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NEW ANTIMICROBIAL DITERPENES FROM THE SPONGE *SPONGIA OFFICINALIS*[1)]

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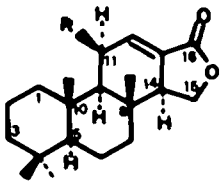
Abstract - The methanol extract of the sponge *Spongia officinalis* from Pt. Güimar, Tenerife (Canary Islands) contained new diterpenoids which inhibited the growth of microbes. The structures of the new diterpenes isolated were determined as: 11β-hydroxyspongi-12-en-16-one (2), 11β-acetoxyspongi-12-en-16-one (3), 7β,11β-dihydroxyspongi-12-en-16-one (5), and 7β,11α-dihydroxyspongi-12-en-16-one (6), by spectral and chemical degradation studies. The previously reported diterpenes isoagatholactone (1) and aplysillin (4) were also found in the extract.

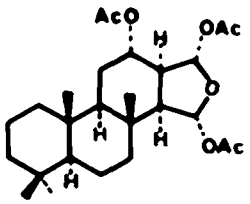
The sponge order Dictyoceratida has proved to be a fertile source of secondary metabolites with diverse and often novel molecular architecture[2)]. The family spongiidae has been particularly well studied and has produced an array of interesting terpenoids[3)], some of which were shown to be active in bioassays employing adult and larval forms of common marine algae and invertebrates[3f)]. Following the earlier isolation[4)] of isoagatholactone (1), a growing group of tetracyclic diterpenes has been more recently isolated from Spongia officinalis[5-7)] whose carbon skeleton of spongian has generated much interest among synthetic chemists[8)].

In the course of studies on the chemical constituents of the Canary Island sponge Spongia officinalis[9)], we became aware that the crude methanol extract of this sponge showed antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus sphaericus in a disk assay. The extract also inhibited HeLa cells with values of ID_{50} 1-5μg/ml. After the crude methanol extract was partitioned between ether and water, concentration of the ether layer yielded a dark brown oil. This oil was separated by being passed through a silica gel column (hexane-EtOAc) and the active fractions were further purified by passing through a prepacked Merck Size C silica gel 60 column (hexane-ether) to give the bioactive compounds[10)] 11β-hydroxyspongi-12-en-16-one (2, 2.5% dry weight), 11β-acetoxyspongi-12-en-16-one (3, 3.6% dry weight), together with the inactive spongi-12-en-16-one (1, 0.6% dry weight), 12α,15α,16α-triacetoxyspongian (4, 0.2% dry weight). No individual testing was carried out for the also isolated mixture of 7β,11β-dihydroxyspongi-12-en-16-one (5) and 7β,11α-dihydroxyspongi-12-en-16-one (6) (1.2% dry weight). Compounds 1 and 4 were respectively identified with the previously reported isoagatholactone[4)] and aplysillin[11)] from spectral data that were identical with literature values.

The major tetracyclic diterpene, 11β-hydroxyspongi-12-en-16-one (2), had the molecular formula $C_{20}H_{30}O_3$, m.p. 199-200°C, $[\alpha]_D$+74.6 (c, 1.22, $CHCl_3$). The IR spectrum showed absorption bands for hydroxyl (3360) and carbonyl (1770 cm^{-1}). The ^{1}H-NMR spectrum showed the presence of four methyl signals at δ0.83 (*s*, 6H), 1.02 (*s*, 3H) and 1.32 (*s*, 3H). The ^{13}C-NMR spectrum contained signals at δ170.5 (*s*) due to the lactone carbonyl, at δ136.5 (*d*) and 127.4 (*s*) for the olefinic carbons at C-12 and C-13, respectively, and at δ65.3 (*d*) and 67.7 (*t*) assigned to C-11 and the γ-carbon on the γ-lactone ring. In the ^{1}H-NMR spectrum, the proton at C-11 gave a signal at δ4.75 (*bs*), the olefinic proton at C-12 gave a signal at δ6.76 (*t*, 1H, *J* = 4Hz), while the methylene protons in the lactone



