3.61 (d, J = 2 Hz, 1 H, C6-H), 3.31 (s, 3 H, C12-H), 2.03 (m, 1 H, OH); ^{13}C NMR[37] (acetone-d_6) δ 203.5 (C5), 163.8 (C3), 139.0 (C7), 135.3 (C8), 134.4 (C4), 128.5 (C9), 120.2 (C10), 108.6 (C11), 94.7 (C2), 65.3 (C1), 54.2 (C12), 53.0 (C6); EIMS 222 (M^+, 15.1), 191 ($M - OCH_3$, 100), 160 (77.1), 103 (65.1), 77 (75.9); HRMS exact mass for $C_{12}H_{14}O_4$, calcd 222.0892, found 222.0889; UV (MeOH) λ_{max} 234 nm (ϵ = 19000); TLC (diethyl ether) R_f = 0.24, coelutes with natural product derivative; crystallographic unit cell constants a = 7.6658 (33) Å, b = 7.7295 (20) Å, c = 9.8184 (39) Å, β = 104.279 (32)°.

Natural product derivative 5:[4] mp 126-128 °C; $[\alpha]_D$ +371.8° (c 0.86, $CHCl_3$); IR ($CDCl_3$) 3690, 3550, 1720, 1215, 1075, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 5.5 Hz, 1 H), 6.85 (ddd, J = 17.0, 10.5, 10.5 Hz, 1 H), 6.30 (dd, J = 10.5, 2.0 Hz, 1 H), 6.17 (d, J = 5.5 Hz, 1 H), 5.39 (d, J = 17.0 Hz, 1 H), 5.36 (d, J = 10.5 Hz, 1 H), 5.33 (s, 1 H), 3.90 (dd, J = 11.0, 3.0 Hz, 1 H), 3.71 (dd, J = 11.0, 7.0 Hz, 1 H), 3.61 (d, J = 2.0 Hz, 1 H), 3.34 (s, 3 H), 2.03 (dd, J = 7.0, 3.0 Hz, exchangeable, 1 H); ^{13}C NMR (acetone-d_6) δ 203.5 (s), 163.7 (d), 138.8 (s), 135.2 (d), 134.4 (d), 128.4 (d), 120.2 (t), 108.6 (d), 94.7 (s), 65.3 (t), 54.2 (q), 52.9 (d); HREIMS 222.0877 (21), 191.0703 ($C_{11}H_{11}O_3$, 100), 160.0525 ($C_{10}H_8O_2$, 78); UV (MeOH) λ_{max} 236 nm (ϵ = 21000); crystallographic unit cell constants a = 7.662 (1) Å, b = 7.709 (1) Å, c = 9.808 (1) Å, β = 104.308 (13)°.

Palladium-mediated tri-*n*-butylvinyltin coupling with (-)-**41** similarly gave (-)-**5**, $[\alpha]^{23}_D$ -245.9° (c 0.270 g/100 mL, $CHCl_3$, ca. 66% ee).

(2*R*,6*R*)-Didemnenones A and B ((+)-1 and -2). Synthetic acetal (+)-**5** (4.31 mg, 19.4 μmol) was dissolved in a mixture of THF (1.00 mL) and H_2O (0.50 mL). The solution was cooled to 0 °C, and 1 M HCl (30 μL) was added. The solution was allowed to warm to room temperature with continued stirring for 2.75 h, at which time TLC showed no remaining **5**. The reaction mixture was recooled to 0 °C, and 0.50 M NaOH (60 μL) was slowly added to the vigorously stirred solution. Concentration by rotary evaporation at room temperature gave an aqueous suspension that was extracted with ethyl acetate (3 × 1 mL). The combined extracts were concentrated, and the residue was chromatographed on Sephadex LH-20 (2 g, ethyl acetate), to afford (+)-**1** and -**2** (1:1 mixture, 2.82 mg, 13.6 μmol, 70%) as an amorphous solid that matched the natural products.

Synthetic (+)-1 and -2: $[\alpha]^{22}_D$ +515° (c 0.081 g/100 mL, DMSO, ca. 89% ee); IR (CH_3CN) 3100-3550, 1720, 1205, 995 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.62 (d, J = 6 Hz, 1 H), 7.55 (d, J = 6 Hz, 1 H), 6.90 (ddd, J = 17, 12, 9 Hz, 2 H), 6.28 (br d, J = 12 Hz, 2 H), 6.21 (d, J = 6 Hz, 1 H), 6.11 (d, J = 6 Hz, 1 H), 5.73 (s, 1 H), 5.50 (br s, 1 H), 5.34 (d, J = 17 Hz, 2 H), 5.28 (d, J = 9 Hz, 2 H), 3.81-3.63 (m, 6 H); EIMS 208 (M^+, 1.7), 190 ($M - H_2O$, 5.8), 177 (6.0), 160 (21.7), 103 (48.9), 77 (100); HRMS exact mass for $C_{11}H_{10}O_3$ ($M - H_2O$), calcd 190.0630, found 190.0626; UV (CH_3CN) λ_{max} 234 nm (ϵ = 13000); TLC (diethyl ether) R_f = 0.09, coelutes with natural product mixture.

