New Polyketides from the Sponge *Plakortis* sp.

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Fractionation of the MeOH extract of the sponge *Plakortis* sp. collected around the Amirantes Islands yielded four novel polyketides (**4**−**7**). The structures have been established by a detailed highfield 2D NMR study.

Marine sponges of the genus *Plakortis* have proven to be a prolific source of polyketides formed from the combination of acetyl-, propionyl-, and/or butyryl-CoA units.[1] Examples include the α,*β*-unsaturated ester **1**,[2,3] the cyclic peroxide **2**,[4] and plakortide F (**3**),[5,6] which was recently found to enhance significantly Ca^{2+} uptake by the cardiac sarcoplasmic reticulum.[5]

In our screening for biologically active metabolites from sponges, an MeOH extract of the sponge *Plakortis* sp. (Plakinidae) collected at St. Francis Atoll (Amirantes Islands) was selected for fractionation and partitioned with hexane−EtOAc−2-propanol−H_2O (2:2:1:4). The organic layer exhibited toxicity toward *Artemia* larvae (LD_{50} 20 mg/L) and was chromatographed on Si gel to obtain six fractions. Further purification of these fractions led to the isolation of four novel polyketides (**4**−**7**), the structure determinations of which are presented in this paper.

Compound **4** had the molecular formula $C_{18}H_{30}O_3$ (by HREIMS). The IR spectrum contained a complex group of bands at 1716, 1689, 1640, and 1626 cm^{-1} and a ultraviolet absorption band at 282 nm (ϵ 10 650) that were reminiscent of the corresponding spectral bands of the unsaturated ester **1**, isolated by Stierle and Faulkner from *Plakortis halichondrioides*.[2] The ^{13}C NMR spectrum of **4** contained four olefinic carbon signals at *δ* 83.8 (CH), 166.9 (C), 140.1 (C), and 139.8 (CH) that were similar to the C-2 (*δ* 84.4), C-3 (*δ* 166.0), C-4 (*δ* 139.7), and C-5 (*δ* 139.9) signals in ester **1**. The ^{13}C NMR spectrum of **4** also contained signals for a carbomethoxy group (*δ* 171.7 and 50.5), a fully substituted oxygen-bearing carbon at *δ* 98.1, and 11 aliphatic carbon atoms (four CH_3, six CH_2, and one CH). The ^1H NMR spectrum contained five methyl signals [*δ* 3.66 (s), 1.15 (t), 0.85(d), 0.84 (t), and 0.78 (t)] and two singlets of 1H each due to olefinic protons (*δ* 6.19 and 4.80). Comparison of these spectroscopic data with those described for **1** together with a complete 2D NMR study at 600 MHz (COSY, HMQC, and HMBC) allowed the assignment of all the NMR signals. The HMBC spectrum was especially useful in establishing structure **4**, as it showed heteronuclear couplings between C-4 and H_2C-13 and H_3C-14 as well as between C-6 and H_2C-15 and H_3C-16. Moreover, clear correlations were observed between C-7 and H_2C-15 and H_3C-17 as well as between C-9 and H_3C-17 and HC-8. The HREIMS of compound **4** contained two intense fragment peaks at *m*/*z* 265.1804 (60%, $C_{16}H_{25}O_3$) and 195.1021 (100%, $C_{11}H_{15}O_3$), resulting from the loss of an ethyl and a C_7H_{15} radical from the molecular ion, respectively. This further indicated that **1** and **4** differed from each other only by the structure of the chain at C-6. The stereochemistry of the 2,3-double bond in **4** was assigned as (*Z*) on the basis of the chemical shift of H-2 (*δ* 4.80), which is similar to that of the corresponding proton in **1**.