1 R = H
2 R = OH
3 R = OAc



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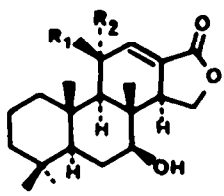
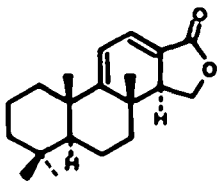


5 R_1 = OH, R_2 = H
6 R_1 = H, R_2 = OH

ring gave signals at δ4.11 and 4.41 (*t*, 1H each, *J* = 10 Hz). Assuming the same skeleton of spongian present in isoagatholactone (**1**), which seemed most likely from the ^{13}C-NMR spectrum (see Experimental), we could place the hydroxyl group at C-11, which was chemically confirmed as follows. Treatment of **2** with Ac_2O/Py gave the also isolated from the extract: acetate **3**. The infrared spectrum of **3** contained bands at 1760 and 1740 cm^{-1} for butenolide and acetate carbonyl groups. The ^{1}H-NMR spectrum contained an acetate signal at δ2.09 (*s*, 3H), an α-acetoxy proton signal at δ5.85 (*m*, 1H), and the butenolide methylene signals at δ4.49 (*t*, 1H, *J* = 11 Hz) and 4.17 (*t*, 1H, *J* = 11 Hz). Treatment of **2** with TosCl/Py gave the diene **7** as a result of concomitant dehydration. Upon oxidation with Jones reagent at 0° compound **2** yields the α,β-unsaturated keto lactone **8**. The infrared spectrum of **8** contained bands at 1770 and 1670 cm^{-1} for butenolide and keto functions. The ^{1}H-NMR spectrum of **8** contained an olefinic proton at δ6.59 (*d*, 1H, *J* = 4 Hz).

The stereochemistry for C-11 (OH) shown in **2** was determined as follows. Hydrogenation of **3** over 10% palladium on charcoal gave a 3:1 mixture of the two isomeric acetoxy γ-lactones **10** and **11**, together with a small amount of the compound from hydrogenolisis **9**. The downfield shift of the methyl groups at C-8 and C-10 observed in the ^{1}H-NMR spectra of **10** and **11** (see Experimental) can be explained in terms of the 1,3-*trans*-diaxial configuration of these two methyls with respect to the C-11 (OH). The C-8 methyl group of **11** was observed at δ1.45 (*s*, 3H), an extremely large downfield shift which is due to the added deshielding effect of the lactone carbonyl. On the basis of the above results, the most plausible alternative for the structure and stereochemistry of these new diterpenes seemed to be: 11β-hydroxyspongi-12-en-16-one and 11β-acetoxyspongi-12-en-16-one as is shown respectively by formulas **2** and **3**. This stereochemistry has been established definitely by the X-ray single-crystal analysis of the pyrazoline derivative **12**[12], a highly crystalline adduct obtained by treatment of **3** with diazomethane in ether.

The more polar fractions of the chromatography gave a cocrystallizing mixture of two hydroxybutenolides, the high-resolution mass indicating it to be an isomeric mixture having molecular formula $C_{20}H_{30}O_4$. Treatment of the mixture with Ac_2O/Py gave a non-crystalline mixture of diacetates which was further treated with diazomethane to give pyrazoline adducts which were isolated by chromatography. The more polar isomer was isolated as a crystalline solid, while the



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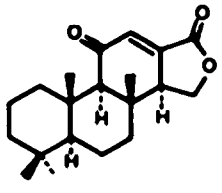

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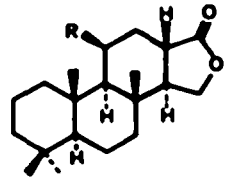
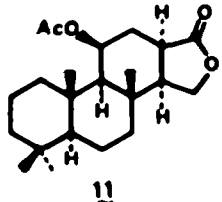


9 R = H
10 R = OAc



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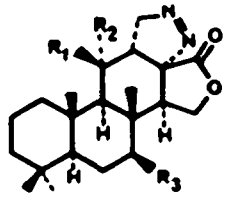

