Marine Sterols. 18.[1] Isolation and Structure of Four Novel Oxygenated Sterols from a Gorgonian Coral *Melithaea ocracea*

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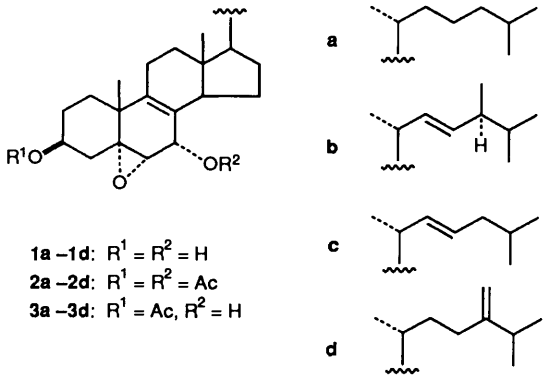
Four new marine polyhydroxysterols, melithasterols A–D (**1a–1d**), were isolated from a gorgonian coral *Melithaea ocracea* of the Okinawa Islands. The ^1H and ^{13}C NMR spectral analyses indicated them to be cholestane and 24-methylcholestane derivatives, having an unprecedented 3β,7α-dihydroxy-5α,6α-epoxy-Δ^8 steroid nucleus. PCC (pyridinium chlorochromate) oxidation of the 3-monoacetate mixture (**3a–d**) afforded the corresponding α,β-unsaturated ketone mixture (**4a–d**). The predominant constituent melithasterol A (**1a**) was identified as its diacetate (**2a**) by direct comparison with the authentic compound, prepared by lead tetraacetate oxidation of cholest-6-ene-3β,5α,8α-triol 3-monoacetate (**5**).

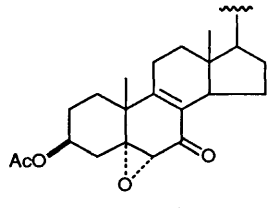
Melithaea ocracea (Linné) is a deep-red gorgonian coral which is ubiquitous in the coral reefs of Indo-Pacific coastal waters. In contrast to their abundant occurrence, until the present time there were few reports regarding their chemical constituents. The reason may be that the glyceride derivatives represent almost all of the constituents of its lipid extract. Repeated chromatography of the lipid extract of *M. ocracea*, collected in the Okinawa Islands, over a column of silica gel afforded a small amount (0.0015% of the wet material) of a mixture of novel sterols (**1a–d**) having a new pattern of oxygenated steroid nucleus. The ^1H and ^{13}C NMR spectra of the mixture showed that it contained compounds having different side chains but a similar steroid nucleus. It gave both a diacetate (**2a–d**) and a monoacetate (**3a–d**) mixture on acetylation.



1a–1d: $R^1 = R^2 = H$
2a–2d: $R^1 = R^2 = Ac$
3a–3d: $R^1 = Ac$, $R^2 = H$

Silver nitrate-impregnated silica gel preparative TLC[2] of the diacetate mixture afforded four major components (**2a–d**). Mild alkaline hydrolysis of **2a–d** afforded four new free sterols, named melithasterols A–D **1a–d**. Comparison of the ^1H and ^{13}C NMR spectra of the purified compounds with those in literature indicated that the major compound **1a** has a cholestane-type side chain,[3,4] while **1b–d** have side chains of 24-methyl-22*E*-dehydrocholestane,[3,4] 22*E*-dehydrocholestane,[3,4] and 24-methylenecholestane,[5] respectively. The side chain signals of 24-methyl-Δ^{22} sterol diacetate **2b** [δ_H 1.009, H-21, δ_C 135.7 (C-22), 18.1 (C-28)], corresponded to those having a (24*S*) configuration.[3,4] The simultaneous presence of a small amount of its (24*R*)-isomer, which was resistant to separation, was also confirmed [δ_H 1.017 (H-21), δ_C 135.5 (C-22), 17.6 (C-28)].[3,4]

