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Further syphonosides from the sea hare *Syphonota geographica* and the sea-grass *Halophila stipulacea*

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Abstract

The unusual structural features of syphonoside (**1**), recently reported from the marine mollusc *Syphonota geographica* and its prey *Halophila stipulacea*, stimulated further investigations on the minor secondary metabolites of both organisms. The three novel macrocyclic glycoterpenoids **2**–**4**, structurally related to the main co-occurring metabolite **1**, have been isolated and chemically characterized mainly by NMR spectroscopic techniques and degradation methods. Compounds **2** and **3** were found only in the mollusc whereas compound **4** was isolated in trace quantities exclusively from the sea-grass.
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Keywords: Marine natural products; *Halophila stipulacea*; *Syphonota geographica*; Molluscs; Macrocycles; Glycoterpenoids

1. Introduction

In the course of our investigations on natural products from marine opisthobranchs,[1–12] we have recently analyzed the secondary metabolite content obtained from a Mediterranean population of the Lessepsian anaspidean mollusc *Syphonota geographica*, collected along the Greek coasts.[9,12] A number of degraded sterols, structurally related to those found in other anaspidean molluscs,[13–15] characterized the skin metabolite pattern of *S. geographica*[9] whereas the main secondary metabolite of the viscera was found to be the glycoterpenoid macrocycle syphonoside (**1**).[12] This compound was also found to be the main component of the butanol extract of the invasive sea-grass *Halophila stipulacea*, collected together with the mollusc.

The presence of syphonoside (**1**) in both organisms supported the trophic relationship between the mollusc and the sea-grass,[12] previously suggested by the detection of *H. stipulacea* fragments in the stomach content of *S. geographica*.[9] From the chemical point of view, syphonoside (**1**) is particularly interesting because it has an unusual framework in which a diterpene moiety is incorporated in a macrocycle along with a residue of 3-hydroxy-3-methyl glutaric acid and a glucopyranosyl moiety. The structure and the absolute stereochemistry of compound **1** were determined by a combination of spectroscopic techniques, chemical degradation methods, and conformational analysis.[12] In a preliminary cytotoxicity evaluation, **1** inhibited high density induced apoptosis in selected human and murine cancer cell lines,[12] suggesting a possible involvement in the regulation of the cell survival and death under specific conditions.

The intriguing structural features of syphonoside (**1**) prompted us to study the minor components of the glycoterpenoid fractions from the extracts of *S. geographica* and *H. stipulacea*. Herein we report the results of this investigation that led to the isolation and the chemical characterization of three novel glycoterpenoid macrocycles, compounds **2**–**4**,

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structurally related to the main metabolite **1**. Compounds **2** and **3** were isolated from the diethyl ether extract of the mollusc, whereas compound **4** co-occurred with the main metabolite **1** in the butanol extract of the sea-grass.

2. Results and discussion

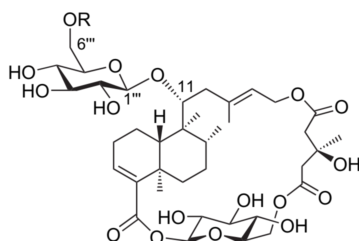
The diethyl ether soluble portion (589 mg) of the acetone extract, obtained as already described from the viscera of seven frozen *S. geographica* individuals,[9,12] was analyzed by TLC. Some polar components that were absent in the mantle of the mollusc were revealed by reaction with cerium sulfate. The extract was submitted to a chromatographic purification on a LH-20 Sephadex column, to afford fractions A and B. Preliminary ^{1}H NMR spectroscopic analysis of these fractions showed that both of them contained glycosyl compounds structurally related to syphonoside (**1**), previously isolated from the *n*-BuOH soluble portion of the same acetone extract.[12] Fractions A and B were further purified on preparative silica gel TLC plates ($CHCl_3$/MeOH eluent system) to obtain syphonosideol (**2**, 12.0 mg), the main component of the ether extract, and a mixture of syphonoside fatty acid ester derivatives **3** (5.4 mg).