12 R_1 = OAc; R_2 = H; R_3 = H
13 R_1 = OH; R_2 = H; R_3 = H
14 R_1 = OAc; R_2 = H; R_3 = OAc
15 R_1 = H; R_2 = OAc; R_3 = OAc

less polar was purified to give an oil. Assuming the spongian skeleton, which seemed most likely from the ^{13}C-NMR spectra and critical comparison with that of compound 12, the most plausible alternative for the structure and stereochemistry of the crystalline adduct is 14, and 15 for the non-crystalline isomer. The ^{1}H-NMR spectra indicated that the proton signal geminal to the C-11 acetoxy group appeared in the β-isomer at δ5.54 (*bs*, 1H) and in the α-isomer at δ5.35 (*bd*, 1H, J = 10 Hz); the geminal proton to the acetoxy group at C-7 appears at δ5.27 (*dd*, 1H, J = 18, 10 Hz) in 14 and at δ5.32 (*dd*, 1H, J = 18, 12 Hz) in 15, which suggests that the C-7 (OAc) is β-equatorial in both isomers.

EXPERIMENTAL

Mps were determined on a Kofler block and are uncorr. Infrared spectra were recorded on Perkin-Elmer Mod. 237 and Mod. 681 spectrophotometers. Optical rotations were determined for solutions in chloroform with a Perkin-Elmer 141 polarimeter. ^{1}H-NMR spectra were recorded on a Perkin-Elmer R-32 (90 MHz) and Bruker Mod. WM 360 spectrometers, chemical shifts are reported relative to Me_4Si (δ0) and coupling constants are given in hertz. ^{13}C-NMR spectra were obtained on a Bruker Mod. WM 360 and the chemical shifts are reported relative to Me_4Si (δ0). Low and high resolution mass spectra were obtained from a VG Micromass ZAB-2F. Column and dry-column chromatography were performed on silica gel G, all Merck products. Silica gel GF_{254} (Type 60) was utilized for thin-layer plates (TLC), and spots were visualized by staining with anisaldehyde-sulfuric acid[13]. Combustion analyses were carried out by the Analytical Laboratory of C.S.I.C. (Madrid). All solvents used were either spectral grade or distilled from glass prior to use. Anhydrous sodium sulphate was used for drying solutions.

Collection, Extraction and Chromatography. The sponge Spongia officinalis was collected by hand using SCUBA (-10 m) at Pt. Güimar (Tenerife, Canary Islands) in April 1979, immediately frozen and then lyophilized. The sponge (42 g) was placed in a Soxhlet apparatus and exhaustively extracted with hexane (2 L) and methanol (3 L). The hexane and methanol extracts were evaporated to give a viscous oil (15.3 g, 36% dry weight) that was judged to contain the majority of secondary metabolites. The oil was preabsorbed onto silica gel and applied to a column of silica gel (500 g) that was eluted with solvents of increasing polarity from hexane to ethyl acetate. One-litre fractions were collected employing the following elution scheme: hexane, fractions 1-6; hexane/ethyl acetate (20/1), fractions 7-10; hexane/ethyl acetate (10/1), fractions 11-14; hexane/ethyl acetate (4/1), fractions 15-18; hexane/ethyl acetate (3/2), fractions 19-24; hexane/ethyl acetate (2/3), fractions 25-30; ethyl acetate, fractions 31-34. Fractions exhibiting similar TLC profiles were combined. A portion of 1.02 g of combined fractions 2-6 (2.32 g) was chromatographed on 120 g of silica gel H using hexane-ethyl acetate (10:1) as solvent and collecting 20 ml fractions. Fractions 6-10 yielded spongi-12-en-16-one (1, 118 mg). Fractions 8-14 (4.42 g) were chromatographed on 300 g of silica gel H using hexane-ethyl acetate (5:1) as solvent and collecting 20 ml fractions; fractions 4-12 yielded 11β-acetoxyspongi-12-en-16-one (3, 1.52 g); fractions 16-18 yielded 12α,15α,16α-triacetoxyspongian (4, 112 mg); fractions 26-34 yielded 11β-hydroxyspongi-12-en-16-one (2, 1.04 g). A portion of 4.8 g of combined fractions 17-22 (5.31 g) was chromatographed on a column (30 x 2 cm) containing silica gel (40 g) in 10% ethyl acetate in hexane and collecting 50 ml fractions. Fractions 18-27 (2.2 g) were rechromatographed on a pre-packed Merck Size C silica gel 60 column in 20% ethyl acetate in hexane, which allowed the isolation of a solid that was recrystallized from hexane-dichloromethane to obtain a mixture of 7β, 11β-dihydroxyspongi-12-en-16-one (5) and 7β,11α-dihydroxyspongi-12-en-16-one (6) (418 mg).

