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SPONGIANE AND *ENT*-ISOCOPALANE DITERPENOIDS FROM THE MEDITERRANEAN SPONGE *SPONGIA ZIMOCCA*

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ABSTRACT.—The Mediterranean sponge *Spongia zimocca* (Dictyoceratida) contains several spongiane and *ent*-isocopalane diterpenoids. Along with seven known compounds (**1–6** and **11**), four new diterpenoids, 12-deacetyl-aplysillin [**13**], 15-hydroxy-*ent*-isocopal-12-en-16-al [**14**], 15,17-diacetoxy-*ent*-isocopal-12-en-16-al [**15**] and 15,16-diacetoxy-11-oxo-*ent*-isocopal-12-ene [**16**], were isolated and characterized. The structures of the new compounds were determined mainly by spectroscopic methods and chemical correlation with known compounds. The absolute stereochemistry of **13** was assigned by applying Mosher's method, while those of **14** and **15** were suggested by cd comparison with polygodial [**17**] and 12-deacetoxyscalaradial [**18**].

Spongiane (1) and *ent*-isocopalane (2) diterpenoids are widely present in marine sponges (3). The Mediterranean *Spongia officinalis* L. yielded the first spongiane diterpenoid, isoagatholactone [**1**] (4), and also some related metabolites possessing either spongiane [**2**] or *ent*-isocopalane [**3–5**] skeletons (5). Some oxidized derivatives of isoagatholactone [**6–9**] were also found in *S. officinalis* from the Canary Islands (6), along with aplysillin [**10**], a spongiane diterpenoid already isolated from the dendroceratid sponge *Aplysilla rosea* from New Zealand (1). Two spongiane diterpenoids related to **10**, 12-*epi*-aplysillin [**11**] and its deacetyl derivative [**12**], were found among the secondary metabolites from the Mediterranean opisthobranch *Chromodoris luteorosea* (7). This paper reports the chemical analysis of the Mediterranean sponge *Spongia zimocca*, Schmidt 1862, which contains four new diterpenoids [**13–16**], together with the previously reported compounds **1–6** and **11**.

RESULTS AND DISSCUSSION

Spongia zimocca was collected by dredging, during the summer of 1991, off Mazara del Vallo (Channel of Sicily). The sponge was frozen at −20° until extraction with Me_2CO. The Et_2O-soluble fraction from the Me_2CO extract of *S. zimocca* was fractionated by repeated Si gel chromatography (C_6H_6-Et_2O, 85:15) yielding, in order of increasing polarity, the following pure terpenoids: **1**, **3**, **2**, **4**, **5**, **6**, **11**, **16**, **15**, **14**, and **13**. The structures of compounds **1–6** and **11** were secured by comparison with the data reported in the literature (4–7), whereas those of the new diterpenoids, **13–16**, were supported by evidence presented in the following paragraphs.

All of the nmr spectral data of **13** (Tables 1 and 2) indicated strong analogies with those of **10** (1). The main difference was the absence in the ^{1}H-nmr spectrum of **13** of the acetyl singlet at δ 2.05 and the shift of H-12 from δ 5.08, observed in the ^{1}H-nmr spectrum of **10**, to δ 4.15, resulting from a deacetylation at C-12. Acetylation of **13** yielded a compound with all obtained data, including optical rotation, identical to those reported for **10**. The absolute stereochemistry was ascertained by applying Mosher's method (8,9) as modified by Kakisawa's group (10,11) and successfully applied by our

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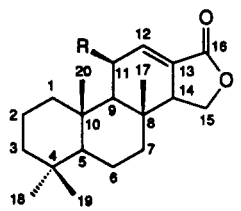

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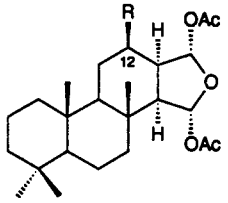
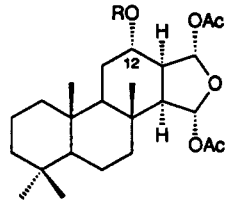
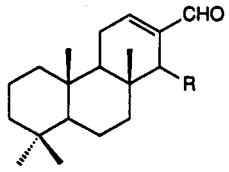
6 R=OAc
7 R=OH