Natural products 1 and 2:[4] $[\alpha]_D$ +576.1° (c 0.49 g/100 mL, DMSO); IR (Nujol) 3100-3500, 1710, 1272, 1255, 1203, 1083, 1045, 1005, 995 cm^{-1}; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.62 (d, J = 5.5 Hz, 1 H), 7.55 (d, J = 5.5 Hz, 1 H), 6.90 (ddd, J = 17.0, 10.8, 9.8 Hz, 2 H), 6.28 (br d, J = 10.8 Hz, 2 H), 6.21 (d, J = 5.5 Hz, 1 H), 6.12 (d, J = 5.5 Hz, 1 H), 5.73 (s, 1 H), 5.51 (br s, 1 H), 5.35 (d, J = 17.0 Hz, 2 H), 5.28 (d, J = 9.8 Hz, 2 H), 3.81-3.62 (m, 6 H); HREIMS 208.0747 ($C_{11}H_{12}O_4$ requires 208.0736, 11.1%), 190.0616 ($C_{11}H_{10}O_3$, 14.8%), 177.0549 ($C_{10}H_9O_3$, 55.7%); UV (CH_3CN) λ_{max} 238 nm (ϵ = 13000).

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Supplementary Material Available: General experimental procedures, full experimental details, and spectral data for compounds **15**, **18-29**, and **32-37**, copies of ^{1}H and ^{13}C NMR spectra of compounds **15** and **32**, ^{1}H NMR spectra of synthetic **1**, **2**, and **5**, difference NOE spectra of **17**, and stereochemical correlation data for **9** (29 pages). Ordering information is given on any current masthead page.

Incisterols, a New Class of Highly Degraded Sterols from the Marine Sponge *Dictyonella incisa*

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Abstract: Four new sterols (**1-4**) with an unprecedented highly degraded skeleton were isolated from the Mediterranean sponge *Dictyonella incisa*. The structures of these new compounds were assigned on the basis of spectral studies, particularly NMR and mass spectrometry. A plausible pathway for the biogenesis of these compounds starting from cholestatrien-3β-ols through a unique in vivo oxidative process is proposed. The entire sterol content of the sponge and the structural elucidation of two new polyhydroxy sterols (**6b** and **6d**) are also reported.

Marine sponges are the richest source of unusual sterols with a bewildering variety of remarkable variations both in the side chain and nucleus, many of which have no terrestrial counterpart.[1-3] Most marine sterols possess novel side-chain alkylation patterns including the exceedingly rare in nature cyclopropene ring. A second class of unusual sterols is characterized by a great variety of oxygenated functionalities, including polyhydroxy, epoxide, and mono- and polyenone systems. Several other sterols include structural modification of the basic carbon skeleton, such as *B*- and *C*-secosterols and (hydroxymethyl)-*A*-norsteranes, the latter composing the entire sterol content of some sponges. A further small class of unusual sterols are the 19-norsterols, which, until now, represent the only example of biodegradation leading

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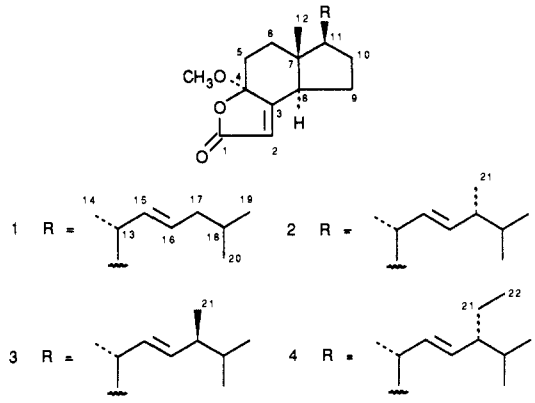
to the loss of carbon atoms from the sterol skeleton. 19-Norsterols are of exceptional interest from basic structural, pharmacological, and economic viewpoints.

Herein we describe the isolation and the structure determination of four strictly related unique norsterols [incisterol (**1**), (17*R*)-17-methylincisterol (**2**), (17*S*)-17-methylincisterol (**3**), (17*R*)-17-ethylincisterol (**4**)], which are derived from a dramatic biodegradation of the sterol nucleus resulting in the loss of all six carbon atoms of the A ring and of the 19-methyl group.



Results and Discussion

The new sterols were isolated from extracts of the Mediterranean sponge *Dictyonella incisa*, family Hymeniacidonidae (Porifera, Demospongiae). The sponge is cushion shaped to massive, up to ~10 cm in diameter, fleshy, rather soft, with an uneven, grooved surface. The color in life is orange and the tissue becomes cream-orange after preservation in alcohol. The spicules form a skeleton of ascending tracts, which ramify toward the surface.

Freshly collected material was stored frozen and subsequently homogenized with methanol. The ether-soluble portion of the methanol extract was fractionated by medium-pressure liquid chromatography (MPLC) on silica gel. Compounds **1**–**4** were isolated from fractions eluted with petroleum ether/ethyl ether (9:1) by silica gel HPLC, followed by reversed-phase HPLC.