Spongi-12-en-16-one (1): mp 153-155°C; $[\alpha]_D$+6.3 (c, 0.9, $CHCl_3$); IR ($CHCl_3$) 1760, 1690 cm^{-1}; ^{1}H-NMR (CCl_4) δ0.79 (*s*, 3H), 0.84 (*s*, 3H), 0.88 (*s*, 3H), 0.94 (*s*, 3H), 2.23 (*m*, 2H), 2.75 (*m*, 1H), 3.93 (*t*, 1H, J = 9 Hz), 4.24 (*t*, 1H, J = 9 Hz), 6.69 (*q*, 1H, J = 3 Hz); ^{13}C-NMR ($CDCl_3$) δ39.7 (C-1), 18.3 (C-2)#, 41.7 (C-3)+, 33.1 C-4), 56.6 (C-5), 18.7 (C-6)#, 40.7 (C-7)+, 34.4 (C-8), 51.1 (C-9), 37.2 (C-10), 24.1 (C-11), 136.2 (C-12), 127.0 (C-13), 54.4 (C-14), 67.70 (C-15), 170.0 (C-16), 14.0 (C-17), 33.4 (C-18), 21.6 (C-19), 24.1 (C-20) (chemical shifts marked with # and + are interchangeable). Mass spectrum, *m/z* 302 (M^+), further peaks are found at *m/z* 287(8), 192(100), 191(40), 177(53), 137(28). Anal. Calcd. for $C_{20}H_{30}O_2$: C, 67.24; H, 8.62. Found: C, 66.91; H, 8.61. The physical and spectroscopic data were identical with those reported for isoagatholactone[4].

11β-Acetoxyspongi-12-en-16-one (3): mp 195-197°C; $[\alpha]_D$+184.1 (c, 1.09, $CHCl_3$); IR ($CHCl_3$) 1760, 1740, 1390, 1370, 1225, 1160 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ0.90 (*s*, 6H), 1.08 (*s*, 3H), 1.22 (*s*, 3H), 2.09 (*s*, 3H), 4.17 (*t*, 1H, J = 11 Hz), 4.49 (*t*, 1H, J = 11 Hz), 5.85 (*m*, 1H), 6.70 (*t*, 1H, J = 4 Hz); ^{13}C-NMR ($CDCl_3$) δ39.3 (C-1), 18.1 (C-2)#, 41.5 (C-3)+, 33.2 (C-4), 57.4 (C-5), 18.6 (C-6)#, 42.2 (C-7)+, 34.3 (C-8), 58.1 (C-9), 38.2 (C-10), 66.6 (C-11), 131.4 (C-12), 129.8 (C-13), 52.1 (C-14), 67.3 (C-15), 169.6 (C-16), 15.7 (C-17), 33.6 (C-18), 21.7 (C-19), 18.5 (C-20), 169.5 (C_{11}-OAc), 21.4 (C_{11}-OAc) (chemical shifts marked with # and + are interchangeable). Mass spectrum, *m/z* 360 (M^+), further peaks are found at *m/z* 318(12), 301(11), 286(42), 192(80), 177(18), 137(21). Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.3; H, 8.88. Found: C, 73.35; H, 9.00.