A selected fraction obtained by LH-20 Sephadex chromatography of an aliquot (500 mg) of *n*-BuOH layer from the acetone extract of the sea-grass sample[12] was found to contain an additional syphonoside related metabolite as revealed by TLC and ^{1}H NMR analysis. This fraction was further purified by preparative silica-gel TLC chromatography ($CHCl_3$/MeOH eluent system) to yield the syphonoside acetyl derivative **4** (8.3 mg).

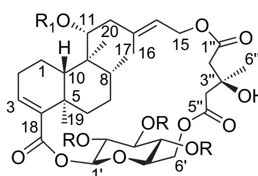
The new compounds **2**–**4** possessed the same macrocycle structure as the co-occurring syphonoside (**1**) and differed in the nature of the substituent at C-11 in the lateral chain of the diterpenoid unit. The structural elucidation of these molecules is described starting from syphonosideol (**2**), which was the main component of the diethyl ether extract from the viscera of the mollusc.

in an ester linkage. Analogously with compound **1**, the proton NMR spectrum of syphonosideol (**2**) displayed five methyl signals at δ 1.78 (3H, br s, H_3-16), 1.39 (3H, s, H_3-6″), 1.26 (3H, s, H_3-19), 1.02 (3H, d, J=7 Hz, H_3-17), and 0.95 (3H, s, H_3-20); two olefinic multiplets at δ 5.36 (1H, m, H-14) and 6.54 (1H, t, J=3 Hz, H-3); a methylene at δ 4.56 (1H, dd, J=9 and 13 Hz, H-15a) and 4.70 (1H, m, H-15b) and a methine at δ 4.00 (1H, dd, J=2 and 8 Hz, H-11) both linked to an oxygen atom (Table 1). The ^{13}C NMR spectrum of **2** showed the presence of 25 sp^{3} carbon signals (five CH_3, nine CH_2, eight CH and three C as deduced by the DEPT sequence), and 7 sp^{2} carbon signals corresponding to two double bonds and three ester carbonyl groups (Table 1), as in syphonoside (**1**). A careful analysis of the 1D and 2D NMR spectra of compound **2** indicated the close similarity to **1**, due to the presence of the same macrocyclic carbon framework constituted by a clerodane acyl moiety oxidized at C-11, C-15, and C-18, a glucopyranosyl unit esterified at both H-1′ and H_2-6′ and a 3-hydroxy-3-methyl-glutaric acyl residue. Similarly to **1**, the α,β-unsaturated acyl function at C-18 of the clerodane moiety esterifies the hydroxyl group at the anomeric position H-1′ of the β-glucose unit, the primary hydroxyl group (6′-OH) of which is in turn linked to the carboxyl group (C-5″) of 3-hydroxy-3-methyl glutaric acid, further esterified at C-1″ with a primary hydroxyl group (15-OH) of the same clerodane, as reported in formula **2**. In fact, diagnostic HMBC correlations were observed between C-18 (δ 167.5) and H-1′ (δ 5.51), between C-5″ (δ 170.6) and H_2-6′ (δ 4.30 and 4.62) as well as between C-1″(δ 172.4) and H_2-15 (δ 4.56 and 4.70). Thus it was suggested that the only difference between compounds **1** and **2** was in the hydroxyl group at C-11, which was free in syphonosideol (**2**) and glycosylated by an additional glucose moiety in **1**.