MS measurements determined the molecular formula $C_{18}H_{32}O_4$ for compound **5**. The IR spectrum had a strong ester band at 1748 cm^{-1} and did not contain other carbonyl or hydroxyl bands. The ^1H NMR contained a 3H singlet at *δ* 3.70 and ^{13}C NMR signals at *δ* 171.6 (s) and 51.9 (t) attributable to a carbomethoxy group. The ^{13}C NMR spectrum also contained two olefinic carbon signals [*δ* 137.6 (s) and 125.2 (d)], two signals at *δ* 83.4 (s) and 76.5 (d) assigned to carbon atoms bearing oxygen, and 12 other aliphatic carbon signals (four CH_3, seven CH_2, and one CH). Moreover, part of the ^1H and ^{13}C NMR signals of compound **5** closely resembled those attributable to the cyclic peroxide moiety of compound **2**[4] (C-1 to C-6 and C-13 to C-16), while the remaining signals resembled those attributable to the aliphatic chain of compound **4** (C-7 to C-12 and C-17). These data, together with a complete 2D NMR study at 600 MHz (COSY, HMQC, and HMBC), allowed the assignment of all the NMR signals. Diagnostic HMBC correlations are represented in Figure 1. Difference NOE experiments did not give conclusive evidence for the relative stereochemistry of the substituents of the peroxide ring.

MS measurements determined the molecular formula $C_{19}H_{34}O_4$ for compound **6**. The ^1H NMR spectrum was identical with that of **5** except that the methyl signal at *δ* 3.70 was replaced by a 2H quadruplet at *δ* 4.08 coupled to a 3H triplet at *δ* 1.20 attributable to a carboethoxy group. Thus, compound **6** is the ethyl ester corresponding to **5**. Further arguments for the structural relationship between esters **5** and **6** were obtained by reduction of both esters with $LiAlH_4$ at room temperature to obtain the same alcohol **9**, which was

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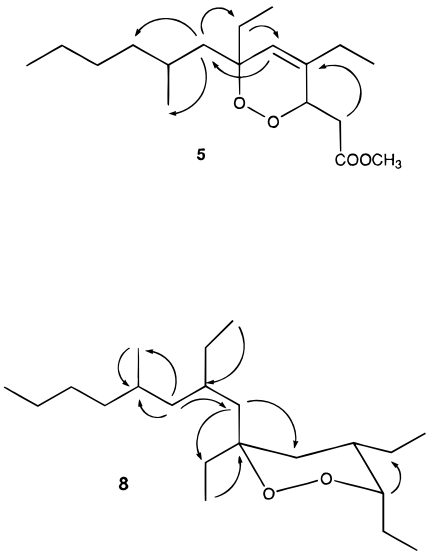


Figure 1. Diagnostic HMBC correlations for compounds **5** and **8**.

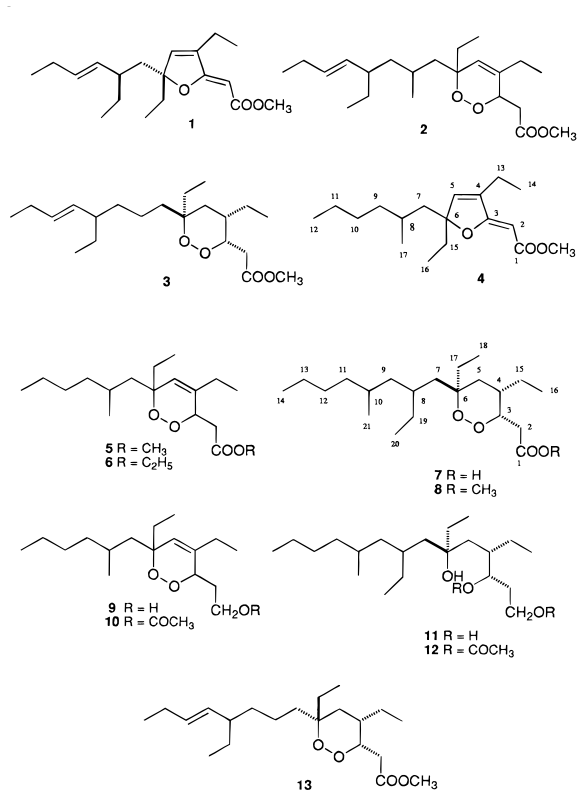
acetylated with Ac_2O in pyridine to yield a monoacetate (**10**). Under these conditions the peroxide function was not reduced.

