Chemical Studies of Egyptian Opisthobranchs: Spongian Diterpenoids from *Glossodoris atromarginata*

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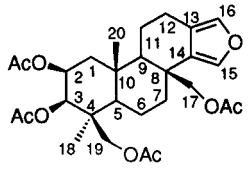
Five new spongian diterpenoids **1**−**5** have been isolated from the mollusk *Glossodoris atromarginata* along with four known related metabolites (**6**−**9**). The structures were solved by extensive spectroscopic analysis and chemical correlation. The absolute stereochemistry of compound **2** was determined by Mosher's method. A dietary origin is inferred for all these molecules on the basis of their structural analogies with known sponge metabolites, but their transfer from the digestive glands to the dorsal skin suggests a defensive role to thwart predation.

Mollusks belonging to the order Nudibranchia have developed a series of effective mechanisms,[1−4] including the use of chemicals, to defend themselves from predators, and the chemical defensive strategy of some genera belonging to the family Chromodorididae (*Cadlina*, *Hypselodoris*, *Chromodoris*, and *Glossodoris*) has been clarified.[5,6] Such organisms, which graze upon sponges, are able to sequester the secondary metabolites from their prey and transfer them to special organs of the mantle, where they are used to thwart predation.[7] Recent studies[8] of Mediterranean nudibranchs have suggested that dorids, belonging to the genera *Cadlina*, *Hypselodoris*, and *Chromodoris*, have highly specialized diets and, consequently, the chemical nature of their defensive allomones varies from one genus to another according to the metabolite pattern of preyed organisms. Up until now, only a few studies[9−11] have been performed on mollusks belonging to the genus *Glossodoris*. It is worth noting that the taxonomy of this genus is quite complex, and for a long time *Glossodoris* was considered synonymous with *Chromodoris* or *Hypselodoris*. Consequently, some published papers, which speak of *Glossodoris*, could concern the other two genera of the family Chromodorididae.[12]

This paper reports the isolation and chemical characterization of secondary metabolites from a population of *Glossodoris atromarginata* Cuvier, 1804 (Chromodorididae) collected off Hurghada (Red Sea). All the identified metabolites (**1**−**9**) possess a spongian skeleton. The structures of the new compounds **1**−**5** were assigned by analysis of their spectroscopic properties and were confirmed by chemical transformation into the known compounds epispongiatriol triacetate (**6**) and spongiatriol triacetate (**10**), previously isolated from an Australian sponge of the genus *Spongia*.[13]



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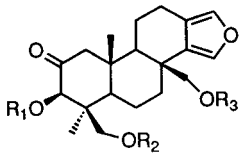
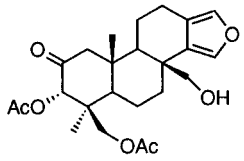


2 R_1=H; R_2=R_3= Ac
3 R_1=R_2=Ac; R_3=H
5 R_1=Ac; R_2=R_3=H
6 R_1=R_2=R_3=Ac
9 R_1=R_2=R_3=H



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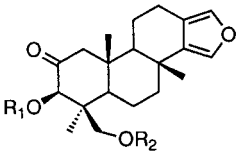

7 R_1=Ac; R_2=H
8 R_1=R_2=H

Results and Discussion

The animals, collected in December 1994, were dissected in order to separate the inner organs (digestive duct, reproductive system, etc.) from the mantle. Comparison of the Et_2O-soluble material from both these sections and mucous secretion by TLC showed several Ehrlich's reagent-positive compounds. Column chromatography of recombined extracts achieved separation into four main fractions. Further purification by reversed phase HPLC gave nine pure spongian compounds (**1**−**9**) together with several other furanoditerpenoids present either in unresolved mixtures or in amounts too small to be characterized.

