*t*, C(15)); 28.60 (*t*, C(16)); 56.70 (*d*, C(17)); 12.06 (*q*, C(18)); 18.95 (*q*, C(19)); 36.47 (*d*, C(20)); 19.12 (*q*, C(21)); 35.49 (*t*, C(22)); 31.21 (*t*, C(23)); 129.68 (*s*, C(24)); 128.19 (*s*, C(25)); 18.06 (*q*, C(26)); 18.86 (*q*, C(27)); 27.50 (*t*, C(28)); 13.62 (*q*, C(29)).

Stigmast-24(25)-en-3-one ((+)-9). $[\alpha]_D^{20} = +20.9$ ($c = 0.72$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.68 (*s*, 3 H–C(18)); 1.00 (*s*, 3 H–C(19)); 0.95 (*d*, $J = 6.8$, 3 H–C(21)); 1.60 (br. *s*, 3 H–C(26), 3 H–C(27)); 2.01 (*q*, $J = 7.4$, 2 H–C(28)); 0.94 (*t*, $J = 7.4$, 3 H–C(29)). ^{1}H-NMR (C_6D_6): 0.40 (br. *ddd*, $J = 10.5, 10.5, 4.0$, H–C(9)); 0.63 (*s*, 3 H–C(18)); 0.62 (*s*, 3 H–C(19)); 1.05 (*d*, $J = 6.6$, 3 H–C(21)); 1.66 (br. *s*, 3 H–C(26)); 1.72 (br. *s*, 3 H–C(27)); 2.14 (*q*, $J = 7.6$, 2 H–C(28)); 1.05 (*t*, $J = 7.6$, 3 H–C(29)); from HETCOR traces: 0.95, 1.55 (H_α–C(1), H_β–C(1)); 1.95–2.25 (2 H–C(2)); 1.9–2.2 (2 H–C(4)); 1.15 (H–C(5)); 1.05 (2 H–C(6)); 0.70, 1.50 (H_α–C(7), H_β–C(7)); 1.15 (H–C(8)); 1.55 (2 H–C(11)); 1.00, 1.95 (H_α–C(12), H_β–C(12)); 0.85 (H–C(14) or H–C(17)); 1.00, 1.55 (H_α–C(15), H_β–C(15)); 1.25, 1.90 (H_α–C(16), H_β–C(16)); 1.10 (H–C(17) or H–C(14)); 1.45 (H–C(20)); 1.25, 1.55 (2 H–C(22)); 1.75, 2.05 (2 H–C(23)). ^{13}C-NMR ($CDCl_3$): 38.57 (*t*, C(1)); 38.22 (*t*, C(2)); 212.29 (*s*, C(3)); 44.75 (*t*, C(4)); 46.71 (*d*, C(5)); 28.98 (*t*, C(6)); 31.73 (*t*, C(7)); 35.39 (*d*, C(8)); 53.78 (*d*, C(9)); 35.65 (*s*, C(10)); 21.45 (*t*, C(11)); 39.87 (*t*, C(12)); 42.62 (*s*, C(13)); 55.94 (*d*, C(14)); 24.27 (*t*, C(15)); 28.22 (*t*, C(16)); 56.25 (*d*, C(17)); 11.48 (*q*, C(18)); 12.08 (*q*, C(19)); 36.10 (*d*, C(20)); 18.77 (*q*, C(21)); 34.98 (*t*, C(22)); 30.72 (*t*, C(23)); 129.39 (*s*, C(24)); 128.06 (*s*, C(25)); 17.85 (*q*, C(26)); 18.60 (*q*, C(27)); 27.00 (*t*, C(28)); 13.20 (*q*, C(29)). ^{13}C-NMR (C_6D_6): 38.55 (*t*, C(1)); 38.22 (*t*, C(2)); 208.65 (*s*, C(3)); 44.79 (*t*, C(4)); 46.53 (*d*, C(5)); 28.08 (*t*, C(6)); 31.95 (*t*, C(7)); 35.50 (*d*, C(8)); 53.85 (*d*, C(9)); 35.59 (*s*, C(10)); 21.62 (*t*, C(11)); 40.21 (*t*, C(12)); 42.83 (*s*, C(13)); 56.34 (*d*, C(14) or C(17)); 24.53 (*t*, C(15)); 28.63 (*t*, C(16)); 56,40 (*d*, C(17) or C(14)); 11.22 (*q*, C(18)); 12.28 (*q*, C(19)); 36.48 (*d*, C(20)); 19.08 (*q*, C(21)); 35.50 (*t*, C(22)); 31.21 (*t*, C(23)); 129. 68 (*s*, C(24)); C(25) submerged by solvent signals; 18.06 (*q*, C(26)); 18.85 (*q*, C(27)); 27,50 (*t*, C(28)); 13.61 (*q*, C(29)).