The IR spectrum of compound **7** displayed a strong band at 1713 cm^{-1} and a very broad absorption in the 3300−2450 cm^{-1} region, which indicated the presence of a carboxylic acid group. The ^1H NMR spectrum at 600 MHz in $CDCl_3$ showed no olefinic signals and only one deshielded multiplet at δ 4.41 attributable to a proton attached to a carbon atom bearing oxygen. In addition, there were a number of methylene multiplets between δ 2.95 and 0.90 and five methyl signals (four triplets and one doublet) in the upfield region. The latter were overlapping in the spectrum obtained in $CDCl_3$ but were well separated in the spectrum obtained in C_6D_6. The ^{13}C NMR spectrum displayed 21 carbon signals (five CH_3, ten CH_2, four CH, and two C). The quaternary carbons were assigned to a carboxyl carbon (δ 177) and to an oxygenated carbon (δ 83.2). A signal at δ 78.4 confirmed the presence of a methine bearing an oxygen. Inasmuch as no significant mass measurements could be obtained on the acid **7** because of decomposition, it was methylated by treatment with ethereal CH_2N_2 to give ester **8**, the molecular formula of which was deduced as $C_{22}H_{42}O_4$ from ^{13}C NMR and mass measurements (SIMSMS and HREIMS). The IR spectrum contained a strong band at 1743 cm^{-1}, and the ^1H NMR spectrum showed a 3H singlet at δ 3.63. COSY and HMQC experiments, together with the data presented above, allowed construction and assignments of several partial structures that could then be interconnected using long-range HMBC data. Important correlations are represented in Figure 1. The NMR data of the atoms constituting the cyclic moiety of the molecule (Table 1) are very similar to those of the corresponding atoms of plakortide F[5] (**3**), indicating that the two compounds differed from each other only by the structure of the hydrocarbon chain. Plakortide F is a cyclic peroxide that had been isolated from Jamaican specimens of *Plakortis halichondrioides*, together with plakortide G (**13**), its epimer at C-6.[5] Although Patil et al.[5] claimed that plakortide F was a novel natural compound, it had already been isolated as the corresponding acid by Rudi and Kashman from another sample of the same sponge.[6] The stereochemistry at C-6 had not been determined for the compound isolated by the latter group. But comparison of the NMR data (Table 1) of this compound with those of plakortides F and G[5] clearly indicates that it is identical to plakortide F rather than to plakortide G. In particular, this conclusion is consistent with the upfield shifts of C-7 in **3** and of C-17 in **13** (Table 1). It has also to be noted that the assignments of C-5 and C-15 in Rudi and Kashman should be reversed.[6]

Table 1. Comparison of the ^{13}C NMR Data of **3**, **8**, and **13**[a]

carbon no.	**3**[5]	**13**[5]	**3**[6]	**8**
1	172.2	172.2	173.6	172.0
2	31.3	31.3	31.1	31.9
3	78.6	78.6	78.3	79.1
4	34.5	34.4	34.3	35.2
5	32.7	33.1	32.6	34.0
6	82.4	82.5	82.6	83.5
7	31.9	35.5	31.9	36.5
15	25.2	25.2	25.1	26.5
16	11.0	11.0	11.0	11.6
17	29.7	24.7	29.6	30.6
18	7.1	7.5	7.1	7.7

[a] $CDCl_3$, δ from TMS.