Preliminary spectral analyses clearly indicated a strong similarity between these four compounds and therefore structural investigation was mainly carried out on the most abundant sterol **4**. Data from high-resolution electron impact mass (*m/z* 360.2728) and ^{13}C NMR spectrometry established a molecular formula of $C_{23}H_{36}O_3$ for compound **4**, which contains the unit A as shown by its UV (λ_{max} 220 nm) and IR (ν_{max} 1754 cm^{-1}) spectra, and by signals in the ^1H [δ 5.76 (1 H, d, J = 1.5 Hz) 3.12 (3 H, s)] and ^{13}C [δ 170.7 (s), 169.1 (s), 114.2 (d), 107.8 (s)] NMR spectra. This assignment was supported by mass spectral fragments at *m/z* 329.2557, 328.2388, and 316.2859, which illustrated characteristic losses of OCH_3, CH_3OH, and CO_2 from the molecular ion, respectively. Consideration of the molecular formula and overall unsaturation delineated from ^{13}C NMR data, which illustrates in addition to the signals relevant to the part structure A and those of a 1,2-disubstituted carbon–carbon double bond only sp^3 carbon resonances (see Table I), showed compound **4** to be tricyclic.

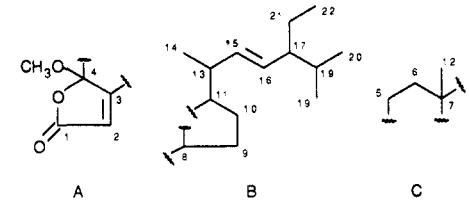
In addition, compound **4** contains some structural features characteristic of C-29 sterols. The sterol-like structure was indicated by ^1H NMR data (see Table I), which showed the presence of a tertiary methyl group and of a 5-ethyl-6-methyl-3-hepten-2-yl moiety, reminiscent of a sterol C-18 methyl group and of a side chain of a typical C-29 Δ^{22}-sterol, respectively. The side-chain signals were assigned as reported in Table I on the basis of sequential ^1H NMR spin–spin decoupling experiments. Additional evidence for the presence of the proposed moiety in compound **4** was obtained from its mass spectrum, which showed diagnostic peaks at *m/z* 317.2073 (M^+ – isopropyl), 221.1181 (M^+ – side chain), and 219.1019 (M^+ – side chain – H_2), and from its ^{13}C NMR spectrum, whose signals at δ 40.3 (d), 21.2 (q), 137.0 (d), 130.5 (d), 50.7 (d), 31.8 (d), 18.9 (q), 21.4 (q), 25.3 (t), and 12.4 (q) closely match the corresponding signals of a 24-ethyl-Δ^{22}-sterol side chain.[4]

Table I. NMR Spectral Data of Compounds **1**, **2**, **3**, and **4**[a]

	Chemical shift, δ_H[b]			4	
pos	1	2	3	δ_H (mult, J in Hz)	δ_C (mult)
1					170.7 (s)[c]
2	5.75	5.75		5.76 (d, 1.5)	114.2 (d)
3					169.1 (s)[c]
4					107.8 (s)
5ax	1.77	1.77		1.77 (ddd, 13.5, 13.5, 4)	34.5 (t)[d]
5eq	2.32	2.33		2.34 (ddd, 13.5, 4, 3)	
6ax				1.57[g]	35.1(t)[d]
6eq	1.94	1.93		1.93 (ddd, 12, 4, 2.5)	
7					48.9 (s)
8	2.41	2.41		2.42 (ddd, 11, 7, 1.5)	50.1 (d)[e]
9a				1.51[g]	21.3 (t)
9b				1.73[g]	
10a				1.94[g]	29.1 (t)
10b				1.48[g]	
11				1.45[g]	55.4 (d)
12	0.62	0.62		0.62 (brs)	11.9 (q)
13	2.06	2.05		2.07 (m)	40.3 (d)
14	1.04	1.04	1.03	1.05 (d, 6.5)	21.2 (q)[f]
15	5.15[h]	5.21[i]		5.17[i]	137.0 (d)
16	5.22[h]	5.32[i]		5.08[i]	130.5 (d)
17	1.88	1.84		1.55[g]	50.7 (d)[e]
18	1.48	1.50		1.55[g]	31.8 (d)
19	0.87	0.83		0.80 (d, 6.5)	18.9 (q)
20	0.87	0.85		0.85 (d, 6.5)	21.4 (q)[f]
21a		0.93	(d, 6.5)	1.44[g]	25.3 (t)
21b				1.19[g]	
22				0.82 (t, 7)	12.4 (q)
OMe	3.12	3.12		3.12 (s)	51.2 (q)

[a] ^1H assignment based on spin–spin decoupling and COSY experiments; ^{13}C assignment based on DEPT and selective decoupling experiments. [b] The multiplicities of resonances relative to compounds **1**, **2**, and **3** are identical with those reported for compound **4**. [c–f] The resonance with the same superscript may be reversed. [g] Submerged by other signals. [h] Further coupled AB system: J_{AB} = 15 Hz, $J_{AX} = J_{BX}$ = 7.5 Hz. [i] Further coupled AB system: J_{AB} = 15.5 Hz, $J_{AX} = J_{BX}$ = 7.5 Hz. [l] Further coupled AB system: J_{AB} = 15.5 Hz, $J_{AX} = J_{BX}$ = 7.5 Hz.