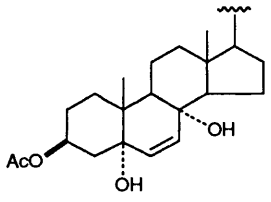
The ^1H NMR spectrum of the free sterol, **1a**, revealed the presence of two secondary hydroxy groups (δ_H 3.95, br m, $W_{\frac{1}{2}}$ 20 Hz; 4.22, m, $W_{\frac{1}{2}}$ 9 Hz), and one trisubstituted epoxide ring (3.32, d, *J* 2.5 Hz). The ^{13}C NMR spectrum showed the presence of a fully substituted double bond in the steroid nucleus (δ_C 123.2 and 137.1). The ^1H NMR decoupling experiment indicated that the epoxide proton was coupled with the hydroxy-methine proton at δ_H 4.22, which showed no further couplings except those with homoallylic protons. This hydroxy-methine proton is adjacent to the double bond since the pyridinium chlorochromate (PCC) oxidation of the 3-monoacetate mixture **3a–d** afforded a mixture of α,β-unsaturated ketone derivatives **4a–d**



4a – 4d

(UV, λ = 265 nm), which showed the signal of the epoxide proton at δ_H 3.30 as a sharp singlet. Assuming the broad hydroxy-methine signal at δ_H 3.95 to be that of the 3α-H of biogenetically common 3β-hydroxy-A/B-*trans* steroids, the epoxide ring was assigned to be 5α,6α and the adjacent secondary hydroxy group to be 7α, since the 4β-proton at δ_H 2.18 (dd, *J* 13.0, 11.5 Hz) was coupled only with 3α-H and 4α-H, and since the NOEs were observed between 19-H (δ_H 1.14) and 4β-H, and between 19-H and 7β-H. Of the two possibilities for the fully substituted double bond [Δ^8 or $\Delta^{8(14)}$], the Δ^8 structure seemed to be favoured from the NOE observed between 7β-H and 19-H. This NOE implies that the B-ring of **1a** adopts a boat-type conformation, as a result of the incorporation of 5α,6α-epoxide moiety and Δ^8 double bond. The lack of coplanarity of the carbonyl group and the Δ^8 double bond of the mixture (**4a–d**) resulted in the rather weak molecular extinction coefficient (ε/dm^3 mol^{-1} cm^{-1} *ca.* 5500). These assignments were supported by ^{13}C NMR spectroscopy since the C-14 signal of its isomer 7α-hydroxy-$\Delta^{8(14)}$-cholestanol is known to occur at δ_C 147.9,[6] which did not coincide with the signals found in **1a**. The calculated chemical shifts[7] of the H-18 and H-19 (H-18, δ_H 0.57; H-19 1.17) of the 3β,7α-dihydroxy-5α,6α-epoxy-Δ^8 steroid are very close to those found in **1a** 18-H, δ_H 0.57; 19-H, 1.14), in contrast to the calculated chemical shifts of its $\Delta^{8(14)}$ isomer (18-H, δ_H 0.83; 19-H, 0.93). The HMBC (heteronuclear multiple bond correlation spectroscopy)[8] spectrum of **2a** supported these assumptions, and the observed correlations between the signals of proton and carbon

atoms, separated by two or three bonds, were as follows: 18-H (C-12,13,14,17), 19-H (C-5,9,10), 6-H (C-5,7,8), 7-H (C-8,9). Lorenc *et al.* have recently oxidized cholest-6-ene-3β,5α,8α-triol 3-monoacetate **5** by lead tetracetate and obtained **2a** as a minor



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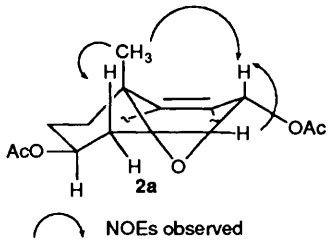

2a

NOEs observed

product.[9] Compound **2a** obtained from *M. ocracea* was identical with the synthetic sample, supplied by Dr. Lorenc, by direct comparisons of their ^1H and ^{13}C NMR spectra, and by TLC. Melithasterols A–D, **1a–d**, were thus shown to have a strained 7α-hydroxy-5α,6α-epoxy Δ^8 moiety, which is unprecedented in the natural steroids previously known. To our knowledge, the steroid constituents of the same soft coral and gorgonian species show less regional variation, unlike other metabolites such as terpenoids. It seems possible that **1a–d** or the structurally related polyhydroxysterols occur in the *M. ocracea* in other districts.