The presence in **8** of a peroxide group was confirmed by $LiAlH_4$ reduction that yielded triol **11**, which was acetylated to diacetate **12**. The relative configuration of ester **8** about the peroxide ring was established by NOE difference experiments and comparison of its ^{13}C NMR data with those of **3** and **13** (Table 1). Saturation of the axial H-4 signal enhanced the equatorial H-3 and the axial H_2-7 methylene signals. Thus H-4 must be on the same face of the peroxide ring as is H-3 and the side chain containing H-7. There is a good correlation between the chemical shifts of the carbon atoms of

compounds **8** and **3** listed in Table 1, except for C-7. This difference of 4.6 ppm can be attributed to the deshielding effect of the ethyl group at C-8 in **8**. The extent of the effect is comparable to that observed (Δ 4.7 ppm) when passing from hexane (C-3 at δ 31.8) to 3-methylheptane (C-4 at δ 36.5).[7] The ester **8** and, consequently, the natural compound **7** have the same relative configuration as plakortide F (**3**) at C-3, C-4, and C-6. The configuration at the two stereogenic carbon atoms in the side chain could not be determined.

Of the four isolated compounds, the dihydrofuran **4** (LD_{50} 7 mg/L) and the acid **7** (LD_{50} 15 mg/L) exhibited toxicity toward *Artemia* larvae. Compound **5** showed low toxicity (LD_{50} 70 mg/L), and compound **6** showed no activity at all (LD_{50} >100 mg/L).

Several sponges of the genus *Plakortis* have already been chemically analyzed.[1] This has resulted in the isolation and identification of a variety of oxidized polyketides.[1] Biogenetically these compounds may be derived from either branched or unbranched[8] long-chain carboxylic acids that are generally oxidized at the carbon atoms close to the carboxyl end. Cyclic peroxides,[5,9] cyclic ethers,[3] or γ-lactones[10,11] are frequent variations of the oxidation pattern. Such a diversity may reflect the ability of these sponges to generate from a common basic biosynthetic pathway a great diversity of secondary metabolites to adjust their defense mechanism to the changing environment. Our finding of four new *Plakortis*-type polyketides in a sample of *Plakortis* sp. from the Amirante Islands strenghtens these views. They also reinforce the idea that these polyketides are valuable taxonomic markers for the genus.

Experimental Section

General Experimental Procedures. HREIMS were performed on a Fisons VG Autospec instrument and GC−EIMS and GC−CIMS analyses on a Finnigan ITD 800 apparatus coupled to a Tracor gas chromatograph equipped with a capillary column (OV1, 25 m, i.d. 0.25 mm, N_2, temperature program: 100 °C to 295 °C at 10 °C/min, collision gas: isobutane). The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument and TMS as internal standard. Some ^{1}H NMR spectra were recorded at 250 MHz on a Bruker WM 250 spectrometer. The IR spectra were obtained on a Bruker IFS 25 instrument as a film on a NaCl disk and the UV−vis spectra on a Philips PU 8700 spectrophotometer in hexane. The optical rotations were measured on a Perkin−Elmer 141 polarimeter (Na-vapor lamp) in a 10-cm cell at room temperature. TLC analyses were performed on 0.25-mm Polygram Si gel SILG/UV_{254} precoated plates (Macherey Nagel). Column chromatographies were performed over Si gel (MN Kieselgel 0.04−0.063 mm), using the flash technique.