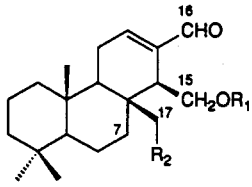
2 R=H
11 R=OAc
12 R=OH



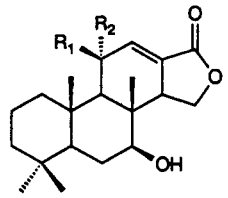
10 R=Ac
13 R=H
20 R=MTPA (*S*)-ester
21 R=MTPA (*R*)-ester



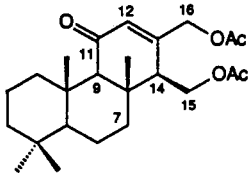
3 R=α-CHO
4 R=β-CHO



5 R_1=Ac, R_2=H
14 R_1=R_2=H
15 R_1=Ac, R_2=OAc



8 R_1=OH, R_2=H
9 R_1=H, R_2=OH



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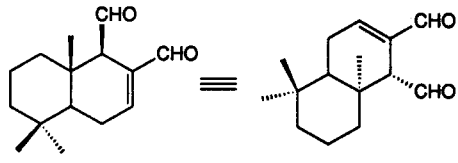

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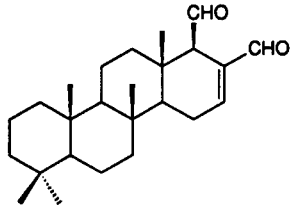

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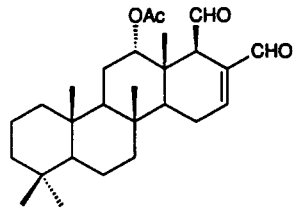

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group to some triterpenoids (12,13) from sponges. The observed Δδ (δ*S*-δ*R*) in the ^{1}H-nmr chemical shifts of the (*S*)- and (*R*)-esters [**20** and **21**] obtained by treating **13**, respectively, with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic (MTPA) chloride (Table 3) suggested an *S* absolute stereochemistry at C-12. The ^{1}H- and ^{13}C-nmr assignments for **13** (12-deacetyl-aplysillin) (Tables 1 and 2) were supported by 2D experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR).

The spectral data of **14** showed strong similarities with those of **5**, differing only by the absence of the acetyl signal and by the upfield ^{1}H-nmr values of H_2-15, resonating as the AB part of an ABX system at δ 3.81 and 3.48. This evidence suggested that **14** is the deacetyl derivative of **5**. Every attempt to acetylate **14** failed. Most likely, the alcoholic proton is stabilized by a strong hydrogen linkage with the aldehyde group as

TABLE 1. ^{1}H-Nmr Data of Compounds **13**–**16**.[a]