Experimental

M.p.s were determined on a Kofler hot stage and are uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter in $CHCl_3$. NMR spectra were determined on a JEOL JMS GX-270 spectrometer at 270 MHz (^1H) and on a JEOL JNM FX-90Q spectrometer at 22.5 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane as an internal standard. *J* and $W_{\frac{1}{2}}$ values are given in Hz. Mass spectra were determined on a JEOL JMS D 300 mass spectrometer.

Isolation of Crude Sterol Mixture.—Partially dried material of *M. ocracea* (5 kg), collected at Iriomote Island, Okinawa, was extracted exhaustively first with MeOH and then with $CHCl_3$–MeOH (1:1, v/v). The combined extract (126.9 g) was concentrated and mixed with $CHCl_3$–MeOH (2:1, v/v, 2 l). The precipitated inorganic salts were filtered off, and the filtrate was concentrated to afford crude extract (63.2 g). This was dissolved in $CHCl_3$ (300 cm^3), mixed with silica gel (*ca.* 70 g), and the mixture was evaporated to dryness. The residue was charged on a column of silica gel (*ca.* 150 g) and eluted (300 cm^3 per fraction) with $CHCl_3$ (fractions 1–3), and then with ethyl acetate–hexane (v/v, 1:2, fraction 4; 1:1, fractions 5, 6; 2:1, fraction 7; 3:1, fraction 8). Combined fractions 7 and 8 were purified again by column chromatography, eluting with ethyl acetate–hexane (2:1, v/v) to give a mixture (78 mg) mainly composed of **1a–d**.

Isolation of Melithasterols A–D, **1a–d**.—The crude sterol mixture was acetylated in the usual manner (Ac_2O–pyridine, room temperature, overnight). The unidentified crystalline material, which was formed in the solution, was filtered off, and the mother liquor was diluted with water and Et_2O. The organic layer was then washed several times with 5% HCl solution, water, and saturated brine. Column chromatography of the evaporation residue with ethyl acetate–hexane (2:1, v/v) gave a diacetate mixture (**2a–d**, 39.1 mg) and a monoacetate mixture (**3a–d**, 11.8 mg). The diacetate mixture was subjected to preparative TLC using 10% silver nitrate-impregnated silica gel, eluting seven times with $CHCl_3$–hexane (2:1, v/v). The four major zones, detected by 366 nm UV lamp, were extracted with ethyl acetate. Each extract was purified again by the same preparative TLC, giving **2a** (18.5 mg), **2b** (4.2 mg), **2c** (3.8 mg) and **2d** (3.0 mg) in the order of their elution. Each compound was hydrolysed with 2.5% KOH-MeOH solution by heating under reflux for 10 min to give the free sterols **1a** (12.1 mg), **1b** (3.8 mg), **1c** (3.3 mg) and **1d** (2.7 mg) after work-up. All compounds were crystallized from MeOH.

Melithasterol A (**1a**). M.p. 175–177 °C; $[\alpha]_D^{26}$ −68° (*c* 2.46); δ_H 0.57 (3 H, s, H-18), 0.860, 0.865 (each 3 H, d, *J* 6.5), 0.92 (3 H, d, *J* 6.5, 21-H), 1.14 (3 H, s, 19-H), 2.18 (1 H, dd, *J* 13.0, 11.5, 4β-H), 3.32 (1 H, d, *J* 2.5, 6β-H), 3.95 (1 H, m, 3α-H) and 4.22 (1 H, m, $W_{\frac{1}{2}}$ 9, 7β-H); δ_C C-1 (30.9), C-2 (30.3), C-3,7 (67.2, 68.4), C-4 (39.2), C-5 (65.7), C-6 (62.7), C-8 (134.5), C-9 (127.0), C-10 (38.0), C-11 (23.5), C-12 (36.2), C-13 (42.3), C-14 (49.6), C-15 (24.0), C-16 (28.9), C-17 (53.9), C-18 (11.2), C-19 (22.9), C-20 (36.2), C-21 (18.8), C-22 (35.9), C-23 (24.0), C-24 (39.6), C-25 (28.1) and C-26,27 (22.6, 22.9); *m/z* 416 (M^+), 398, 383, 380, 365, 355, 337, 313, 295, 285 and 267; HRMS (Found: 398.3192. Calc. for $C_{27}H_{42}O_2$ (*M* − H_2O): 398.3185).