Biological Material. The sponge belongs to a novel yet undescribed species of the genus *Plakortis* (class Demospongiae, order Homosclerophorida, family Plakinidae). It was collected in January 1992, on the west rim of St. Francis Atoll, Amirante Islands, at a depth of 10 m. It was a grayish black lobate mass, with small, round, flush oscules on the lobes. The surface was smooth and the consistency firm. The sides and the interior were of a lighter, beige color. The skeleton formed a confused mass of spicules arranged without order around the canals, with a distinctly denser peripheral spicule mass. The spicules include diods in a wide size range, 40−330 × 1−15 μm, rare triods with rays 65−80 μm and cladome 120 μm, and peculiarly twisted and knobbed microdiods of 4−15 × 0.5 μm. The latter spicules have been described from *Plakortis lita*, but that is a light-colored species with the consistency of liver, unlike the present material. From a survey of all extant *Plakortis* species, it is clear that the upper size range of the diods (330 μm) exceeds all diod sizes recorded from previously described species of *Plakortis*. We conclude that this sponge is new to science, and it will be described in due course. A voucher fragment is kept in the collections of the Zoological Museum of the University of Amsterdam under the register no. ZMA POR 10336.

Extraction, Isolation, and Spectral Properties. Samples of *Plakortis* sp (25 g dry wt) stored in EtOH were exhaustively extracted with CH_2Cl_2−MeOH 50:50. The extracts were evaporated in vacuo and the residue (8.2 g) partitioned with hexane−EtOAc−2-propanol−H_2O 2:2:1:4. The organic phase was evaporated to obtain a gum (2.5 g) that exhibited toxicity toward *Artemia* larvae (LD_{50} 20 mg/L). Typically, the active extract (505 mg) was flash chromatographed over a Si gel column with hexane−Me_2CO (100:0 to 50:50) to afford six fractions (F1 to F6). Fraction F1 (223 mg) was flash chromatographed over a Si gel column with toluene−EtOAc (100:0 to 50:50). This gave compound **4** (35 mg). Then, the remaining fractions were combined and flash chromatographed over Si gel columns with hexane−toluene−EtOAc (50:50:0 to 0:50:50) and hexane−Me_2CO (98:2) successively to afford compound **5** (57 mg) and **6** (15 mg). Fraction F6 (112 mg) was flash chromatographed over a Si gel column using hexane−Me_2CO (100:0 to 0:100) as eluent. This afforded acid **7** (13 mg), which was esterified by treatment with ethereal CH_2N_2. The resulting methyl ester **8** was further purified by flash chromatography over a Si gel column using hexane−Me_2CO 9:1 as eluent.

Compound 4: amorphous solid; $[\alpha]^{20}_D$ −91° (hexane; *c* 0.1); IR (film) 1716, 1689, 1640, 1626, 1162 cm^{-1}; UV (hexane) λ_{max} 282 (10 650); NMR [600 MHz; $CDCl_3$; COSY, HMQC, HMBC] (δ ^{1}H, *J* in Hz; δ ^{13}C) C-3 (171.7), C-1 (166.9), C-4 (140.1), HC-5 (6.19 s; 139.8), C-6 (98.1), HC-2 (4.80 s; 83.8), H_3C-18 (3.66 s; 50.5), H_2C-7 (1.69 m; 44.6), H_2C-9 (1.10 and 1.22 m; 38.1), H_2C-15 (1.70 m and 1.82 dq, 14, 7; 31.9), H_2C-10 (1.20 m; 29.2), HC-8 (1.30 m; 28.7), H_2C-11 (1.20 m; 22.8), H_3C-17 (0.85 d, 7; 21.2), H_2C-13 (2.16 q, 7; 18.6), H_3C-12 (0.84 t, 7; 14.1), H_3C-14 (1.15 t, 7; 12.1), H_3C-16 (0.78 t, 7; 8.0); HREIMS *m*/*z* 294.2195 [M^+] (12), 265.1804 (60, calcd for $C_{16}H_{25}O_3$, 265.1803), 195.1021 (100, calcd for $C_{11}H_{15}O_3$, 195.1021), 181.0865 (23, calcd for $C_{10}H_{13}O_3$, 181.0864); HREIMS *m*/*z* 294.2195 (calcd for $C_{18}H_{30}O_3$, 294.2195).