Position	Compound				
	5	**13**	**14**	**15**	**16**
	δ^1H^b m (*J* in Hz)	δ^1H^b m (*J* in Hz)	δ^1H^c m (*J* in Hz)	δ^1H^b m (*J* in Hz)	δ^1H^b m (*J* in Hz)
1	0.85 m 1.65 m	0.82 m 1.62 m	0.85 m 1.63 m	0.81 m 1.68 m	0.74 m 2.53 m
2	1.38 m, 1.60 m [d]	1.40 m, 1.58 m [e]	1.40 m 1.60 m	1.43 m 1.61 m	1.37 m, 1.64 m [g]
3	1.14 m 1.40 m	1.13 ddd (13.2, 13.9, 4.3) 1.39 m	1.14 ddd (14.2, 13.6, 4.8) 1.40 m	1.13 ddd (13.4, 13.2, 3.9) 1.39 m	1.13 ddd (14.1, 13.9, 4.6) 1.38 m
5	0.83 m	0.88 m	0.85 m	0.89 dd (12.1, 1.9)	0.78 m
6	1.37 m, 1.58 m [d]	1.35 m, 1.57 m [e]	1.40 m 1.63 m	1.26 m 1.61 m	1.40 m, 1.62 m [g]
7	1.16 m 2.06 m	1.20 ddd (13.4, 12.8, 3.5) 1.93 ddd (12.8, 3.0, 3.1)	1.36 m 2.15 m	1.04 ddd (13.4, 13.5, 3.9) 2.43 ddd (13.5, 3.2, 3.2)	1.51 ddd (13.2, 12.9, 3.5) 2.07 m
9	1.14 m	1.28 dd (12.1, 3.7)	1.20 dd (11.9, 5.0)	1.31 dd (12.1, 5.5)	2.08 s
11	2.25 m 2.35 m	1.60 m 1.67 ddd (14.7, 12.1, 4.3)	2.27 m 2.40 m	2.33 m	
12	6.90 m	4.15 dd (7.5, 3.8)	7.02 m	6.94 m	5.96 br s
13		2.59 m			
14	2.41 m	2.18 d (8.3)	2.15 m	2.49 m	2.63 m
15	4.41 d (11.6) 4.62 dd (11.6, 5.7)	6.15 s	3.48 m 3.81 dd (11.9, 11.6)	4.44 dd (11.5, 1.8) 4.69 dd (11.5, 5.5)	4.11 dd (12.0, 7.2) 4.42 dd (12.0, 3.0)
16	9.42 s	6.10 d (6.5)	9.36 s	9.42 s	4.71 ABq (15.7)
17	0.95 s	0.91 s	0.91[f] s	3.99 d (11.8) 4.34 d (11.8)	0.94 s
18	0.88 s	0.85 s	0.88 s	0.87 s	0.86 s
19	0.82 s	0.81 s	0.83 s	0.81 s	0.83 s
20	0.84 s	0.85 s	0.69[f] s	0.99 s	1.16 s
CH_3CO	1.94 s	2.06 s		1.88 s	2.07 s
CH_3CO		2.10 s		1.97 s	2.12 s
-OH			4.67 dd (11.6, 3.0)		

[a]500 MHz; $CDCl_3$; values refer to $CHCl_3$ (δ 7.26) as internal standard.
[b]By ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR experiments.
[c]By ^{1}H-^{1}H COSY.
[d–g]Values with identical superscript in each column may be reversed.

TABLE 2. ^{13}C-Nmr Data of Compounds **13–16**.[a]

Carbon	Compound									
	5		**13**		**14**		**15**		**16**	
	δ ^{13}C[b]	m[d]	δ ^{13}C[b]	m[d]	δ ^{13}C[c]	m[d]	δ ^{13}C[b]	m[d]	δ ^{13}C[b]	m[d]
1	39.9	t	39.7	t	39.8	t	39.8	t	39.8	t
2	18.4[e]	t	18.4[g]	t	18.4[i]	t	18.4	t	18.3[o]	t
3	41.8	t	41.9	t	41.7	t	41.6	t	41.9	t
4	33.2	s	33.3	s	n.d.		33.1	s	33.2	s
5	56.2	d	56.7	d	56.0[l]	d	56.5	d	55.6	d
6	18.5[e]	t	18.1[g]	t	18.6[i]	t	18.6	t	18.1[o]	t
7	41.3	t	42.7	t	40.2	t	35.6	t	40.6	t
8	36.2[f]	s	34.7[h]	s	n.d.		38.5[n]	s	42.5	s
9	53.9	d	49.6	d	53.7[l]	d	53.8	d	67.7	d
10	37.2[f]	s	37.0[h]	s	n.d.		37.4[n]	s	37.2	s
11	24.0	t	26.9	t	24.9	t	23.6	t	199.4	s
12	153.0	d	65.1	d	158.1	d	152.7	d	127.5	d
13	140.0	s	48.9	d	n.d.		139.7	s	151.2	s
14	49.3	d	57.1	d	55.2[l]	d	48.5	d	53.0	d
15	60.1	t	100.0	d	60.8	t	60.2	t	61.2	t
16	194.0	d	101.3	d	197.6	d	193.5	d	63.4	t
17	15.9	q	17.2	q	15.6[m]	q	63.0	t	16.6	q
18	33.4	q	33.3	q	33.4	q	33.3	q	33.5	q
19	21.6	q	21.4	q	21.6	q	21.6	q	21.7	q
20	15.4	q	16.1	q	14.8[m]	q	16.0	q	16.2	q
*C*OCH$_3$	170.8	s	170.6	s			170.9	s	170.6	s
*C*OCH$_3$			169.8	s			170.7	s	170.2	s
-COCH$_3$	21.0	q	21.3	q			20.8	q	21.0	q
-COCH$_3$			21.3	q			20.9	q	20.8	q