10. *Oxidation of* (+)-**8**. A soln. of (+)-**8** (22 mg, 0.054 mmol) in AcOH (3 ml) was vigorously stirred in an open 100 ml flask at r.t. in the dark. After 5 h, all (+)-**8** had disappeared while more polar (**10**) and similarly polar ((–)-**3**) compounds could be detected by TLC. After addition of C_6H_6 (10 ml), the solvent was evaporated; this was repeated 4 times to remove all AcOH. The residue was dissolved in Et_2O (2 ml), PPh_3 added (*ca.* 20 mg), and the mixture stirred for 15 min and then subjected to FC (first hexane/Et_2O 4:1, then Et_2O). The Et_2O eluate was evaporated and the residue subjected to *CN* HPLC (hexane/EtOH 19:1, 10 ml/min, $\lambda = 254$ nm): (+)-**4** (3.2 mg, 14%; $t_R = 3.5$ min), (–)-**3** (9.8 mg, 43%; $t_R = 4.5$ min), (+)-**11** (3.5 mg, 15%; $t_R = 5.7$ min).

6β-Hydroxystigma-4,24(25)-dien-3-one ((+)-**11**). $[\alpha]^{20} = +54.7$ (589), +61.9 (577), +68.6 (546; $c = 0.21$, $CHCl_3$). UV ($CHCl_3$): 242 (10400). ^{1}H-NMR ($CDCl_3$): 1.75 (br. *ddd*, $J = 13.5, 13.5, 5.5$, H_α–C(2)); 6.17 (*dd*, $J(4,2\alpha) = 1.9$, $J(4,6) = 0.6$, H–C(4)); 4.32 (br. *dd*, $J = 12.0, 3.5$, H–C(6)); 2.17 (*ddd*, $J_{gem} = 12.0$, $J(7\beta,8) = 5.5$, $J(7\beta,6) = 3.5$, H_β–C(7)); 0.71 (*s*, 3 H–C(18)); 1.18 (*s*, 3 H–C(19)); 0.96 (*d*, $J = 6.8$, 3 H–C(21)); 1.61 (br. *s*, 3 H–C(26), 3 H–C(27)); 2.01 (*q*, $J = 7.4$, 2 H–C(28)); 0.95 (*t*, $J = 7.4$, 3 H–C(29)); from COSY maps: 2.05 (H_β–C(1)); 2.3–2.5 (2 H–C(2)); 1.05 (H_α–C(7)); 1.6 (H–C(8)). ^{13}C-NMR ($CDCl_3$): 36.27 (*t*, C(1)); 33.82 (*t*, C(2)); 199.47 (*s*, C(3)); 119.65 (*d*, C(4)); 171.45 (*s*, C(5)); 68.71 (*d*, C(6)); 41.49 (*t*, C(7)); 34.14 (*d*, C(8)); 53.73 (*d*, C(9)); 39.04 (*s*, C(10)); 21.02 (*t*, C(11)); 39.40 (*t*, C(12)); 42.47 (*s*. C(13)); 55.51 (*d*, C(14)); 24.21 (*t*, C(15)); 28.09 (*t*, C(16)); 55.73 (*d*, C(17)); 11.95 (*q*, C(18)); 18.73 (*q*, C(19)); 36.02 (*d*, C(20)); 18.29 (*q*, C(21)); 34.90 (*t*, C(22)); 30.68 (*t*, C(23)); 129.48 (*s*, C(24)); 127.93 (*s*, C(25)); 17.85 (*q*, C(26)); 18.60 (*q*, C(27)); 27.00 (*t*, C(28)); 13.32 (*q*, C(29)).

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