Compound 5: amorphous solid; $[\alpha]^{20}_D$ −20° (hexane; *c* 0.1); IR (film) 1748 cm^{-1}; NMR [600 MHz; $CDCl_3$; COSY, HMQC, HMBC] (δ ^{1}H, *J* in Hz; δ ^{13}C) C-1 (171.6), C-4 (137.6), HC-5 (5.48 brs; 125.2), C-6 (83.4), HC-3 (4.59 brd, 8; 76.5), H_3C-18 (3.70 s; 51.9), H_2C-7 (1.35 and 1.43 m; 42.4), H_2C-9 (1.04 and 1.24 m; 38.3), H_2C-2 (2.55 dd, 2, 16 and 2.85 dd, 9, 16; 37.2), H_2C-15 (1.62

dq, 14, 7 and 1.73 dq, 14, 7; 30.9), H_2C-10 (1.23 m; 29.3), HC-8 (1.45 m; 28.4), H_2C-13 (2.00 m; 24.9), H_2C-11 (1.23 m; 22.9), H_3C-17 (0.85 d, 7; 22.0), H_3C-12 (0.85 t, 7; 14.1), H_3C-14 (1.05 t, 7; 11.8), H_3C-16 (0.85 t, 7; 8.2); GC−EIMS (OV1, t_R 15.1 min) no molecular ion, fragment ions at *m/z* 294 (22, $M^+ - H_2O$), 265 (100, $M^+ - H_2O - C_2H_5$), 195 (95, $M^+ - H_2O - C_7H_{15}$), 181 (49); GC−CIMS *m/z* 313 $(M + H)^+$; positive SIMSMS *m/z* 313 $(M + H)^+$; negative SIMSMS *m/z* 311 $(M - H)^-$.

Compound 6: amorphous solid; $[\alpha]^{20}_D$ − 25° (hexane; *c* 0.1); IR (film) 1738, 1154 cm^{-1}; ^1H NMR [250 MHz; $CDCl_3$] δ 5.48 (1H s), 4.53 (1H d, 8), 4.08 (2H q, 7), 2.78 (1H dd, 9, 16), 2.49 (1H dd, 2, 16), 1.95 (2H m), 1.20 (3H t, 7), 1.01 (3H t, 7), 0.80 (9H m); GC−EIMS (OV1, t_R 15.5 min) no molecular ion, fragment ions at *m/z* 308 (18, $M^+ - H_2O$), 279 (100, $M^+ - H_2O - C_2H_5$), 209 (77, $M^+ - H_2O - C_7H_{15}$), 195 (35), 137 (50); GC−CIMS *m/z* 327 $(M + H)^+$; HREIMS no molecular ion, fragment ions at *m/z* 279.1951 (6, calcd for $C_{17}H_{27}O_3$, 279.1960), 209.1183 (6, calcd for $C_{12}H_{17}O_3$, 209.1178).