[a]500 MHz; $CDCl_3$; values refer to $CDCl_3$ (δ 77.0).
[b]Assignments aided by ^{1}H-^{13}C HETCOR experiment.
[c]By comparison with **5**.
[d]By DEPT sequence.
[e–o]Values with identical superscript in each column may be reversed.

supported by the slow exchange of the alcoholic proton observed by recording the ^{1}H-nmr spectrum in $CDCl_3$ with a few drops of D_2O. The instability of **14** when dissolved in all common solvents prevented the use of nmr experiments requiring long acquisition times (^{1}H-^{13}C HETCOR, etc.). However, almost all the ^{1}H- and ^{13}C-nmr values of **14** were assigned (Tables 1 and 2) by one- and some two-dimensional nmr experiments (^{1}H-^{1}H COSY) and by an extensive analysis of both ^{1}H- and ^{13}C-nmr spectra of **5** (Tables 1 and 2), which were only partially reported previously (5). Finally, methanolysis of **5** with Na_2CO_3/MeOH (anhydrous) yielded **14** (15-hydroxy-*ent*-isocopal-12-en-16-al).

The ^{1}H-nmr spectrum of **15** (Table 1) exhibited, by analogy with **5**, resonances at δ 9.42 and 6.94, attributable to an α,β-conjugated aldehyde system, an ABX system at δ 4.69, 4.44, and 2.49, and a 3H singlet at δ 1.97 (CH-CH$_2$-OAc group). However, only three upfield methyl singlets were observed, whereas an isolated methylene displayed resonances at δ 3.99 and 4.34, and a second acetyl singlet was present at δ 1.88. These data, confirmed by the elemental composition $C_{24}H_{36}O_5$, suggested an acetoxy functionalization of one of the tertiary methyls. The ^{13}C-nmr chemical shift of C-7 (δ 35.6) was diagnostic to localize this functionalization at C-17. All ^{1}H- and ^{13}C-nmr resonances for **15** (15,17-diacetoxy-*ent*-isocopal-12-en-16-al) were supported by 2D experiments (Tables 1 and 2).

TABLE 3. Selected δ ^{1}H-Nmr Values for Mosher's Esters of Compound **13**.[a]

Position	δ $^1H^b$ (*S*)-MTPA ester [**20**]	δ $^1H^b$ (*R*)-MTPA-ester [**21**]	Δδ (δ*S*-δ*R*)
1	0.34	0.56	−0.22
	1.40	1.54	−0.14
7	1.13	1.12	+0.01
	1.94	1.93	+0.01
9	1.00	1.09	−0.09
11	1.65	1.70	−0.05
	1.70	1.78	−0.08
13	2.66	2.62	+0.04
14	2.01	1.94	+0.07
15	6.12	6.11	+0.01
16	6.17	6.16	+0.01
20	0.79	0.82	−0.03

[a]500 MHz, $CDCl_3$; chemical shifts are referred to $CHCl_3$ (δ 7.26).
[b]Assignments were aided by ^{1}H-^{1}H COSY experiment.

The elemental composition of **16** was identical to that of **15** ($C_{24}H_{36}O_5$). The ^{1}H-nmr spectrum of **16** exhibited, by analogy with **15**, signals attributable to two acetoxy groups (δ 2.07 and 2.12), to an ABX system [δ 4.42 and 4.11 (H_2-15) and δ 2.63 (H-14)] and to an isolated AB system [δ 4.71 (H_2-16)]. The absence in the ^{1}H-nmr spectrum of **16** of the aldehydic proton and the presence in the ^{13}C-nmr spectrum of a signal at δ 199.4 suggested an α,β-unsaturated carbonyl group. The multiplicity of the olefinic proton allowed the correct localization of all the functionalizations. In fact, H-12 resonates as a broad singlet at δ 5.96, allylically coupled with the isolated methylene (H_2-16) at δ 4.71 (AB quartet) and with the methine (H-14) at δ 2.63. The sharp singlet (H-9) at δ 2.08 confirmed the oxidation of C-11. All ^{1}H- and ^{13}C-nmr resonances were assigned by 2D experiments (Tables 1 and 2). The relative stereochemistry at C-14 of **16** (15,16-diacetoxy-11-oxo-*ent*-isocopal-12-ene) was supported by the ^{13}C-nmr chemical shift of C-7 (δ 40.6), similar to that recorded for **5**.