Further consideration of ^1H NMR data, including COSY plot and extensive decoupling and decoupling difference experiments, allowed us to extend the side chain up to the carbon C-8, through the protonated carbons C-9, C-10, and C-11 to form the partial structure B (see Table I). Evidence to combine the part structures A and B was provided by long-range coupling between H-2 and H-8, indicative of their allylic relationship, thus establishing the C-3/C-8 linkage.



Taking into account the molecular formula and the partial structure A–B, two methylenes and a methyl group linked to a quaternary carbon atom remained to be positioned. That all these functionalities form a second isolated spin system was inferred from ^1H NMR decoupling data, which showed that the two methylenes were connected and most likely part of a six-membered ring (see Table I). Decoupling and COSY NMR data further demonstrated a small 4σ coupling between the methylene proton resonating at δ 1.57 (H-6ax) and the 3 H signal due to the tertiary methyl group. This indicated that the methylene (C-6) and methyl group (C-12) must be connected to the quaternary carbon atom, thus leading to partial structure C.

Considering that part structures A–B and C represent two isolated spin systems, they could be unambigously combined. The quaternary carbon atom (C-7) must be linked to C-8 and C-11, while the C-5 methylene group must be linked to C-4 to close a six-membered carbocycle as shown in structure **4**.

(4) Wright, J. L. C.; Mc Innes, A. G.; Shimizu, S.; Smith, D. G.; Walter, J. A. *Can. J. Chem.* **1976**, *56*, 1898–1903.

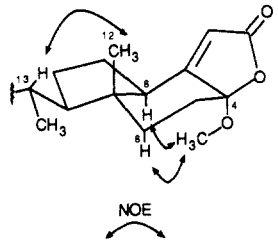


Figure 1.

The relative stereochemistry of the rigid part of the molecule was deduced by NOESY NMR experiments, which demonstrated the NOEs reported in Figure 1. These data indicated that the methyl group at C-7 and the H-13 methine are oriented on the same side (β), while the axial H-6, the OCH_3 at C-4, and the axial proton at C-8 are all oriented on the opposite face of the molecule (α). The stereochemistry of the double bond at C-15 was assigned as *E* on the basis of the large vicinal coupling H-15/H-16 (J = 15.5 Hz) observed. Tentative assignment for the C-17 *R* configuration was based on the chemical shift differences between H_3-19 and H_3-22 ($\Delta\delta$ 0.03) and between H_3-20 and H_3-22 ($\Delta\delta$ 0.02), which are characteristic of 24-ethyl-Δ^{22}-sterol side chains having the *R* configuration at C-24.[5] An assignment of the resonances in the ^{13}C NMR spectrum of **4** (see Table I), which were based on selective decoupling and DEPT experiments and comparison with model compounds, further supported the proposed structure.

Sterol **1** was obtained in minute amounts as an oil that gave a parent ion in the HREIMS at m/z 332.2312, in agreement with a molecular formula of $C_{21}H_{32}O_3$. ^1H NMR resonances (see Table I), assigned on the basis of COSY measurements, single-frequency spin decoupling, and spin decoupling difference experiments, together with UV (λ_{max} 220 nm) and IR (ν_{max} 1755 cm^{-1}) absorptions, revealed that **1** has the same skeleton as **4**. This suggested that the only difference between the two sterols was limited to the side chain, which on the basis of the molecular formula for **1** must contain two less carbon atoms.

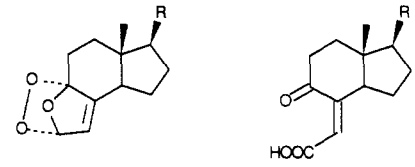
The structure of the entire side chain, including the stereochemistry of the double bond, was established through ^1H NMR spectroscopic analysis. By irradiation at δ 1.48 (H-18), the 6 H doublet at δ 0.87 (H-19 and H-20) collapsed into a singlet and the multiplet at δ 1.88 (H-17) was simplified. Conversely, by irradiation at δ 1.88 (H-17), the multiplet at δ 5.22 (H-16) was simplified in the A part of an AB system. Finally, saturation of the proton signal at δ 2.06 (H-13) caused the 3 H doublet at δ 1.04 (H-14) to collapse into a singlet and the multiplet at δ 5.15 (H-15) to converge to a B part of an AB system. The *E* configuration of the double bond was clearly indicated by the large coupling constant (J = 15 Hz) between H-15 and H-16.

The molecular formula of **2**, deduced from HRMS analysis, corresponds to $C_{22}H_{34}O_3$. Spectral data (see Table I and Experimental Section) indicated a strong similarity between **1** and **2**, the only significative difference being the presence in **2** of a 3 H doublet at δ 0.93 due to a methyl group. Extensive decoupling experiments showed the methyl group was located on the side chain at C-17, which defined the entire side-chain sequence from C-14 to C-20 and C-21 (Table I).

Compound **3** showed IR, UV, and mass spectra identical with those of **2**, thus suggesting that the two compounds were C-24 epimers. This was supported by comparison of their ^1H NMR spectra, which were superimposable apart from the difference between the resonances of the H_3-14 doublets (**2**, δ 1.04; **3**, δ 1.03). Since it has been reported that the signal of the 21-methyl of (22*E*, 24*R*)-24-methyl-Δ^{22}-sterols appears at lower fields than that of their 24*S* analogues,[6] we assigned the 17*R* configuration to compound **2**, and the 17*S* configuration to compound **3**.