Compound 7: amorphous solid; $[\alpha]^{20}_D$ − 168° (CH_2Cl_2; *c* 1.5); IR (film) 3300−2450, 1713 cm^{-1}; ^1H NMR [600 MHz; $CDCl_3$] δ 4.41 (1H m), 2.95 (1H dd, 9,16), 2.35 (1H dd, 3,16), 2.12 (1H m), 1.91 (1H dd, 8, 15), 1.54 (1H m), 1.48 (1H, m), 1.45−0.90 (18H), 0.86 (3H t, 7), 0.81 (3H), 0.78 (9H); ^{13}C NMR [150.87 MHz; $CDCl_3$] δ 177.0 (C), 83.2 (C), 78.4 (CH), 42.8 (CH_2), 37.0 (CH_2), 35.7 (CH_2), 34.5 (CH), 33.5 (CH_2), 31.6 (CH), 31.3 (CH_2), 30.2 (CH), 29.7 (CH_2), 29.3 (CH_2), 26.5 (CH_2), 25.2 (CH_2), 23.0 (CH_2), 20.1 (CH_3), 14.1 (CH_3), 11.0 (CH_3), 10.2 (CH_3), 7.2 (CH_3); ^1H NMR [600 MHz; C_6D_6] δ 4.48 (1H m), 3.00 (1H dd, 9, 16), 2.15 (1H dd, 3, 16), 2.13 (1H, m), 1.92 (1H, m), 1.84 (1H, m), 1.65 (1H, m), 1.50 (3H, m), 1.40−1.02 (13H), 0.99 (3H t, 7), 0.90 (6H t + d, 7), 0.80 (2H m), 0.75 (3H t, 7), 0.64 (3H t, 7); ^{13}C NMR [150.87 MHz; C_6D_6] δ 177.7 (C), 82.9 (C), 78.7 (CH), 43.5 (CH_2), 37.7 (CH_2), 36.0 (CH_2), 34.9 (CH), 34.1 (CH_2), 32.2 (CH), 31.9 (CH_2), 30.7 (CH), 30.0 (CH_2), 29.8 (CH_2), 26.9 (CH_2), 25.4 (CH_2), 23.6 (CH_2), 20.5 (CH_3), 14.5 (CH_3), 11.1 (CH_3), 10.6 (CH_3), 7.5 (CH_3).

Compound 8: amorphous solid; IR (film) 1743, 1159 cm^{-1}; NMR [600 MHz; $CDCl_3$; COSY, HMQC] (δ ^1H, *J* in Hz; δ ^{13}C) C-1 (172.0), C-6 (83.5), HC-3 (4.43 ddd, 9.5, 5.5, 4; 79.1), H_3C-22 (3.63 s; 52.4), H_2C-9 (1.05 and 1.00 m; 43.4), H_2C-11 (1.23 and 1.10 m; 37.5), H_2C-7 (1.90 dd, 15, 8 and 1.25 dd, 15, 3.5; 36.5), HC-4 (2.10 m; 35.2), H_2C-5 (1.40 and 1.20 m; 34.0), HC-8 (1.41 m; 32.2), H_2C-2 (2.94 dd, 16, 9.5 and 2.30 dd, 16, 4; 31.9), HC-10 (1.32 m; 30.7), H_2C-17 (1.47 and 1.20 m; 30.6), H_2C-12 (1.20 m; 30.3), H_2C-19 (1.52 and 1.18 m; 27.0), H_2C-15 (1.15 and 1.05 m; 26.5), H_2C-13 (1.25 m; 23.6), H_3C-21 (0.76 d, 7; 20.7), H_3C-14 (0.81 t, 7; 14.7), H_3C-16 (0.84 t, 7; 11.6), H_3C-20 (0.77 t, 7; 10.7), H_3C-18 (0.86 t, 7; 7.7); NMR [600 MHz; C_6D_6; COSY, HMQC, HMBC, NOE] (δ ^1H, *J* in Hz; δ ^{13}C) C-1 (171.7), C-6 (82.7), HC-3 (4.60 ddd, 9, 4, 4; 78.9), H_3C-22 (3.34 s; 51.3), H_2C-9 (1.20 and 1.15 m; 43.3), H_2C-11 (1.31 and 1.12 m; 37.5), H_2C-7 (2.17 dd, 15, 7 and 1.24 m; 35.9), HC-4 (1.96 m; 34.8), H_2C-5 (1.22 and 1.02 m; 34.1), HC-8 (1.66 m; 32.0), H_2C-2 (3.05 dd, 16, 9.5 and 2.16 dd, 16, 4; 31.7), HC-10 (1.49 m; 30.6), H_2C-17 (1.51 and 1.30 m; 29.9), H_2C-12 (1.30 m; 29.7), H_2C-19 (1.86 and 1.52 m; 26.8), H_2C-15 (0.85 and 0.78 m; 25.3), H_2C-13 (1.27 m; 23.4), H_3C-21 (0.90 d, 6.5; 20.3), H_3C-14 (0.90 t, 6.5; 14.4), H_3C-16 (0.66 t, 7; 11.0), H_3C-20 (0.99 t, 7.5; 10.5), H_3C-18 (0.77 t, 7.5; 7.4); positive SIMSMS *m/z* 371 $(M + H)^+$; negative SIMSMS *m/z* 369 $(M - H)^-$; HREIMS no molecular ion, fragment ions at *m/z* 352.2986 (0.3, calcd for $C_{22}H_{40}O_3$, 352.2977), 341.2704 (0.7, calcd for $C_{20}H_{37}O_4$, 341.2692), 338.2826 (0.4, calcd for $C_{21}H_{38}O_3$, 338.2821), 323.2588 (3, calcd for $C_{20}H_{35}O_3$, 323.2586), 309.2438 (5, calc for $C_{19}H_{33}O_3$, 309.2429), 249.2227 (5, calcd for $C_{17}H_{29}O_6$, 249.2218), 235.2070 (4, calcd for $C_{16}H_{27}O_6$, 235.2062), 215.1293 (8, calcd for $C_{11}H_{19}O_4$, 215.1283), 197.1185 (6, calcd for $C_{11}H_{17}O_3$, 197.1178), 183.1749 (7, calcd for $C_{12}H_{23}O_6$, 183.1749), 141.0923 (100, calcd for $C_8H_{13}O_2$, 141.0915).