12α,15α,16α-Triacetoxyspongian (4): mp 169-171°C; $[\alpha]_D$+13 (c, 0.5, $CHCl_3$); IR ($CHCl_3$) 1740, 1440, 1360, 1350, 1230, 1150, 940, 880, 740 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ0.80 (*s*, 3H), 0.82 (*s*, 3H), 0.84 (*s*, 3H), 0.92 (*s*, 3H), 2.04 (*s*, 3H), 2.05 (*s*, 3H), 2.08 (*s*, 3H), 2.58 (*m*, 1H), 5.08 (*m*, 1H), 6.07 (*s*, 1H), 6.08 (*d*, 1H, J = 8 Hz). The physical and spectroscopic data were identical with those reported for aplysillin[11].

11β-Hydroxyspongi-12-en-16-one (2): mp 199-200°C; $[\alpha]_D$+74.6 (c, 1.22, $CHCl_3$); IR (KBr) 3360, 1770, 1690, 1430, 1385, 1375, 1220 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ0.83 (*s*, 6H), 1.02 (*s*, 3H), 1.32 (*s*, 3H), 4.11 (*t*, 1H, J = 10 Hz), 4.41 (*t*, 1H, J = 10 Hz), 4.75 (*bs*, 1H), 6.76 (*t*, 1H, J = 4 Hz); ^{13}C-NMR $CDCl_3$ δ39.6 (C-1), 18.2 (C-2), 41.8 (C-3), 33.2 (C-4), 57.3 (C-5), 18.7 (C-6)#, 42.6 (C-7)+, 34.7 (C-8), 59.3 (C-9), 38.5 (C-10), 65.3 (C-11), 136.5 (C-12), 127.4 (C-13), 52.4 (C-14), 67.7 (C-15), 170.5 (C-16), 16.0 (C-17), 33.7 (C-18), 21.8 (C-19), 17.9 (C-20) (chemical shifts marked with #

and + are interchangeable). Mass spectrum, *m/z* 318 (M^+), further peaks are found at *m/z* 301(10), 286(22), 192(80), 177(52), 137(12). Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.47; H, 9.43. Found: C, 75.34, H, 9.38.

7β,11β-Dihydroxyspongi-12-en-16-one (5) and 7β,11α-dihydroxyspongi-12-en-16-one (6): The diastereoisomeric mixture of 5 and 6 was crystallized from acetone to give crystals of mp 60°C; IR (KBr) 3350, 1770, 1750, 1690, 1390, 1220, 1100, 1020 cm^{-1}. High resolution mass measurements: Found: 334.2239; Calcd. for $C_{20}H_{30}O_4$: 334.2244. Anal. Calcd. for $C_{20}H_{30}O_4$: C, 71.86; H, 8.98. Found: C, 71.88; H, 9.00.

Acetylation of 2: A solution of 11β-hydroxyspongi-12-en-16-one (2) (10 mg, 0.035 mmol) and acetic anhydride (0.5 ml) in pyridine (0.5 ml) was stirred at 25°C for 7 h. The reagents were evaporated under high vacuum. The residue was dissolved in dichloromethane, and the solution filtered through a short column of silica gel to obtain 11β-acetoxyspongi-12-en-16-one (3) (7 mg, 62% theoretical) with spectral data identical with the natural product.