Compound **1** is closely related to the metabolite (**11**) isolated from an Australian sponge,[14] but it differs from the latter compound in being completely acetylated, as suggested by the HREIMS parent ion at m/z 518.2520 corresponding to the molecular formula $C_{28}H_{38}O_9$. Its ^{1}H- and ^{13}C-NMR spectra showed only two tertiary methyl groups (δ 1.23, H_3-18; δ 1.08, H_3-20), typical signals of a β,β'-disubstituted furan ring (δ 7.10 and 7.09), and two downfield methylene resonances at δ 2.81 and 2.52, attributable to the protons at C-12, which in turn were coupled with two protons (H_2-11) resonating at δ 1.73. Four NMR sharp singlets at δ 2.09, 2.08, 2.02, and 2.00, each integrating for three protons, and two bands at 1746 and 1741 cm^{-1} in the IR spectrum of **1** supported the presence of four acetyl groups. Two isolated AB systems at δ 4.49, 4.40 (J = 10.7 Hz, H_2-

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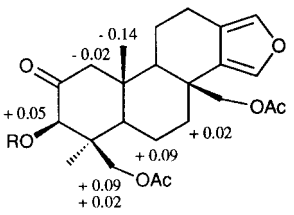


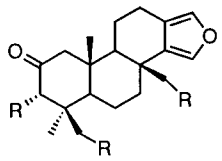
R = (*S*)-MTPA, **2a**
R = (*R*)-MTPA, **2b**

Figure 1. ^{1}H chemical shift difference ($\Delta\delta = \delta_S - \delta_R$) of MTPA esters of **2** (values given in hertz).

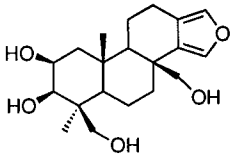
19) and δ 4.40, 4.10 (J = 10.7 Hz, H_2-17) suggested that two acetyl moieties were present as acetoxymethyls (65.7 and 64.3 ppm in the ^{13}C-NMR spectrum), whereas the remaining two acetyl groups were assigned to two vicinal carbinol centers, showing mutually coupled resonances at δ 5.32 (H-2) and δ 4.77 (H-3). Accordingly, the C-1 protons (δ 2.12 and 1.44) occurred at higher field than in **6** as a result of the absence of the C-2 carbonyl moiety. COSY experiments indicated a further coupling of the signal at δ 5.32 with the methylene protons (H_2-1) at δ 2.12 and 1.44, supporting the evidence of an isolated $CH_2CH(OAc)CH(OAc)$ system between two quaternary carbons. These results were only compatible with the structure **1**. Assuming a chair conformation of ring A, the small coupling constants between H_2-1 and H-2 strongly suggested an equatorial orientation for H-2. The β-configuration for the acetoxy group at C-2 was also supported by the highfield resonance of H_3-20 (δ 1.08) in agreement with a 1,3 diaxial relationship between the substituents at C-2 and C-10.[15] Moreover, the highfield value of H-3 (δ 4.77) and the ^{13}C resonances at 68.8 ppm (C-2) and 76.7 (C-3), which accorded with the presence of the *cis* diacetoxy function,[14,15] suggested an equatorial orientation for the group in 3.

The identity of the products **2**−**5** was established by comparison with the spectral data reported in the literature.[13,16] In particular, **2**, **3**, and **5** are derived formally from epispongiatriol (**9**), whereas compound **4** is a diacetate derivative of spongiatriol (**12**).

Compound **2**, $C_{24}H_{32}O_7$ by HREIMS, showed hydroxyl (3452 cm^{-1}), ester (1738 cm^{-1}), and keto (1709 cm^{-1}) absorptions in its IR spectrum. The ^{1}H- and ^{13}C-NMR spectra revealed the signals of a β,β'-disubstituted furan ring (δ 7.14 and 7.11), two angular methyls (δ 0.96, H_3-20; δ 1.32, H_3-18), and two acetyl (δ 2.06 and 2.01) groups. Two low-field AB systems (δ 4.02 and 3.90; δ 4.38 and 4.07), correlated to ^{13}C resonances at 64.7 and 63.9 ppm, were assigned to the acetoxymethyl functions at C-19 and C-17, respectively. A ^{13}C-NMR signal at 209.1 ppm, together with the isolated doublets at δ 2.77 and 2.15 (H_2-1), were consistent with an A-ring substitution pattern similar to that of epispongiatriol (**9**),[13] where the hydroxy group is β. Compound **2** is epimeric at C-3 with **13**, previously isolated from a population of *Glossodoris atromarginata* from Sri Lanka.[11] The *R* absolute configuration of the chiral carbinol center of **2** was determined by Mosher's method[17−19] (Figure 1). The deduced stereochemistry is in agreement with the previously proposed configuration of epispongiatriol (**9**), inferred indirectly by CD and ORD measurements on the epimeric spongiatriol (**12**).[13]