In order to confirm the proposed structure and the relative stereochemistry of **2**, some degradation and derivatization reactions were performed. Initially, a sample of syphonosideol (**2**) was acetylated to give the tetra-acetyl derivative **5**, which



1 R = H
4 R = -Ac



2 R = R_1 = H
3 R = H, R_1 = $CH_3(CH_2)_{14}CO$- or $CH_3(CH_2)_{16}CO$-
5 R = R_1 = Ac

Syphonosideol (**2**) displayed a pseudo-molecular peak at *m/z* 647 in the HRESI-MS spectrum, indicating the molecular formula $C_{32}H_{48}O_{12}$, which was consistent with a structure containing one glucose unit less than **1**. Consistently, the ^{1}H NMR spectrum of **2** contained only one set of glycosyl signals resonating at δ 3.49–4.62 along with a doublet at δ 5.51 (J=8 Hz) due to the anomeric sugar proton (H-1′) involved

was fully characterized (Table 1). Diagnostic acylation shifts were observed for the protons of the three carbinol groups (H-2′, H-3′, and H-4′) of the glucose unit as well as for H-11 of the clerodane portion, confirming the presence of the free hydroxyl at C-11 in compound **2**.

Analogously with syphonoside (**1**), the alkaline hydrolysis (Na, MeOH anhyd) of compound **2** was very informative

Table 1
NMR data[a,b] of compounds **2**, **5**, and **6**

Position	**2**		**5**		**6**	
	δ ^{1}H m, *J*, Hz	δ ^{13}C m[c]	δ ^{1}H m, *J*, Hz	δ ^{13}C m[c]	δ ^{1}H m, *J*, Hz	δ ^{13}C m[c]
1	1.58 m	18.6 t	1.55 m	18.7 t	1.62 m	18.2 t
			1.82 m			
2	2.17 m	26.7 t	2.15 m	27.0 t	2.23 m	27.3 t
			2.28 br t, 5			
3	6.54 t, 3	139.4 d	6.65 t, 3	138.8 d	6.58 t, 3	136.2 d
4	—	142.4 s	—	141.5 s	—	142.8 s
5	—	37.5 s	—	37.5 s	—	38.0 s
6	1.09 ddd, 4, 11, 12	35.2 t	1.04 m	35.1 t	1.12 ddd, 4, 11, 12	35.8 t
	2.14 m		2.19 m		2.24 m	
7	1.38 m	28.3 t	1.42 m	27.0 t	1.50 m	28.4 t
8	1.90 m	34.4 d	1.96 m	34.6 d	1.60 m	35.9 d
9	—	44.3 s	—	44.4 s	—	42.9 s
10	1.45 m	45.2 d	1.51 m	45.4 d	1.41 m	46.5 d
11	4.00 dd, 2, 8	74.3 d	5.37 dd, 3, 10	74.7 d	3.77 dd, 2, 8	74.2 d
12	2.27 m	45.2 t	2.11 m	42.3 t	2.08 m	42.6 t
	2.71 m		2.69 m		2.32 m	
13	—	139.6 s	—	138.7 s	—	137.8 s
14	5.36 m	121.8 d	5.44 br t, 7	122.6 d	5.52 t, 7	126.9 d
15	4.56 dd, 9, 13	61.5 t	4.58 m	61.3[d] t	4.19 d, 7	59.3 t
	4.70 m					
16	1.78 br s	16.2 q	1.79 br s	16.1 q	1.70 br s	16.4 q
17	1.02 d, 7	18.6 q	0.99 d, 7	17.8 q	0.98 d, 7	18.6 q
18	—	167.5 s	—	166.3 s	—	167.9 s
19	1.26 s	21.3 q	1.15 s	21.3 q	1.32 s	21.0 q
20	0.95 s	12.5 q	0.74 s	12.1 q	0.96 s	12.5 q
1′	5.51 d, 8	94.5 d	5.66 d, 8	91.9 d		
2′	3.49 m	73.1 d	5.17 dd, 8, 9	70.2 d		
3′	3.65 m	76.8 d	5.25 dd, 9, 9	74.7 d		
4′	3.51 m	69.9 d	5.04 dd, 9, 1	68.6 d		
5′	3.69 m	74.5 d	3.87 m	72.8 d		
6′	4.30 dd, 6, 12	62.2 t	4.14 dd, 2, 12	61.5[d] t		
	4.62 dd, 2, 12		4.32 dd, 7, 12			
1″	—	172.4 s	—	172.2 s		
2″	2.60 s	44.0 t	2.57 s	44.2 t		
3″	—	69.9 s	—	69.6 s		
4″	2.62 d, 14	47.1 t	2.62 d, 15	47.0 t		
	2.65 d, 14		2.67 d, 15			
5″	—	170.6 s	—	169.7 s		
6″	1.39 s	27.0 q	1.40 s	27.1 q		
$-OCH_3$					3.68 s	51.2 q
$-OCOCH_3$			2.05 s	21.1[e] q		
			2.05 s	20.6[e] q		
			2.01 s	20.5[e] q		
			2.00 s	20.5[e] q		
$-OCOCH_3$			—	170.0 s		
			—	169.8 s		
			—	169.3 s		
			—	168.8 s		