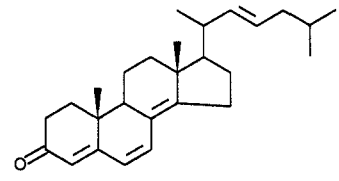
The structural features of compounds **1**, **2**–**3**, and **4** point to their biogenetic origin from an unprecedented degradative process starting from C-27, C-28, and C-29 sterols, respectively. Although many biosynthetic ways leading to sterols **1**–**4** could be outlined, all of them must implicate an oxidative process on the ring B. In this connection, it is relevant to mention that oxidation of the ring B is a common feature for sponge sterols.

Methoxy butenolides are common metabolites of marine organisms[7] and some have been observed to be artifacts resulting from methanol incorporation during extraction processes.[8] Since we utilized methanol in the extraction of *D. incisa*, there is indeed the possibility that **1**–**4** are artifacts of methanol incorporation. This further suggests that *D. incisa* contains a solvent-reactive intermediate, perhaps the endoperoxide **a** or the keto acid **b** which decompose in the extraction solvent to yield the observed lactones. Although *D. incisa* is a rare sponge collected with difficulty, experiments to probe this interesting question are planned.



a b

These considerations prompted us to examine the overall sterol composition of *D. incisa* in order to obtain information about the precursors and the intermediate biosynthetic steps leading to the sterols **1**–**4**. A preliminary spectroscopic examination of the eluates emerging from the SiO_2 column of the crude extract allowed us to single out four sterol-rich fractions, one containing compound **5**, whose isolation and structural determination has been recently reported.[9] A second fraction containing $\Delta^{5,7,22}$-sterols (fraction A) and two more polar fractions eluted with ethyl acetate (fractions B and C) were also observed.



5

Fraction A was separated by HPLC into four pure compounds: (22*E*)-cholesta-5,7,22-trien-3β-ol (82 mg), (22*E*,24*R*)-24-methylcholesta-5,7,22-trien-3β-ol (30 mg), (22*E*,24*S*)-24-methylcholesta-5,7,22-trien-3β-ol (41 mg), and (22*E*,24*R*)-24-ethylcholesta-5,7,22-trien-3β-ol (130 mg), which were identified by comparison of their spectral properties with those reported in the literature.[5,6]

Reversed-phase HPLC separation of fraction B (see Experimental Section) gave four pure compounds (**6a**–**6d**). From physical and spectroscopic data, two of these compounds [(22*E*)-6β-methoxy-5α-cholesta-7,22-diene-3β,5-diol (**6a**; 0.6 mg) and (22*E*, 24*S*)-24-methyl-6β-methoxy-5α-cholesta-7,22-diene-3β,5-diol (**6c**; 0.3 mg)] were found to be known products recently isolated from the marine sponge *Spongia agaricina*.[10]

Compound **6b** (0.1 mg, $C_{29}H_{48}O_3$ from HRMS) differed from **6c** only in the stereochemistry at C-24. This conclusion resulted from a comparison of their ^1H NMR spectra, which were identical

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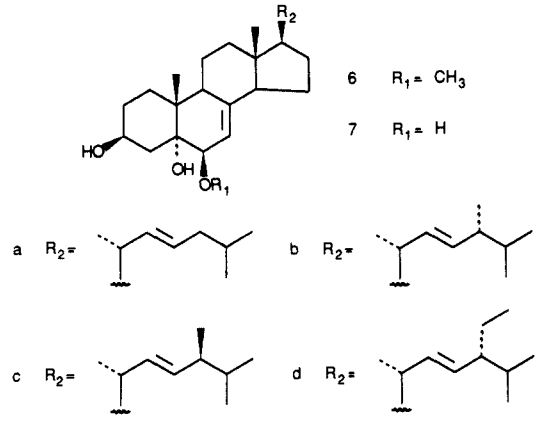
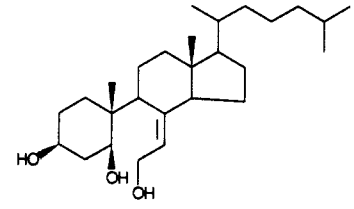
except for the resonances of the H_3-21 methyls (δ 1.03 in **6b** and δ 1.02 in **6c**) and those of the side-chain olefinic protons (H-22, δ 5.17 in **6b** and 5.14 in **6c**; H-23, δ 5.23 in **6b** and 5.21 in **6c**). The cooccurrence of the epimeric pair **6b–6c** allowed us to assign the absolute stereochemistry 24*R* and 24*S* to compounds **6b** and **6c**, respectively, on the basis of the same empirical rule used for compounds **2** and **3**.[6] Consequently, the previously unassigned configuration at C-24 of the corresponding sterol elaborated by the sponge *S. agaricina* can be now established as *S*.