R = OAc; **10**
R = OH; **12**



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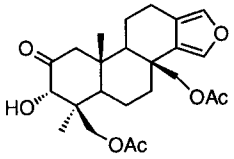

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Compounds **3** and **4**, which were isolated in very low amounts, have similar spectroscopic data: Both compounds showed characteristics for two acetyl and two angular methyl groups, as well as for the presence of one hydroxymethyl and one acetoxymethyl moiety (see Table 1). All major resonances for **3** and **4** were very similar (see Table 1), with the exception that the chemical shift value of H-3 occurred at δ 4.98 in **3** and at δ 5.47 in **4**. As reported in the literature,[16] these data suggested that the C-3 acetoxy group is β in **3** and α in **4**. Finally, the substitution pattern of **3** and **4** was inferred by chemical conversion of the natural compounds into epispongiatriol triacetate (**6**) and spongiatriol triacetate (**10**), respectively. Acetylation of **4** induced the downfield shifts of the signals at δ 3.81 and δ 3.49, whereas the AB system at δ 4.03 and δ 3.97 did not experience any change. Comparison of these data with those of spongiatriol triacetate (**10**) (δ 4.36 and 4.13, H_2-17; δ 4.02 and δ 3.97, H_2-19)[13] led to assignment of the resonances at δ 3.81 and δ 3.49 to H_2-17, and those at δ 4.03 and δ 3.97 to H_2-19. In the same way, the mutually coupled signals at δ 3.77 and 3.48, and at δ 4.11 and 4.07 of compound **3** were attributable to H_2-17 and H_2-19, respectively.

Product **5** exhibited an HREIMS parent ion at m/z 390.2051 corresponding to the molecular formula $C_{22}H_{30}O_6$. The mass spectrum showed two main fragments corresponding to the successive loss of MeOH (m/z 359) and HOAc (m/z 299). IR absorption supported the presence of hydroxy (3442 cm^{-1}), ester (1758 cm^{-1}), and keto (1728 cm^{-1}) groups, while the ^{1}H-NMR spectrum was consistent with the presence of two tertiary methyl groups (δ 0.90, H_3-20; δ 1.25, H_3-18) and an acetoxy group at C-3 (δ 5.01) with an axial orientation. The sharp singlet of an acetyl group at δ 2.21, together with AB systems, at δ 3.78 and 3.45 (H_2-19 or H_2-17) and a broad signal at 3.47 (H_2-19 or H_2-17), led to assignment of the structure as **5**. This conclusion was confirmed by further data in the ^{13}C-NMR spectrum, which showed a carbonyl function (δ 203.0) in a six-membered ring and the presence of an acetate group (δ 169.8, 20.7), as well as three oxygen-bearing carbons (δ 84.8, C-3; δ 63.8 and 61.6, C-17 and C-19 or vice versa).

Finally, the structures of the known metabolites **6**−**9** were ascertained by comparison of their data (^{1}H-NMR and mass spectra) with those reported in the literature.[13,16] The structural and stereochemical assignments were supported by the chemical transformation of the isolated products into epispongiatriol triacetate (**6**) or spongiatriol triacetate (**10**).