Spongi-12-en-11,16-dione (8): A solution of 2 (30 mg, 0.1 mmol) in acetone (10 ml) was treated with CrO_3 (90 mg)-H_2O (0.15 ml)-H_2SO_4 (0.15 ml) and the ice-cooled mixture stirred for 1 h. After usual work-up the product was chromatographed, elution with light petroleum yielding colourless crystals of 8 (21 mg, 67% theoretical), mp 221-223°C; $[\alpha]_D$ +34.5 (c, 1.08, $CHCl_3$); IR (KBr) 1770, 1670, 1450, 1370, 1350, 1170, 1050, 1000 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.89 (*s*, 3H), 0.92 (*s*, 3H), 1.01 (*s*, 3H), 1.19 (*s*, 3H), 2.15 (*s*, 1H), 3.32 (*dt*, 1H, *J* = 10, 4 Hz), 4.20 (*t*, 1H, *J* = 10 Hz), 4.60 (*t*, 1H, *J* = 10 Hz), 6.59 (*d*, 1H, *J* = 4 Hz). Mass spectrum, *m/z* 316 (M^+), further peaks are found at *m/z* 301(15), 286(22), 258(59), 275(32), 220(18), 202(27), 192(100), 167(24), 122(15), 107(37). High resolution mass measurement: Found: 316.2033; Calcd. for $C_{20}H_{28}O_3$: 316.2039. Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.95; H, 8.86. Found: C, 75.63; H, 8.79.

Spongi-9(11),12-dien-16-one (7): A solution of 11β-hydroxyspongi-12-en-16-one (2) (40 mg, 0.14 mmol) and tosyl chloride (1 ml) in pyridine (0.5 ml) was stirred at 25°C for 12 h. The mixture was poured into water, extracted with ether and the crude product chromatographed using hexane-ethyl acetate (5:1) as eluent. Recrystallization gave 7 (12 mg, 32% theoretical), as colourless crystals, mp 124-126°C; $[\alpha]_D$ +118 (c, 0.34, $CHCl_3$); IR (KBr) 1740, 1650, 1540, 1450, 1380, 1370, 1230 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.92 (*s*, 3H), 0.95 (*s*, 6H), 1.21 (*s*, 3H), 3.05 (*m*, 1H), 4.28 (*t*, 1H, *J* = 10 Hz), 4.52 (*t*, 1H, *J* = 10 Hz), 6.15 (*d*, 1H, *J* = 6 Hz), 7.00 (*m*, 1H). High resolution mass measurement: Found: 300.2092; Calcd. for $C_{20}H_{28}O_2$: 300.2089.

Hydrogenation of acetate 3: A solution of 3 (240 mg, 0.67 mmol) in methanol (30 ml) containing 10% palladium on charcoal catalyst (4 mg) was stirred under an atmosphere of hydrogen for 12 h. The catalyst was removed by filtration and the solvent evaporated to obtain a solid residue (234 mg). The solid obtained was applied to small preparative plates and these were eluted with light petroleum-ether (20:1). The band with highest R_f afforded the non-crystalline compound 9 (18 mg, 7.5% theoretical). *Spongian-16-one (9)*; $[\alpha]_D$ +14.2 (c, 0.09, $CHCl_3$); IR (KBr) 1775, 1440, 1370, 1180, 980, 745 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.78 (*s*, 6H), 0.80 (*s*, 3H), 0.82 (*s*, 3H), 2.30 (*m*, 2H), 4.05 (*t*, 2H, *J* = 3 Hz). Mass spectrum, *m/z* 304 (M^+), further peaks are found at *m/z* 289(14), 287(10), 260(49), 247 (32), 205(21), 191(100), 177. High resolution mass measurement: Found: 304.2412; Calcd. for $C_{20}H_{32}O_2$: 304.2402. The second band afforded the acetate 10 (110 mg, 46%) as a crystalline compound, mp 188-190°C; $[\alpha]_D$ +162 (c, 0.15, $CHCl_3$); IR (KBr) 1775, 1690, 1460, 1390, 1380, 1170, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.78 (*s*, 3H), 0.80 (*s*, 3H), 0.98 (*s*, 3H), 1.25 (*s*, 3H), 1.95 (*s*, 3H), 2.35 (*m*, 2H), 2.58 (*s*, 1H), 4.25 (*t*, 2H, *J* = 5 Hz), 5.45 (*m*, 1H). Mass spectrum, *m/z* 362 (M^+), further peaks are found at *m/z* 302(12), 287(41), 256(24), 218(15), 205(21), 191(100), 177(40), 152(38), 147(24), 136(32). Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.93; H, 9.39. Found: C, 72.90; H, 9.35. The third band afforded the acetate 11, which was isolated as a crystalline substance (40 mg, 16.7%); mp 141-142°C; $[\alpha]_D$ +181 (c, 0.16, $CHCl_3$). IR (KBr) 1775, 1690, 1470, 1390, 1380, 1170, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.80 (*s*, 6H), 1.04 (*s*, 3H), 1.45 (*s*, 3H), 1.98 (*s*, 3H), 2.40 (*m*, 2H), 4.80 (*m*, 2H), 5.75 (*m*, 1H). Mass spectrum, *m/z* 362 (M^+), further peaks are found at *m/z* 302(18), 287(48), 256(25), 218(24), 205(30), 191(100), 177(35), 152(41), 137(22). Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.93; H, 9.39. Found: C, 72.86; H, 9.27.