$LiAlH_4$ Reductions of 5 and 6 and Acetylation of 9. Reduction of compound **5** (10 mg) with $LiAlH_4$ (30 mg) was performed in anhydrous THF (5 mL) at room temperature. The progress of the reduction was monitored by TLC. After completion of the reaction, H_2O (5 mL) was added and the solution extracted with CH_2Cl_2. Evaporation in vacuo of the organic phase yielded crude alcohol **9** that was purified by flash chromatography over a Si gel column using hexane−Me_2CO 9:1 as eluent. Reduction of compound **6** using the same conditions furnished an alcohol identical to **9** (R_f, ^1H NMR). Compound **9**: amorphous solid; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.47 (1H brs), 4.31 (1H brd, 10), 3.84 (2H m), 1.07 (3H t, 7); EIMS no molecular ion, fragment ions at *m/z* 269 (10), 255 (68), 239 (24), 237 (22), 185 (55), 153 (52), 139 (63), 125 (100), 111 (84). The acetylation of **9** was performed at room temperature during 24 h, using a 1:1 mixture of Ac_2O and pyridine (2 mL). After usual work up the monoacetate **10** was isolated and purified by flash chromatography over a Si gel column using hexane−Me_2CO 9:1 as eluent. Compound **10**: amorphous solid; IR (film) 1743, 1238 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.40 (1H brs), 4.18 (3H m), 1.98 (3H s), 1.03 (3H t, 7).

$LiAlH_4$ Reduction of 7 and Acetylation of 11. Reduction of compound **7** (10 mg) with $LiAlH_4$ (5 mg) in refluxing anhydrous THF furnished after usual workup a triol (**11**) that was purified by flash chromatography over Si gel using hexane−Me_2CO 75:25 as eluent. Then the triol was acetylated with a 1:1 mixture of pyridine−Ac_2O anhydride (2 mL) at room temperature during 24 h. After usual work up the diacetate **12** was isolated and purified by flash chromatography over a silica gel column using hexane−Me_2CO 98:2 as eluent. Compound **12**: amorphous solid; IR (film) 3541, 1744 and 1232 cm^{-1}; EIMS no molecular ion, fragment ions at *m/z* 399 (tr), 353 (1), 339 (43), 325 (11), 279 (5), 261 (3), 213 (100), 156 (46), 153 (52); ^1H NMR ($CDCl_3$, 250 MHz) δ 5.21 (1H m), 4.06 (2H m), 2.02 (3H s), 2.01 (3H s).

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