Table 1. ^{1}H-NMR (500 MHz, $CDCl_3$) Data of New Spongian Diterpenoids Isolated from *G. atromarginata*

position	compound **1** (δ, m)	compound **2** (δ, m)	compound **3** (δ, m)	compound **4** (δ, m)	compound **5** (δ, m)
1	2.12, m	2.77, d	2.66, d	2.61, d	2.65, d
	1.44, brd	2.15, d	2.21, d	2.03, d	2.22, d
2	5.32, br s				
3	4.77, br s	3.95, d	4.98, s	5.47, s	5.01, s
4					
5[a]	1.31, m	1.72, m	n.a.[d]	n.a.	n.a.
6	1.73, m	1.80, m	n.a.	n.a.	1.70, m
7	2.36, brd	2.40, brd	2.54, m	2.51, m	2.56, m
	1.34, m	1.38, m	1.34, m	n.a.	1.40, m
8					
9[a]	1.37, m	1.72, d	n.a.	n.a.	1.82, m
10					
11	1.73, m	1.69, m	n.a.	n.a.	1.70, m
12	2.81, dd	2.84, brd	2.82, dd	2.82, dd	2.81,m
	2.52, m	2.52, m	2.54, m	2.51, mt	2.56, m
13					
14					
15[b]	7.10, s	7.11, br s	7.21, s	7.20, d	7.19, br s
16[b]	7.09, s	7.14, br s	7.18, br s	7.18, br s	7.21, br s
17	4.40, d	4.38, d	3.77, d	3.81, d	3.78, d[c]
	4.10, d	4.07, d	3.48, br d	3.49, d	3.45, d[c]
18	1.23, s	1.32, s	1.21, s	1.25, s	1.25, s
19	4.49, d	4.02, d	4.11, d	4.03, d	3.47, d[c]
	4.40, d	3.90, d	4.07, d	3.97, d	
20	1.08, s	0.96, s	0.95, s	1.02, s	0.90, s
CH$_3$CO	2.09, s	2.06, s	2.17, s	2.16, s	2.21, s
CH$_3$CO	2.08, s	2.01, s	2.06, s	2.08, s	
CH$_3$CO	2.02, s				
CH$_3$CO	2.00, s				

[a–c] Values marked with the same letter are interchangeable. [d] n.a. means not assigned.

In order to define the metabolite distribution in *G. atromarginata*, the Et_2O extracts of dissected parts (inner organs, mantle, and mucus) were compared with purified diterpenes **1**−**9** by TLC. We noted a similar composition in all extracts, although two aspects were very diagnostic: (a) all the products were more abundant in the mantle, and, (b) the more polar compounds, such as **5** and **9**, were mainly found in the soluble material obtained from the mantle and from the mucus. The mucus contained exclusively the furanoditerpenoids **5** and **9**.

In conclusion, the distribution of spongian diterpenoids **1**−**9** in the Egyptian population of *G. atromarginata* supports the hypothesis that the general defensive strategy of Chromodorididae mollusks is to use dietary products as chemical deterrents.[2,7,8] Unfortunately, due to the low levels of the metabolites in the animals, no direct proof of this function is furnished. It is likely that such mollusks must prey upon sponges of the genus *Spongia*, although we were unable to find evidence to support this hypothesis. As already reported for a collection of *G. atromarginata* from Sri Lanka,[11] the isolated allomones are related to spongian diterpenoids. As might be expected of spongivorous nudibranchs, the compounds from the mantle of our population of *G. atromarginata* differ slightly from those found in the Indian specimens. Of interest from a chemotaxonomic point of view is the difference between the allomonic pattern of *G. atromarginata* and other species of *Glossodoris* studied[9,10] previously, which are characterized by sesterterpenes belonging to the scalarane series. The assignment of an *R* absolute configuration at C-3 of compound **2** by Mosher's method is, to the best of our knowledge, the first direct verification of the absolute stereochemistry of epispongiatriol.

Experimental Section

General Experimental Procedures. HPLC was performed by a Waters liquid chromatography unit, using a Waters R41 differential refractometer. MS were recorded on a Kratos MS 50. Precoated TLC plates (Merck Si gel 60 F_{254}) were used for analytical TLC, and Merck kieselgel 60 powder was used for preparative column chromatography. ^{1}H- and ^{13}C-NMR spectra were recorded by Bruker AMX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million referred to $CHCl_3$ used as an internal standard. Coupling constants (*J* values) are reported in Hertz. Optical rotations were determined by JASCO DIP 370 polarimeter. IR spectra were measured with a BIO-RAD FTS-7 FTIR spectrophotometer.