[a] Bruker DPX-300, DPX-600, and AVANCE 400 MHz spectrometers; $CDCl_3$; chemical shifts (ppm) referred to $CHCl_3$ (δ 7.26) and to $CDCl_3$ (δ 77.0).
[b] Assignments made by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.
[c] By DEPT sequence.
[d,e] Values with identical superscripts in the same column may be reversed.

leading by fragmentation of the molecule to: (a) free aglycone methyl ester **6**, (b) dimethyl ester derivative of 3-hydroxy-3-methyl glutaric acid, and (c) glucose. Compound **6** was fully characterized by NMR spectroscopy (Table 1) confirming the relative stereochemistry of the bicyclic system proposed for the corresponding part in syphonoside (**1**). The diagnostic ^{13}C NMR chemical shifts for C-6 (δ 35.8), C-20 (δ 12.5), and C-19 (δ 21.0), which were comparable with those of the same carbon atoms in syphonoside (**1**),[12] were consistent with a *trans*-clerodane framework exhibiting H_3-17 equatorial and H_3-20 axially oriented. A series of NOE effects, observed between H-10 and the protons H-8, H-11, and H_2-12 in a NOESY experiment, further confirmed the suggested relative stereochemistry. The configuration at carbinol C-11 in the side chain was assigned to be the same as syphonoside (**1**) by obvious biogenetical considerations, even though some attempt to obtain

a suitable derivative for NMR stereochemical study was undertaken. In particular, compound **6** was submitted to acylation reaction by MTPA chlorides under different conditions in order to get the corresponding Mosher esters. Unfortunately, due to a rapid dehydration reaction that was observed to occur in the acylation conditions, every attempt was unsuccessful. The *ent* absolute stereochemistry of clerodane skeleton that had been determined in syphonoside (**1**) by a conformational analysis study[12] was in the present work further supported by comparison of the optical rotation value of compound **6** with those of literature data for related compounds. In particular, the negative $[\alpha]_D$ value of **6** was comparable (Fig. 1) with those of a series of *ent*-clerodanes, the absolute stereochemistry of which has been secured by different methods, including stereospecific synthesis.[16–19]

Analogously with the previously reported procedure,[12] 3-hydroxy-3-methyl glutaric acid was recovered as dimethyl ester derivative in the organic phase of alkaline hydrolysis of compound **2** and identified by comparison with a standard, whereas the glucose moiety, which was esterified in **2** at both C-1′ and C-6′, was recovered in the aqueous phase. The conversion of glucose into the corresponding α and β pentabenzoyl derivatives and the subsequent comparison of their CD curves with those of standards confirmed the same absolute stereochemistry of D-glucose as in syphonoside-A (**1**).