The structure of the remaining compound **6d** (0.4 mg) was determined on the basis of the following evidence. The high-resolution mass spectrum for **6d** showed a molecular formula of $C_{30}H_{50}O_3$. The ^1H NMR spectrum exhibited a 3 H triplet at δ 0.81 indicative of an ethyl group. All other NMR data were similar to those of **6a–6c** including signals due to H_3-18, H_3-19, H-3, H-6, and H-7. It follows that sterol **6d** has a structure analogous to those of **6a–6c**, except for the nature of the side chain. Consistent with this hypothesis was the fragmentation pattern of **6d** in the mass spectrum, which showed the same prominent peaks as **6a–6c** at *m/z* 301.2151 derived by the loss of the side chain in conjunction with the loss of water from the molecular ion. The structure of the side chain of **6d** was ascertained through a COSY NMR and double-irradiation experiments [irradiation at δ 2.00 (H-20) collapsed the double doublet at δ 5.17 (H-22) to a doublet (J = 14 Hz) and the doublet at δ 1.03 (H_3-21) to a singlet; further irradiation at δ 1.50 (H-25) simplified the 3 H doublets at δ 0.84 and 0.79 (H_3-26 and H_3-27) into two sharp singlets]. These data established the part structure C-20/C-23 and the presence of an isopropyl group; as a consequence, the ethyl must be located at C-24. The *R* configuration of this carbon atom was tentatively assigned as for **4** on the basis of the chemical shift differences between H_3-26 and H_3-29 ($\Delta\delta$ 0.03) and between H_3-27 and H_3-29 ($\Delta\delta$ 0.02).[5]

Finally, the more polar sterol fraction C was subjected to preparative reversed-phase HPLC on a C-18 column. The resulting four pure compounds were identified as (22*E*)-5α-cholesta-7,22-diene-3β,5,6β-triol (**7a**; 1.3 mg), (22*E*,24*R*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**7b**; 0.4 mg), (22*E*,24*S*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**7c**; 0.5 mg), and (22*E*,24*R*)-24-ethyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**7d**; 0.7 mg), on the basis of their spectral properties and those of their acetyl derivatives when compared with those previously reported.[11,12] The C-24 configuration for compound **7d** has been assigned as *R* once more on the basis of the chemical shifts of H_3-21, H_3-26, and H_3-27, since in the previous paper[12] it was undetermined.

When one considers that in the sponge *D. incisa* $\Delta^{5,7,22}$-sterols represent by far the most abundant sterol compounds, it is tempting to postulate that the genesis of compounds **1–4** is initiated by an initial cleavage of the 5,6 π bond of these compounds. The possibility of an oxidative attack at this double bond in the sponge seems to be likely based on the cooccurrence of compounds **7a–7d**. A successive oxidative cleavage of the Δ^5 bond could afford a seco compound closely related to hipposterol **8**, recently isolated by Sica et al. from the sponge *Hippospongia communis*.[13] The final steps of this biogenetic hypothesis could be the oxidation of C-6 and the cleavage of the 9,10 bond, resulting in the removal of the entire ring A including the angular 19-methyl.



8

Although the above mechanism is not the only possible one, it nicely explains the formation of compounds **1–4**. Along with further studies of the chemistry of *D. incisa*, we intend to perform feeding experiments with appropriately labeled precursors to verify the above biosynthetic scheme.

Experimental Section

General Methods. EIMSs were obtained at 70 eV on a Kratos MS50 mass spectrometer. FT-IR spectra were recorded on a Bruker IFS-48 spectrophotometer in chloroform solution. UV spectra were performed on a Beckman DU70 spectrometer in ethanol solution.

^1H and ^{13}C NMR spectra were determined on a Bruker AC-400 spectrometer in $CDCl_3$. Proton chemical shifts were referenced to the residual chloroform signal (δ 7.26). ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at 77.0 ppm. The multiplicities of ^{13}C resonances were determined by DEPT experiments, which were performed with polarization transfer pulses of 90 and 135°, obtaining in the first case only signals for CH groups and in the other case positive signals for CH and CH_3 and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average C–H coupling of 135 Hz.

Medium-pressure liquid chromatography (MPLC) was performed on a Büchi 861 apparatus using SiO_2 (230–400 mesh) column. High-performance liquid chromatographies (HPLC) were performed on a Varian 5020 apparatus equipped with an RI-3 refractive index detector.

Collection and Extraction. Specimens of *D. incisa* were collected from the rocky areas of the Portofino Promontory (eastern Ligurian coast) around 10-m depth, during November 1988. They were frozen when still alive at −18 °C and then dispatched to the laboratory. Reference specimens are deposited at the Istituto di Zoologia dell'Università di Genova. Freshly collected animals (40 g of dry weight after extraction) were homogenized and extracted with MeOH (500 × 5 mL) at room temperature. The methanolic extracts were evaporated in vacuo to give an aqueous phase, which was extracted with Et_2O. Evaporation of the combined Et_2O extracts afforded 1.5 g of an oily residue, which was chromatographed by MPLC on an SiO_2 column using a solvent gradient system from petroleum ether to Et_2O and then to EtOAc.

Isolation of 1–4. Fractions eluted with petroleum ether/ethyl ether (9:1) afforded a mixture (27 mg) containing compounds **1–4**, which was rechromatographed by high-pressure liquid chromatography using a Hibar LiChrosorb Si60 (4 × 250 mm) column with a mobile phase of *n*-hexane/AcOEt (85:15). Final separation was achieved by HPLC on a Hibar Superspher RP-18 column, using MeOH as eluent.