Animal Material. *G. atromarginata* (5 specimens) was collected off Hurghada (Red Sea) at a depth of 12 m in December 1994. The mollusks and the white mucus released by the collected animals were frozen at −20 °C and stored until the day of the analysis at −80 °C. A voucher specimen (no. SN RS1995−21) is deposited at ICMIB. Taxonomic classification was carried out by Dr. A. Valdes at the University of Oviedo, Spain.

Extraction and Isolation. Frozen *G. atromarginata* (4 specimens) was directly dissected in order to separate internal organs (digestive gland, reproductive apparatus, digestive duct, etc.) from the mantle. Dissected parts and frozen mucus were separately extracted with Me_2CO, and, after removing the organic solvent, the aqueous residue was diluted with H_2O (ca. 5 mL) and partitioned with Et_2O (3 × 10 mL). The soluble Et_2O fractions were concentrated at reduced pressure to give 80 mg of Et_2O extract from internal organs, 120 mg from mantle and 40 mg from mucus.

TLC of the extracts was carried out in petroleum ether−Et_2O, and the metabolites were visualized with Ehrlich's reagent. A small amount of each extract was reserved as reference, and the rest of the sample was reconstituted to give a yellow oil (220 mg) that was fractionated on Si gel into seven major fractions. Each of the fractions positive to Ehrlich's reagent—4 (34 mg, crude **1**−**4**), 5 (23 mg, crude **6**−**8**), 6 (18 mg, crude **5**), and 7 (47 mg, crude **5** and **9**)—was purified further to give compounds **1**−**9**.

Purification of Compounds 1−4 and 6−8. Fractions 4 and 5 were separated on reversed-phase HPLC (Spherisorb S5 ODS-2 column; Detector UV at 210 nm; flow 3 mL/min) by using a MeOH−H_2O gradient (from 65% up to 80% in MeOH in 45 min) to give pure **1**−**4** and **6**−**8**. Spectroscopic data of products **6**−**8** were identical with those reported in the literature.

Compound 1. 2α,3β,17,19-Tetracetoxyspongia-13-(16),14-diene (2.0 mg) was obtained as an oil: $[\alpha]_D$ −1.5 (*c* 0.1, $CHCl_3$) (mean value of four measurements); IR (film) ν_{max} 3443 br, 2925 (CH), 2858 (CH), 1746 (C=O, ester), 1741 (C=O, ester), 1380, 1229 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR ($CDCl_3$, 500 MHz) δ 181.5 (s, CH_3CO−), 170.8 (s, CH_3CO−), 170.1 (s, CH_3CO−), 137.7 (d, C-15 or C-16), 137.2 (d, C-16 or C-15), 131.1 (s, C-), 129.7 (s, C-), 128.6 (s, C-)119.3 (s, C-), 76.7 (d, C-3), 68.8 (d, C-2), 65.7 (t, C-17 or C-19), 64.3 (t, C-19 or C-17), 57.5 (d, C-5 and C-9), 41.7 (t, C-1), 41.0 (s, C-), 38.2 (s,

C-), 36.9 (s, C-), 35.9 (t, C-7), 23.0 (q, C-20), 21.2 (q, CH_3CO-), 21.0 (q, CH_3CO-), 21.0 (q, CH_3CO-), 20.8 (q, CH_3CO-), 20.4 (t, C-12), 19.2 (t, C-6 and C-11), 18.2 (q, C-18); EIMS (*m/z*) 518 (M^+, 5), 462 ($M^+ - 56$, 15), 458 ($M^+ - 60$, 10), 445 ($M^+ - 73$, 20), 360 (40), 325 (35), 283 (85), 265 (100); HREIMS *m/z* 518.2520; calcd for $C_{28}H_{38}O_9$, 518.2516.