Analysis of the spectral data (see Section 4) of compound **3** clearly showed that it was a mixture of fatty acyl derivatives of compound **2**. In fact, the ^{1}H NMR spectrum of **3** differed from that of **2** in the chemical shift of H-11 resonating at δ 5.37 and in the additional presence of typical fatty acyl signals (δ 1.26 and 0.88). This suggested that the hydroxyl function at C-11 was in **3** esterified by a fatty acid. The LC-HRESI-MS spectrum indicated the presence of two major components with pseudo-molecular peaks (M+Na) at *m/z* 885 ($C_{48}H_{78}O_{13}Na$) and 913 ($C_{50}H_{82}O_{13}Na$). Analysis of these data led to the identification of the two fatty acid residues as palmitic and stearic acid.

The NMR spectra of compound **4**, which was isolated from the *n*-BuOH extract of the sea-grass and was not detected in the mollusc extracts, were similar to those of syphonoside (**1**), differing only in the presence of signals that were attributed to an additional acetyl group in the molecule. In the HRESI-MS spectrum the sodiated-molecular peak was observed at *m/z* 851 $(M+Na)^+$, which was consistent with the molecular formula $C_{40}O_{18}H_{60}$. Spectral analysis revealed that the acetyl residue had esterified the hydroxyl group at C-6‴. All proton and carbon resonances were assigned as reported in Section 4.

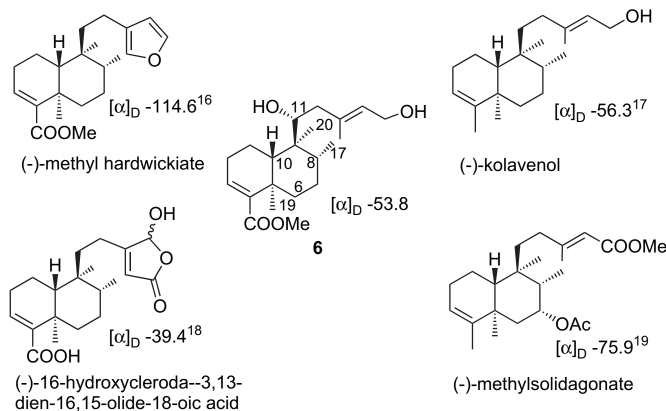


Figure 1. Comparison of the $[\alpha]_D$ values of aglycone **6** and known *ent*-clerodanes.

3. Conclusion

Following our previous study on the chemical constituents of the Lessepsian anaspidean *S. geographica* and its prey, the sea-grass *H. stipulacea*,[12] that had resulted in the isolation of a glucosyl terpenoid macrocycle, syphonoside (**1**), the related secondary metabolites of both organisms have been investigated. The diethyl ether extract of the mollusc was found to contain syphonosideol (**2**) and a mixture of syphonoside esters (**3**), whereas the 6‴-acetyl syphonoside (**4**) was isolated from the sea-grass. Compounds **2** and **3** were absent in the extract of the plant, suggesting that they might be obtained by a bio-transformation in the mollusc of the main metabolite syphonoside (**1**). Furthermore, the relative amount of syphonoside (**1**) in the extract of *S. geographica* was much higher with respect to that observed for *H. stipulacea*, thus indicating that this molecule is in the mollusc as a typical dietary accumulation product.

4. Experimental section

4.1. General procedures

General experimental procedures were performed as previously reported.[9]

4.2. Biological material

The collection of biological material (seven individuals of *S. geographica* and a sample of *H. stipulacea*) has been described in Refs. 9 and 12.

4.3. Isolation procedure

The Et_2O extract (589 mg) of internal organs was fractionated by Sephadex LH-20 chromatography eluted with chloroform/methanol (1:1) to give two glycoterpenoid-containing fractions, A (21.2 mg) and B (80.2 mg), which were further purified on preparative silica-gel TLC (90 and 80% chloroform/methanol, respectively). The mixture of fatty acid ester derivatives **3** (5.4 mg) was obtained from fraction A whereas pure compound **2** (12.0 mg) was obtained from fraction B. The *n*-BuOH soluble part of the sea-grass sample was processed as previously reported,[12] yielding a fraction from which pure compound **4** (8.3 mg) was obtained, after silica-gel preparative TLC purification (90% chloroform/methanol).