Incisterol (1): 0.5 mg; UV (*n*-hexane) λ_{max} 220 nm (ϵ 6200); IR ($CHCl_3$) ν_{max} 1755 cm^{-1}; HRMS, *m/z* (assignment, relative intensities) 332.2312 (M^+, $C_{21}H_{32}O_3$, 48), 301.2103 ($M^+ - OCH_3$, $C_{20}H_{29}O_2$, 23), 300.2064 ($M^+ - CH_3OH$, $C_{20}H_{28}O_2$, 35), 289.1742 (M^+ – isopropyl, $C_{18}H_{25}O_3$, 9), 288.2395 ($M^+ - CO_2$, $C_{20}H_{32}O$, 5), 257.1486 (M^+ – isopropyl – CH_3OH, $C_{17}H_{21}O_2$, 100), 221.1153 (M^+ – side chain, $C_{13}H_{17}O_3$, 45), 219.1003 (M^+ – side chain – H_2, $C_{13}H_{15}O_3$, 55), 189.0913 (M^+ – side chain–CH_3OH, $C_{12}H_{13}O_2$, 23), 187.0725 (M^+ – side chain – CH_3OH – H_2, $C_{12}H_{11}O_2$, 14); ^1H and ^{13}C NMR data are reported in Table I.

(17*R*)-17-Methylincisterol (2): 0.3 mg; UV (*n*-hexane) λ_{max} 220 nm (ϵ 6100); IR ($CHCl_3$) ν_{max} 1755 cm^{-1}; HRMS, *m/z* (assignment, relative intensities) 346.2428 (M^+, $C_{22}H_{34}O_3$, 48), 315.2278 ($M^+ - OCH_3$, $C_{21}H_{31}O_2$, 22), 314.2206 ($M^+ - CH_3OH$, $C_{21}H_{30}O_2$, 35) 303.1914 (M^+ – isopropyl, $C_{19}H_{27}O_3$, 9), 302.2547 ($M^+ - CO_2$, $C_{21}H_{34}O$, 6), 271.1678 (M^+ – isopropyl – CH_3OH, $C_{18}H_{23}O_2$, 100), 221.1162 (M^+ – side chain

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(12) Cafieri, F.; Fattorusso, E.; Gavagnin, M.; Santacroce, C. *J. Nat. Prod.* **1985**, *48*, 944–947.
(13) Madaio, A.; Piccialli, V.; Sica, D. *Tetrahedron Lett.* **1988**, *29*, 5999–6000.

$C_{13}H_{17}O_3$, 40), 219.1001 (M^+ – side chain – H_2, $C_{13}H_{15}O_3$, 53), 189.0925 (M^+ – side chain, CH_3OH, $C_{12}H_{13}O_2$, 22), 187.0732 (M^+ – side chain – CH_3OH – H_2, $C_{12}H_{11}O_2$, 16); ^1H and ^{13}C NMR data are reported in Table I.

(17*S*)-17-Methylincisterol (3): 0.4 mg; spectroscopic properties identical with those of **2**, except for the ^1H NMR spectrum (see Table I).

(17*R*)-17-Ethylincisterol (4): 1 mg; UV (*n*-hexane) λ_{max} 220 nm (ϵ 6200); IR ($CHCl_3$) ν_{max} 1754 cm^{-1}; HRMS, *m/z* (assignment, relative intensities) 360.2728 (M^+, $C_{23}H_{36}O_3$, 50), 329.2557 (M^+ – OCH_3, $C_{22}H_{33}O_2$, 23), 328.2388 (M^+ – CH_3OH, $C_{22}H_{32}O_2$, 33), 317.2073 (M^+ – isopropyl, $C_{20}H_{29}O_3$, 7), 316.2859 (M^+ – CO_2, $C_{22}H_{36}O$, 5), 285.1846 (M^+ – isopropyl – CH_3OH, $C_{19}H_{25}O_2$, 100), 221.1181 (M^+ – side chain $C_{13}H_{17}O_3$, 43), 219.1019 (M^+ – side chain – H_2, $C_{13}H_{15}O_3$, 55), 189.0902 (M^+ – side chain – CH_3OH, $C_{12}H_{13}O_2$, 23), 187.0729 (M^+ – side chain – CH_3OH – H_2, $C_{12}H_{11}O_2$, 14); ^1H and ^{13}C NMR data are reported in Table I.

Identification of $\Delta^{5,7,22}$-Sterols. The crude sterol fraction (310 mg, R_f equals cholesterol by TLC) eluted with petroleum ether/ethyl ether (6:4) was purified by HPLC using a reversed-phase Hibar RP-18 (10 × 250 mm) column with a mobile phase of methanol, thus obtaining four pure compounds, which were identified as (22*E*)-cholesta-5,7,22-trien-3β-ol (82 mg), (22*E*,24*R*)-24-methylcholesta-5,7,22-trien-3β-ol (30 mg), (22*E*,24*S*)-24-methylcholesta-5,7,22-trien-3β-ol (41 mg), and (22*E*,24*R*)-24-ethylcholesta-5,7,22-trien-3β-ol (130 mg) by comparison of their physical and spectral properties with those previously reported.[5,6]

Isolation of 6a–6d. The earlier fractions eluted with ethyl acetate gave 5 mg of an oily residue that was subjected to HPLC with a Hibar Superspher C-18 (4 × 250 mm) column and MeOH/H_2O (95:5) as eluent, thus obtaining pure compounds **6a–6d**. Compound **6a** [0.6 mg; (22*E*)-6β-methoxy-5α-cholesta-7,22-diene-3β,5-diol] and **6c** [0.3 mg; (22*E*,24*S*)-24-methyl-6β-methoxy-5α-cholesta-7,22-diene-3β,5-diol] were identified by comparison of their spectral data with those of authentic samples.