Melithasterol A Diacetate (**2a**). M.p. 177–178 °C; $[\alpha]_D^{27}$ −100° (*c* 0.92); δ_H 0.56 (3 H, s, 18-H), 0.86, 0.87 (each 3 H, d, *J* 6.5, 26-, 27-H), 0.92 (3 H, d, *J* 6.5, 21-H), 1.17 (3 H, s, 19-H), 2.02, 2.13 (each 3 H, s), 2.26 (1 H, dd, *J* 12.5, 11.5, 4β-H), 3.37 (1 H, d, *J* 2.5, 6β-H), 4.95 (1 H, m, 3α-H) and 5.53 (1 H, br s, $W_{\frac{1}{2}}$ 6, 7β-H); δ_C C-1 (29.9), C-2 (27.0), C-3 (71.0), C-4 (35.3), C-5 (63.9), C-6 (59.4), C-7 (70.3), C-8 (137.2), C-9 (123.1), C-10 (38.4), C-11 (23.1), C-12 (35.7), C-13 (42.5), C-14 (49.2), C-15 (24.0), C-16 (28.9), C-17 (53.9), C-18 (11.2), C-19 (22.5), C-20 (36.1), C-21 (18.8), C-22 (36.1), C-23 (23.9), C-24 (39.6), C-25 (28.1), C-26,27 (22.6, 22.9) and OAc (21.4, 170.2, 171.0); *m/z* 440 (M^+ − AcOH), 380.

Melithasterol B (**1b**). M.p. 174–175 °C, $[\alpha]_D^{26}$ −51° (*c* 0.76); δ_H 0.59 (3 H, s, 18-H), 0.82, 0.84 (each 3 H, d, *J* 7.0, 26-, 27-H), 0.91 (3 H, d, *J* 6.5, 28-H), 1.013 (major), 1.021 (minor) (total 3 H, each d, *J* 6.5 21-H) and 5.16–5.21 (2 H, m, 22-, 23-H). Other signals, see **1a**; *m/z* 428 (M^+), 410, 395, 392, 377, 349, 285 and 267; HRMS (Found: M^+, 428.3276. Calc. for $C_{28}H_{44}O_3$: *M*, 428.3291).

Melithasterol B Diacetate (**2b**). M.p. 162–163 °C; $[\alpha]_D^{27}$ −98° (γ 0.84); δ_H 0.57 (3 H, s, 18-H), 0.81, 0.83 (each 3 H, d, *J* 6.5, 26-, 27-H), 0.904 (minor) and 0.908 (major) (total 3 H, d, *J* 7.0, 28-H), 1.009 (major), 1.017 (minor) (total 3 H, d, *J* 6.5, 21-H) and 5.1–5.2 (2 H, m, 22-, 23-H). Other signals, see **2a**; δ_C C-16 (29.1, 29.4), C-17 (53.7), C-18 (11.4), C-20 (40.4, 40.5), C-21 (21.0), C-22 (135.5, 135.7), C-23 (132.3), C-24 (42.9, 43.2), C-25 (33.2, 33.3), C-26,27 (19.7, 20.2, 20.5) and C-28 (17.6, 18.1). Other signals, see **2a**; *m/z* 452 (M^+ − AcOH), 392.