Treatment of 11β-acetoxyspongi-12-en-16-one (3) with diazomethane: Compound 3 (200 mg, 0.56 mmol) with diazomethane in ether was allowed to stand overnight at room temp. Usual work-up gave 12 in colourless crystals (187 mg, 84% theoretical); mp 175-177°C; $[\alpha]_D$-94 (c, 1.0, $CHCl_3$); IR (KBr) 1720, 1650, 1370, 1360, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.87 (*s*, 6H), 1.11 (*s*, 3H), 1.24 (*s*, 3H), 2.03 (*s*, 3H), 2.52 (*t*, 1H, *J* = 11 Hz), 4.11 (*dd*, 1H, *J* = 18, 11 Hz), 5.35 (*dd*, 1H, *J* = 18, 10 Hz), 4.56 (*d*, 1H, *J* = 10 Hz), 4.88 (*d*, 1H, *J* = 10, 6 Hz), 5.51 (*s*, 1H). ^{13}C-NMR ($CDCl_3$) δ40.6(C-1), 18.3(C-2)#, 41.5(C-3), 33.3(C-4)#, 57.5(C-5), 18.4(C-6)#, 43.7(C-7), 34.6(C-8)+, 55.2 (C-9), 38.2(C-10), 66.3(C-11), 37.9 (C-12), 91.6(C-13), 53.1(C-14), 67.8(C-15), 174.0 (C-16), 17.1(C-17), 33.7(C-18), 21.7(C-19), 17.8 (C-20), 82.5(C-21), 170.0 (C_{11}-OAc), 21.6 (C_{11}-OAc). Mass spectrum *m/z* 374 (M^+-N_2), further peaks are found at *m/z* 332(45), 314(59), 299(21), 271(48), 192(95), 177(37), 137(23). Treatment of acetate 12 (35 mg) in acetone with potassium carbonate (100 mg) gave after 2 h the corresponding alcohol 13. After usual work-up the product was chromatographed, elution with light petroleum-ethyl acetate (5:1) gave pure 13 (97 mg), which was crystallized from acetone to give long crystals: mp 165-167°C; $[\alpha]_D$-40.6 (c, 0.37, $CHCl_3$). IR (KBr) 3490, 1755, 1435, 1380, 1360, 1150, 1105, 960cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.84 (*s*, 6H), 1.26 (*s*, 3H), 1.30 (*s*,3H), 2.55 (*t*, 1H, *J* = 11 Hz), 3.99 (*dd*, 1H, *J* = 10, 0.5 Hz), 4.52 (*dd*, 1H, *J* = 10, 0.5 Hz), 4.55 (*s*, 1H), 4.82 (*dd*, 1H, *J* = 10, 6 Hz), 5.22 (*dd*, 1H, *J* = 18, 10 Hz). Mass spectrum *m/z* 332 (M^+-N_2), further peaks are found at *m/z* 317(58), 314(41), 192 (24), 177(49), 137(32).