Most likely, the carbon skeletons of all metabolites from *S. zimocca* possess the same absolute stereochemistry as **1**. This suggestion has been proven for **13** by applying Mosher's method, whereas the cd curves of *ent*-isocopal-12-en-15,16 diol [**4**] and related compounds [**5** and **15**] were opposite to the cd curves of polygodial [**17**], of scalaradial [**19**], and of the recently isolated 12-deacetoxy-scalaradial [**18**] (14), well supporting the suggested *ent*-isocopalane skeleton.

It is worth noting that the chemistry of two different, but closely related, dictyoceratid sponges offers quite confusing data from a taxonomic point-of-view. In fact, two chemically distinct specimens of *S. officinalis* contain either furanoterpenoids (15) and sesterterpenoids (16) or tri- and tetracyclic diterpenoids (4–6). By analogy, two chemically distinct specimens of *S. zimocca* contain either typical algal metabolites (17,18) or spongiane and *ent*-isocopalane diterpenoids. Further comparative chemical and taxonomic studies are necessary.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Si gel chromatography was performed using precoated Merck F_{254} plates and Merck Kieselgel 60 powder. The ir spectra were taken on Nicolet FT 5DxB and Bio-Rad FTS-7 spectrometers. The uv spectra were recorded on a Varian DMS 90 spectrophotometer. Optical rotations were measured on a Jasco DIP 370 polarimeter and cd curves were obtained on a Jasco 710 spectropolarimeter. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM 500 spectrometer; chemical shifts are reported in

ppm referenced to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon). Mass spectra were obtained on Kratos MS50 and VG TRIO 2000 instruments.

BIOLOGICAL MATERIAL.—*Spongia zimocca* was collected by dredging, during the summer of 1991, along the coast of Mazara del Vallo (Channel of Sicily), and identified by Dr. M. Uriz (Centro de Estudios Avanzados de Blanes, Spain). The fresh sponge was immediately frozen and kept at −20° until extracted with Me_2CO. A voucher sample is available both at the Istituto per la Chimica di Molecole di Interesse Biologico, Arco Felice (Na) and at the Centro de Estudios Avanzados de Blanes.

EXTRACTION AND ISOLATION.—*Spongia zimocca* (66 g dry wt) was extracted thoroughly with Me_2CO (1 liter). After removal of the organic solvent, the residual H_2O was extracted with Et_2O (700 ml) and with *n*-BuOH (300 ml) successively. The Et_2O phase was concentrated under reduced pressure to give 1.17 g of crude material. An aliquot (514 mg) of the Et_2O-soluble fraction was submitted to a Si gel column using petroleum ether with increasing amounts of Et_2O as eluent: (a) the fraction (38 mg) eluted with petroleum ether-Et_2O (9:1) was further purified by Si gel flash chromatography (C_6H_6 with increasing amounts of Et_2O) to give in order of increasing polarity compounds **1** (5 mg), **3** (2.5 mg), **2** (2 mg), and **5** (18 mg); (b) the fraction (42 mg) eluted with petroleum ether-Et_2O (8:2) was again chromatographed on a Si gel flash column using the same eluent to afford **4** (20 mg) and **6** (8 mg); (c) the fraction (85 mg) eluted with petroleum ether-Et_2O (7:3) was applied on Si gel tlc plates [C_6H_6-Et_2O, (85:15)], affording **16** (7 mg), **11** (22 mg), **15** (15 mg), and **14** (4.5 mg); (d) finally, the fraction (73 mg) eluted with petroleum ether-Et_2O (1:1) was further purified on a Si gel flash column [C_6H_6-Et_2O (9:1)] to give **13** (23 mg). All known compounds (**1–6** and **11**) were identified by comparison of spectral and physical data (^{1}H-nmr and [α]D) with those reported in the literature (4–7).