Compound 2. 3β-Hydroxy-17,19-diacetoxyspongia-13(16),14-dien-2-one (1.4 mg) was obtained as an oil: $[\alpha]_D$ +2.1 (*c* 0.14, $CHCl_3$) (mean value of three measurements); IR (film) ν_{max} 3452 (OH), 2967 (CH), 2919 (CH), 2849 (CH), 1738 (C=O, ester), 1709 (C=O, ketone), 1496, 1432, 1382, 1244, 1046 cm^{-1}; ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$, 500 MHz) δ 209.1 (s, C-2), 170.6 (s, CH_3CO-), 170.6 (s, CH_3CO-), 137.9 (d, C-15 or C-16), 137.2 (d, C-16 or C-15), 128.8 (s, C-13 or C-14), 119.3 (s, C-14 or C-13), 82.2 (d, C-3), 64.7 (t, C-17 or C-19), 63.9 (t, C-19 or C-17), 56.7 (d, C-5 or C-9), 55.1 (d, C-9 or C-5), 53.2 (t, C-1), 47.8 (s, C-4), 42.8 (s, C-10), 38.4 (s, C-8), 35.6 (t, C-7), 24.2 (q, C-18), 21.0 (q, CH_3CO-), 21.0 (q, CH_3CO-), 20.3 (t, C-12), 19.1 (t, C-6 or C-11), 18.2 (t, C-11 or C-6), 17.3 (q, C-20); EIMS *m/z* 432 (M^+, 5), 372 ($M^+ - 60$, 20), 167 (85), 149 (100); HREIMS *m/z* 432.2154, calcd for $C_{24}H_{32}O_7$ 432.2147.

Compound 3. 17-Hydroxy-3β,19-diacetoxyspongia-13(16),14-dien-2-one (trace amount) was obtained as an oil: ^1H NMR: see Table 1; EIMS (*m/z*) 432 (M^+, 10), 401 ($M^+ - 31$, 15), 459 ($M^+ - 73$, 15), 281 (30), 149 (75), 83 (100); HREIMS *m/z* 432.2150, calcd for $C_{24}H_{32}O_7$, 432.2147.

Compound 4. 17-Hydroxy-3α,19-diacetoxyspongia-13(16),14-dien-2-one (0.5 mg) was obtained as an oil: ^1H NMR, see Table 1; EIMS (*m/z*) 432 (M^+, 10), 401 ($M^+ - 31$, 15), 372 ($M^+ - 60$, 5), 459 ($M^+ - 73$, 15), 299 (25), 281 (30), 149 (75), 85 (90), 83 (100); HREIMS *m/z* 432.2163, calcd for $C_{24}H_{32}O_7$ 432.2147.

Purification of Compounds 5 and 9. Fractions 6 and 7 were further fractionated on a SiO_2 column with a gradient of iPrOH in $CHCl_3$. The column fractions positive to the Ehrlich's reagent were separated on HPLC with a Sperisorb S5W column by eluting with EtOAc to give pure **5** and **9**. Spectroscopic data (^1H NMR, IR, and optical activity) of **9** were identical to those reported in literature.

Compound 5. 17, 19-Dihydroxy-3β-acetoxyspongia-13(16),14-dien-2-one (1.0 mg) was obtained as an oil: $[\alpha]_D$ +8.3 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3442 (OH), 2958 (CH), 2918 (CH), 2859 (CH, aliphatic), 1758 (C=O, ester), 1728 (C=O, ketone), 1373, 1230, 1071, 1037, 973 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR ($CDCl_3$, 500 MHz) δ 203.0 (s, C-2), 169.8 (s, CH_3CO-), 137.9 (d, C-16), 137.2 (d, C-15), 84.8 (d, C-3), 63.8 (t, C-17 or C-19), 61.6 (t, C-19 or C-17), 56.3 (d, C-9 or C-5), 56.2 (d, C-5 or C-9), 54.0 (t, C-1), 48.1 (s, C-4), 42.7 (s, C-10), 40.6 (s, C-8), 34.4 (t, C-7), 23.4 (q, C-18), 20.7 (q, CH_3CO-), 20.9 (t, C-12), 18.7 (t, C-6 or C-11), 18.0 (t, C-11 or C-6), 17.4 (q, C-20); EIMS (*m/z*) 390 (M^+, 10), 359 ($M^+ - 31$, 30), 317 (15), 299 (15), 281 (30), 243 (20), 149 (80), 83 (100); HREIMS *m/z* 390.2051, calcd for $C_{22}H_{30}O_6$ 390.2042.