Acetylation and reaction with diazomethane of the diastereoisomeric mixture of 7β,11β-dihydroxyspongi-12-en-16-one (5) and 7β,11α-dihydroxyspongi-12-en-16-one (6): A solution of the diastereoisomeric mixture of 5 and 6 (400 mg, 1.2 mmol) and acetic anhydride (2.0 ml) in pyridine (2.0 ml) was stirred at 25°C for 12 h. The reagents were evaporated under high vacuum. The residue was dissolved in dichloromethane and the solution filtered through a short column of silica gel to obtain a diastereoisomeric mixture of diacetates as an oil (387 mg), which without further purification was dissolved in ether and allowed to stand with diazomethane overnight at room temp. Usual work-up gave the mixture of 14 and 15 as a crystalline solid (328 mg). The residue obtained was applied to two large preparative plates and these were eluted four times with light petroleum-ether (20:1). The major

band afforded compound 14 (123 mg) as a crystalline solid, mp 170-172°C, $[\alpha]_D$-72.6 (c, 1.24, $CHCl_3$); IR (KBr) 1780, 1730, 1400, 1390, 1380, 1020, 745 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.81 (*s*, 3H), 0.86 (*s*, 3H), 1.09 (*s*, 3H), 1.37 (*s*, 3H), 2.04 (*s*, 3H), 2.12 (*s*, 3H), 2.53 (*t*, 1H, *J* = 10 Hz), 4.02 (*dd*, 1H, *J* = 18, 10 Hz), 4.50 (*dd*, 1H, *J* = 18, 10 Hz), 4.70 (*m*, 1H), 5.27 (*dd*, 1H, *J* = 18, 10 Hz), 5.54 (*bs*, 1H). ^{13}C-NMR ($CDCl_3$): δ40.3(C-1), 18.4(C-2), 41.1(C-3), 33.4(C-4), 51.4(C-5), 25.0(C-6), 84.5(C-7), 39.2 (C-8), 54.2(C-9), 38.3(C-10), 65.4(C-1), 37.8(C-12), 90.9(C-13), 53.0(C-14), 68.6(C-15), 173.2(C-16), 12.40(C-17), 33.4(C-18), 21.7(C-19), 17.4(C-20), 81.5(C-21), 169.7(C_{11}-OAc)#, 164.43(C_7-OAc)#, 21.5 C_{11}-OAc)+, 21.6(C_7-OAc)+ (chemical shifts marked with # and + are interchangeable). Mass spectrum *m/z* 432 (M^+-N_2), further peaks are found at *m/z* 390(52), 372(27), 330(69), 312(100), 297(43).

A minor band afforded compound 15 (93 mg) as an oil; $[\alpha]_D$-77 (c, 1.69, $CHCl_3$); IR (neat) 1780, 1730, 1410, 1390, 1380 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ0.82 (*s*, 3H), 1.12 (*s*, 3H), 0.87 (*s*, 6H), 2.07 (*s*, 3H), 2.13 (*s*, 3H), 2.55 (*t*, 1H, *J* = 10 Hz), 4.31 (*dd*, 1H, *J* = 18, 10 Hz), 4.50 (*dd*, 1H, *J* = 18, 10 Hz), 4.90 (*d*, 1H, *J* = 5 Hz), 5.32 (*dd*, 1H, *J* = 18, 12 Hz), 5.35 (*bd*, 1H, *J* = 10 Hz). ^{13}C-NMR ($CDCl_3$) δ40.5(C-1), 18.2(C-2), 40.2(C-3), 34.0(C-4), 52.1(C-5), 26.2(C-6), 84.6(C-7), 39.8(C-8), 53.7(C-9), 39.1(C-10), 62.4(C-11), 39.3(C-12), 90.8(C-13), 52.8(C-14), 67.8(C-15), 174.0(C-16), 12.45(C-17), 33.2(C-18), 22.1(C-19), 19.2(C-20), 81.3(C-21), 170.0(C_{11}-OAc)#, 164.5 (C_7-OAc)#, 21.3 C_{11}-OAc)+, 22.0 (C_7-OAc)+ (chemical shifts marked with # and + are interchangeable). Mass spectrum *m/z* 432 (M^+-N_2), further peaks are found at *m/z* 390(58), 372(21), 330(74), 312(100), 297(38).

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