12-Deacetyl-aplysillin [**13**].—$[\alpha]_D^{25}$ +7.5° ($CHCl_3$, *c*=1.33); cd $[\theta]_{223}$ (EtOH) +1,200; ir ν max (liquid film) 1746 cm^{-1}; ^{1}H nmr shown in Table 1; ^{13}C nmr shown in Table 2; hreims *m/z* 362.2450 (M^+−AcOH, $C_{22}H_{34}O_4$ requires 362.2457); eims *m/z* 362 (M^+−AcOH, 1), 344 (M^+−AcOH−H_2O, 1.2), 320 (2.5), 303 (base peak), 285 (11), 269 (15), 191 (65).

15-Hydroxy-ent-*isocopal-12-en-16-al* [**14**].—Ir ν max (liquid film) 1672 cm^{-1}; ^{1}H nmr shown in Table 1; ^{13}C nmr shown in Table 2; hreims *m/z* 304.2410 ($C_{20}H_{32}O_2$ requires 304.2402); eims *m/z* 304 (M^+, 3), 303 (8), 286 (M^+−H_2O, 9), 271 (4), 191 (36), 177 (40), 133 (22), 69 (base peak).

15,17-Diacetoxy-ent-*isocopal-12-en-16-al* [**15**].—$[\alpha]_D^{25}$ −3.8° ($CHCl_3$, *c*=1.4); cd $[\theta]_{215}$ (EtOH) +24,400; ir ν max (liquid film) 1734 and 1685 cm^{-1}; uv λ max (CH_3OH) 227 (ε 6,100); ^{1}H nmr shown in Table 1; ^{13}C nmr shown in Table 2; hreims *m/z* 344.2344 (M^+−AcOH, $C_{22}H_{32}O_3$ requires 344.2351); eims *m/z* 344 (M^+−AcOH, 15), 302 (95), 284 (M^+−2 AcOH, 26), 271 (base peak), 177 (31), 105 (90), 91 (85).

15,16-Diacetoxy-11-oxo--ent-*isocopal-12-ene* [**16**].—$[\alpha]_D^{25}$ −54.2° ($CHCl_3$, *c*=0.36); cd $[\theta]_{342}$ (EtOH) −3,500; ir ν max (liquid film) 1746 and 1675 cm^{-1}; uv λ max (CH_3OH) 229 (ε 10,600); ^{1}H nmr shown in Table 1; ^{13}C nmr shown in Table 2; hreims *m/z* 404.2551 ($C_{24}H_{36}O_5$ requires 404.2563); eims *m/z* 404 (M^+, 2), 362 (M^+−CH_2CO, 4), 344 (M^+−AcOH, 6), 284 (M^+−2 AcOH, 12), 269 (5), 193 (base peak), 151 (65), 120 (60).

ACETYLATION OF **13**.—12-Deacetyl-aplysillin (**13**, 3.0 mg) was dissolved in dry pyridine (0.5 ml) and 2 drops of Ac_2O were added under stirring. The reaction was allowed to stand at room temperature overnight. After the usual workup, 2.6 mg of a pure product was isolated and identified by spectral data (^{1}H-nmr and [α]D) as aplysillin [**10**].

METHANOLYSIS OF **5**.—Compound **5** (1.4 mg) was dissolved in anhydrous MeOH (1 ml) and an excess of Na_2CO_3 was added. The solution was stirred at room temperature overnight, filtered, and the solvent evaporated. The residue was chromatographed on a Si gel tlc plate [C_6H_6-Et_2O (75:25)] to afford 0.8 mg of a pure product that was identical to **14** (^{1}H nmr, [α]D).

PREPARATION OF (*S*)- AND (*R*)-MTPA ESTERS OF **13**.—(*S*)- and (*R*)-MTPA esters of 12-deacetylaplysillin [**13**] were prepared by treating two aliquots of 3.0 mg each of **13** respectively with (*R*)-and (*S*)-MTPA chloride (an excess) in dry pyridine (1 ml), for 40 h at room temperature under stirring. The esters were purified by chromatography on a Si gel column [petroleum ether-Et_2O (8:2)], obtaining 2.1 mg of **20** and 1.8 mg of **21**. All ^{1}H-nmr resonances of esters **20** and **21** (significant selected data are reported in Table 3) were assigned by ^{1}H-^{1}H COSY nmr experiments.

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