*4.3.1. Syphonosideol (**2**)*

TLC ($CHCl_3$/MeOH, 9:1 v/v): R_f=0.4; $[\alpha]_D$ +3.78 (*c* 0.19, $CHCl_3$); CD (EtOH), θ_{237} −3888; ^1H and ^{13}C NMR in Table 1; IR (liquid film): ν_{max} 3444.4, 2924.5, 1713.6, 758.0 cm^{-1}; UV (MeOH): λ_{max} 205 (ε=6340); HRMS (ESI): $(M+Na)^+$, found 647.3033 $(C_{32}H_{48}O_{12}Na)^+$ requires 647.3043.

*4.3.2. Syphonoside esters (**3**)*

TLC ($CHCl_3$/MeOH, 9:1 v/v): R_f=0.55; selected ^1H NMR chemical shifts (400 MHz, $CDCl_3$): δ 6.58 (m, H-3), 5.51 (d, *J*=8 Hz, H-1′), 5.40 (m, H-14), 5.37 (m, H-11), 4.64 (m, H-6′a), 4.58 (m, H_2-15), 4.33 (dd, *J*=6 and 12 Hz, H-6′b), 3.68−3.49 (overlapping signals, glucose protons), 1.80 (br s, H_3-16), 1.39 (s, H_3-6″), 1.26 (H_2-fatty acid), 1.00 (d, *J*=7 Hz, H_3-17), 0.88 (t, *J*=7 Hz, H_3-fatty acid); HRMS (ESI): $(M+Na)^+$, found 885.5322 $(C_{48}H_{78}O_{13}Na)^+$ requires 885.5340; found 913.5624 $(C_{50}H_{82}O_{13}Na)^+$ requires 913.5653.

*4.3.3. Compound **4***

TLC ($CHCl_3$/MeOH, 7:3 v/v): R_f=0.65; $[\alpha]_D$ −15.5 (*c* 0.3, MeOH); CD (EtOH) θ_{206} 12700, θ_{239} −5340; ^1H NMR (400 MHz, CD_3OD): δ 6.43 (1H, t, *J*=3 Hz, H-3), 5.51 (1H, d, *J*=8 Hz, H-1′), 5.46 (1H, br t, *J*=6 Hz, H-14), 4.65 (2H, m, H_2-15), 4.55 (1H, m, H-6′a), 4.52 (1H, m, H-6‴a), 4.30 (1H, d, *J*=8 Hz, H-1‴), 4.27 (2H, m, H-6′b and H-6‴b), 4.12 (1H, br d, *J*=9 Hz, H-11), 3.59 (1H, m, H-5′), 3.46−3.26 (6H, overlapping signals, H-2′, H-3′, H-3‴, H-4′, H-4‴and H-5‴), 3.19 (1H, t, *J*=8 Hz, H-2‴), 2.74 (1H, m, H-12a), 2.638 (2H, s, H_2-4″), 2.649 (1H, d, *J*=13 Hz, H_2-2″a), 2.57 (1H, d, *J*=13 Hz, H_2-2″b), 2.35 (1H, m, H-12b), 2.20 (2H, m, H_2-2), 2.18 (1H, m, H-6a), 2.05 (3H, s, $-COCH_3$), 2.00 (1H, m, H-8), 1.86 (3H, br s, H_3-16), 1.70 (2H, m, H_2-1), 1.55 (1H, m, H-10), 1.46 (3H, s, H_3-6″), 1.39 (2H, m, H_2-7), 1.34 (3H, s, H_3-19), 1.17 (1H, m, H-6b), 1.03 (3H, d, *J*=7 Hz, H_3-17), 0.99 (3H, s, H_3-20); ^{13}C NMR (300 MHz, CD_3OD): δ 172.8 (s, C-1″), 172.6 (s, *CO*CH3), 171.8 (s, C-5″), 169.3 (s, C-18), 144.6 (s, C-4), 140.8 (s, C-13), 138.5 (d, C-3), 123.5 (d, C-14), 102.1 (d, C-1‴), 95.5 (d, C-1′), 81.1 (d, C-11), 78.4 (d, C-3′or C-3‴or C-5‴), 78.1 (d, C-3‴or C-3′or C-5‴), 77.8 (d, C-5‴or C-3′or C-3″), 76.2 (d, C-5′), 75.1 (d, C-2‴), 74.0 (C-2′), 72.1 (d, C-4‴or C-4′), 71.6 (d, C-4′or C-4‴), 71.0 (s, C-3″), 63.9 (t, C-6′or C-6‴), 63.1 (t, C-6‴or C-6′), 62.4 (t, C-15), 49.6 (t, C-4″), 46.6 (d, C-10), 46.3 (t, C-2″), 45.6 (s, C-9), 42.4 (t, C-12), 38.7 (s, C-5), 36.5 (t, C-6), 35.7 (d, C-8), 30.7 (q, C-6″), 29.4 (t, C-7), 27.6 (t, C-2), 21.8 (q, C-19), 20.9 (q, $COCH_3$) 19.9 (t, C-1), 19.0 (q, C-17), 16.4 (q, C-16), 13.5 (q, C-20); IR (liquid film): ν_{max} 3584, 2922, 1714 cm^{-1}; UV (MeOH): λ_{max} 207 (ε=11,131); HRMS (ESI): $(M+Na)^+$, found 851.3680 $(C_{40}H_{60}O_{18}Na)^+$ requires 851.3677.