(22*E*,24*R*)-24-Methyl-6β-methoxy-5α-cholesta-7,22-diene-3β,5-diol (6b): 0.1 mg; IR ($CHCl_3$) ν_{max} 3500 cm^{-1}; HRMS, *m/z* (M^+) 444.3622, calcd for $C_{29}H_{48}O_3$ 444.3591; ^1H NMR ($CDCl_3$) δ 0.60 (s, H_3-18), 0.82 and 0.84 (d's, J = 6.5 Hz, H_3-26 and H_3-27), 0.82 (t, J = 7 Hz, H_3-29), 1.00 (s, H_3-19), 1.03 (d, J = 6.5 Hz, H_3-21), 3.18 (br d, J = 5 Hz, H-6), 3.39 (s, OMe), 4.07 (m, H-3), 5.17 and 5.23 (further coupled AB system, J_{AB} = 14 Hz, H-22 and H-23, respectively), 5.42 (br d, J = 5 Hz, H-7).

(22*E*,24*R*)-24-Ethyl-6β-methoxy-5α-cholesta-7,22-diene-3β,5-diol (6d): 0.4 mg; IR ($CHCl_3$) ν_{max} 3500 cm^{-1}; HRMS, *m/z* (M^+) 458.3702, calcd for $C_{30}H_{50}O_3$ 458.3747; ^1H NMR ($CDCl_3$) δ 0.60 (s, H_3-18), 0.79 and 0.84 (d's, J = 6.5 Hz, H_3-26 and H_3-27), 0.81 (t, J = 7 Hz, H_3-29), 1.00 (s, H_3-19), 1.03 (d, J = 6.5 Hz, H_3-21), 3.17 (br d, J = 5 Hz, H-6), 3.39 (s, OMe), 4.05 (m, H-3), 5.03 and 5.17 (further coupled AB system, J_{AB} = 14 Hz, H-23 and H-22, respectively), 5.40 (br d, J = 5 Hz, H-7).

Isolation of 7a–7d. The combined more polar fractions eluted with ethyl acetate gave 9 mg of an oily residue that contained a mixture of polyhydroxysterols. HPLC with a Hibar Superspher C-18 (4 × 250 mm) column and MeOH/H_2O (92:8) as eluent afforded four pure compounds: (22*E*)-5α-cholesta-7,22-diene-3β,5,6β-triol (**7a**; 1.3 mg), (22*E*,24*R*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**7b**; 0.4 mg), (22*E*,24*S*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**7c**; 0.5 mg), and (22*E*,24*R*)-24-ethyl-5α-cholesta-7,22-diene-3β,5,6β-triol (**7d**; 0.7 mg), which were identified by comparison of their spectral data and/or those of their acetyl derivatives with those reported in literature.[11,12]

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Outer-Sphere Dissociative Electron Transfer to Organic Molecules: A Source of Radicals or Carbanions? Direct and Indirect Electrochemistry of Perfluoroalkyl Bromides and Iodides

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Abstract: As an example of the general problem posed in the title, the reduction of CF_3Br, CF_3I, $C_6F_{13}I$, and $C_8F_{17}I$ by outer-sphere heterogeneous (glassy-carbon electrodes) and homogeneous (aromatic anion radicals) reagents is investigated, in aprotic solvents containing tetralkylammonium salts, by cyclic voltammetry and preparative-scale electrolysis. A $R_F^\bullet$ chemistry is thus triggered in all cases with the exception of CF_3Br where a mixed $R_F^\bullet/R_F^-$ chemistry is obtained by direct electrochemical reduction or by electron transfer from aromatic anion radicals having a close reduction potential. Quantitative analysis of the reduction kinetics of R_FX (X = Br, I) and of $R_F^\bullet$ led to the following conclusions. R_FX most likely undergoes a concerted electron-transfer–bond-breaking reduction involving a substantial overpotential (ca. 1.5 V for C–Br and 1.0 V for C–I), mainly governed by the C–X bond dissociation energy. $R_F^\bullet$ radicals are strongly stabilized by interaction with the solvent but are nevertheless easier to reduce than alkyl radicals on thermodynamical grounds, exhibiting however a high intrinsic barrier of the same order as for alkyl radicals. Unlike alkyl radicals, but similar to aryl radicals, $R_F^\bullet$ radicals are good H-atom scavengers and do not couple with aromatic anion radicals.

Electron transfer from heterogeneous and homogeneous outer-sphere reagents to frangible organic substrates provides a means of generating either radicals or carbanions according to the reduction characteristics of both the substrate and the radical/carbanion couple. Investigation and rationalization of these characteristics should thus allow one to predict what type of

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