Melithasterol C (**1c**). M.p. 165–166 °C, $[\alpha]_D^{26}$ −66° (*c* 0.66); δ_H 0.59 (3 H, s, 18-H), 0.86 (3 H, d, *J* 7.0, 26-, 27-H), 1.02 (3 H, d, *J* 6.5, 21-H), 5.21 (1 H, dd, *J* 15.0, 7.5, 22-H) and 5.30 (1 H, dt, *J* 15.0, 6.5, 23-H). Other signals, see **1a**; *m/z* 414 (M^+), 396, 381, 378, 363, 353, 335, 285 and 267; HRMS (Found: M^+, 414.3131. Calc. for $C_{27}H_{42}O_3$: *M*, 414.3134).

Melithasterol C Diacetate (**2c**). M.p. 180–182 °C; $[\alpha]_D^{27}$ −105° (*c* 0.76); δ_H 0.57 (3 H, s), 0.85, 0.86 (each 3 H, d, *J* 6.5, 26-, 27-H), 1.01 (3 H, d, *J* 6.5, 21-H), 5.19 (1 H, dd, *J* 15.0, 8.0, 22-H), 5.29 (1 H, dt, *J* 15.0, 8.0, 23-H). Other signals, see **2a**; δ_C C-16 (29.3), C-17 (53.6), C-18 (11.4), C-20 (40.4), C-21 (20.9), C-22 (137.7), C-23 (126.7), C-24 (42.1), C-25 (28.7) and C-26,27 (22.4). Other signals see **2a**; *m/z* 438 (M^+ − AcOH), 378.

Melithasterol D (**1d**). M.p. 164–165 °C; $[\alpha]_D^{26}$ −59° (*c* 0.54); δ_H 0.58 (3 H, s, 18-H), 0.96 (3 H, d, *J* 6.5, 21-H), 1.02, 1.03 (each 3 H, d, *J* 7.0, 26-, 27-H), 4.66 and 4.72 (each 1 H, br s, $W_{\frac{1}{2}}$ 4.5, 28-H). Other signals, see **1a**; *m/z* 428 (M^+), 410, 395, 392, 377, 367, 349, 335, 311, 285 and 267; HRMS (Found: M^+, 428.3286. Calc. for $C_{28}H_{44}O_3$: *M*, 428.3290).

Melithasterol D Diacetate (**2d**). M.p. 170–171 °C; $[\alpha]_D^{27}$ −97° (*c* 0.60); δ_H 0.96 (3 H, d, *J* 6.5, 21-H), 1.020, 1.025 (each 3 H, d, *J* 7.0, 26-, 27-H), 2.22 (1 H, sept, *J* 7.0, 25-H, overlapped by 4β-H signal), 4.65, 4.72 (each 1 H, br s, $W_{\frac{1}{2}}$ 4.5, 28-H). Other signals, see **2a**; δ_C C-22 (34.7), C-23 (31.2), C-24 (156.8), C-25 (34.0), C-26,27 (22.0, 22.1) and C-28 (106.1). Other signals, see **2a**; *m/z* 452 (M^+ − AcOH), 392.

PCC Oxidation of Melithasterols A–D 3-Monoacetate mixture **3**.—A solution of the monoacetate mixture **3** (5 mg, *ca.* 0.01 mmol) in CH_2Cl_2 (1 cm^3) was stirred with PCC (11 mg, 0.05 mmol) at room temperature for 20 min, after which the mixture diluted with water and extracted with Et_2O. The Et_2O solution was washed with water and saturated brine, and the solvent evaporated. Column chromatography of the residue with ethyl acetate–hexane (1:9, v/v) gave a mixture (2.5 mg) of **4a–d**; δ_H 0.59 (s, 18-H, major), 0.59 (s, 18-H, minor), 1.26 (3 H, s, 19-H), 2.02 (3 H, s), 2.34 (1 H, dd, *J* 13.0, 12.0, 4β-H), 3.30 (1 H, s, 6β-H), 4.99 (1 H, m, 3α-H); λ_{max}(95% EtOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 5500); *m/z* 468 (M^+), 456 (M^+), 454 (M^+), 396, 343 (M^+ − side chain), 283, 265 and 255.

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