*4.3.4. Acetylation of **2***

Pure syphonosideol (**2**, 2.0 mg) was dissolved in 0.5 ml of dry pyridine and treated with 0.2 ml of acetic anhydride at room temperature. The reaction mixture was stirred overnight at room temperature. After this period, the solvent was evaporated and the crude material was subjected to silica gel column chromatography to afford 2.0 mg of the tetracetyl derivative (**5**). TLC ($CHCl_3$/MeOH, 9:1 v/v): R_f=0.8; ^1H and ^{13}C NMR in Table 1; HRMS (ESI): $(M+Na)^+$, found 815.3458 $(C_{40}H_{56}O_{16}Na)^+$ requires 815.3466.

*4.3.5. Alkaline methanolysis of **2***

Compound **2** (10.0 mg) was dissolved in 0.5 M solution of MeONa in MeOH (1.5 mL) and the resulting solution was stirred at room temperature for 1.5 h. The reaction mixture was initially chromatographed on a DOWEX-50W column eluted with methanol (100 mL) and then extracted with Et_2O to afford 6.0 and 5.6 mg of ethereal and aqueous phase, respectively. Analogously to **1**, LC−MS analysis of an aliquot of ethereal layer confirmed the identity of 3-hydroxy-3-methyl glutaric dimethyl ester.[12] The remaining ethereal phase was purified on preparative TLC ($CHCl_3$/MeOH, 97:3 v/v) to give 3.0 mg of pure aglycone **6**. TLC ($CHCl_3$/MeOH, 95:5 v/v): R_f=0.65; $[\alpha]_D$ −53.8 (*c* 0.2, $CHCl_3$); CD (*n*-hexane) θ_{209} −2231, θ_{243} −1436; ^1H and ^{13}C NMR in Table 1; IR (liquid film): ν_{max} 1713.6, 1381.7 cm^{-1}; MS (ESI): $(M+Na)^+$ 373.

The aqueous phase from the work-up was concentrated and analyzed by ^1H NMR, which proved the presence of glucose. The absolute stereochemistry of D-glucose was determined by analysis of ^1H NMR and CD spectra of the corresponding benzoate derivates, as previously described for **1**.[12]

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