***(S)*-MTPA Ester of 2 (2a).** Compound **2** (0.5 mg) was treated in dry pyridine (0.3 mL) with (*R*)-MTPA chloride (0.8 μL). After being stirred under an argon atmosphere for 4 h, the reaction was stopped by adding MeOH (0.5 mL), and the organic solvent was evaporated under reduced pressure. The (*S*)-MTPA ester **2a** was purified on HPLC using a Sperisorb S5W column with *n*-hexane−2-propanol 95:5: ^1H NMR ($CDCl_3$, 500 MHz) 7.64 (2H, m, MTPA), 7.40 (3H, m, MTPA), 7.15 (1H, d, *J* = 1.5 Hz, H-16), 7.13 (1H, br d, *J* = 1.2 Hz, H-15), 5.13 (1H, s, H-3), 4.37 (1H, d, *J* = 10.7 Hz, H-17a), 4.09 (1H, d, *J* = 10.7 Hz, H-17b), 4.04 (1H, d, *J* = 12.4 Hz, H-19a), 3.97 (1H, d, *J* = 12.4, H-19b), 3.55 (3H, s, $-OCH_3$), 2.85 (1H, br d, *J* = 16.9 Hz, H-12a), 2.74 (1H, br d, 12.7 Hz, H-1a), 2.54 (1H, m, H-12b), 2.43 (1H, dt, *J* = 13.7, 2.9, 2.9 Hz, H-7a), 2.27 (1H, d, *J* = 12.7 Hz, H-1b), 2.01 (3H, s, CH_3CO-), 1.97 (3H, s, CH_3CO-), 1.87 (2H, m), 1.79 (1H, m, H-6), 1.70 (3H, m), 1.27 (3H, s, Hs-20), 0.98 (3H, s, Hs-18).

***(R)*-MTPA Ester of 2 (2b).** Compound **2** (0.5 mg) was treated with (*S*)-MTPA-Cl as above described. The (*R*)-MTPA ester **2b** was purified on analytical TLC by eluting with *n*-hexane−EtOAc 6:4: ^1H NMR ($CDCl_3$, 500 MHz) 7.71 (2H, m, MTPA), 7.42 (3H, m, MTPA), 7.14 (1H, br s, H-16), 7.12 (1H, br s, H-15), 5.08 (1H, s, H-3), 4.36 (1H, d, *J* = 10.8 Hz, H-17a), 4.08 (1H, d, *J* = 10.8 Hz, H-17b), 3.95 (2H, br s, Hs-19), 3.63 (3H, s, $-OCH_3$), 2.85 (1H, br d, *J* = 16.3 Hz, H-12a), 2.74 (1H, d, *J* = 12.7 Hz, H-1a), 2.54 (1H, m, H-12b), 2.41 (1H, dt, *J* = 13.7, 2.7, 2.7 Hz, H-7a), 2.29 (1H, d, *J* = 12.7 Hz, H-1b), 2.01 (3H, s, CH_3CO-), 1.85 (3H, s, CH_3CO-), 1.82 (1H, m), 1.70 (3H, m), 1.41 (3H, s, Hs-20), 0.98 (3H, s, Hs-18).

Acetylation of 2−5. The pure compounds were separately acetylated by treatment with AC_2O (0.3 mL) in dry pyridine (1.2 mL). The reactions were stirred at room temperature overnight and then quenched with MeOH. The mixture was dried under reduced pressure. The $[\alpha]_D$, CD, and ^1H-NMR ($CDCl_3$, 500 MHz) data of peracetylated compounds **2, 3,** and **5** were identical to those reported in the literature for epispongiatriol triacetate (**6**),[13] whereas those of peracetyl **4** fitted with the spectroscopic data of spongiatriol triacetate (**10**).[13]

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Supporting Information Available: Copies of ^1H-NMR spectra of compounds **1**−**5** (5 pages). Ordering information is given on any current masthead page.

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