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Blurring the Boundary between Bio- and Geohopanoids: Plakohopanoid, a C_{32} Biohopanoid Ester from *Plakortis* cf. *lita*

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Dedicated to the memory of Ernesto Fattorusso

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Plakohopanoid (**3a**), a new type of hopanoid derivative composed of a C_{32} hopanoid acid ester linked to a mannosyl-*myo*-inositol, was isolated from the sponge *Plakortis* cf. *lita* as its peracetyl derivative **3b**. The structure of **3b** was determined by a combination of spectroscopic analysis and microscale chemical degradation. Even though plakohopanoid was isolated from a sponge, its component parts are clearly of bacterial origin, and its bacterial biosynthesis is very likely. Until now, C_{32} hopanoic acids have been considered to be geohopanoids, i.e., diagenetic products that are formed through abiotic degradation of the biohopanoids present in bacteria. The presence of **3a** in a marine living organism shows that there is a biosynthetic pathway to C_{32} hopanoic acids, and these substances should therefore no longer with certainty be considered to be geohopanoids.

Introduction

Hopanoids (e.g., bacteriohopanetetrol, or BHT; **1a**) are bacterial metabolites composed of a pentacyclic C_{30} hopane triterpene linked to a sugar-derived polyfunctionalized C_5 chain (Scheme 1). They play a similar role in bacterial membranes to that played by sterols in eukaryotes. The pentacyclic ring moiety of hopanoids is very stable and not readily degraded, but after the death of the bacterial cell, the functionalized side-chain can undergo various abiotic degradative processes, including reduction (as far as alkanes), loss of carbon atoms, and epimerization. Degraded hopanoids are ubiquitous in sediments, rocks, and crude oil,[1] and, because the characteristic pentacyclic moiety is preserved, they can easily be recognized and may be used as biomarkers in geochemical studies. Consequently, hopanoids are generally classified into two classes: biohopanoids, produced in bacteria by biosynthetic processes, and geohopanoids, derived from abiotic degradation of biohopanoids.

There are a few hopanoids that escape this classification. Among them is 32,35-anhydro-BHT (**2**), which was first isolated in our laboratory from the Caribbean sponge *Plakortis simplex*,[2] and was later shown to be present in the cells of the bacterial symbionts of this sponge,[3] and should therefore be considered to be a biohopanoid (Scheme 1). Very recently, however, Schaeffer et al. demonstrated that anhydro-BHT can be formed from BHT and other hopanoids by geochemical processes,[4] so that the anhydro-BHT that is present (sometimes predominantly) in sediments may be of abiotic origin.

In this paper, we describe the isolation and the structural elucidation of a new biohopanoid, plakohopanoid (**3a**) from the Indonesian sponge *Plakortis* cf. *lita*. Plakohopanoid is an ester composed of a C_{32} hopanoic acid, a well-known hopanoid that has, until now, been considered to be a typical example of a geohopanoid, and mannosyl-*myo*-inositol, a building block of phosphatidylinositol mannosides, which are characteristic of Mycobacteria and related species.

Results and Discussion

The MeOH extract of an Indonesian specimen of *Plakortis* cf. *lita* was partitioned between BuOH and H_2O, and the organic phase was purified by reverse-phase chromatography followed by normal-phase chromatography to give a crude fraction mainly composed of glycolipids, but also containing hopanoids. This fraction was acetylated with Ac_2O in pyridine. The composition of the peracetylated glycolipid fraction was analyzed using normal-

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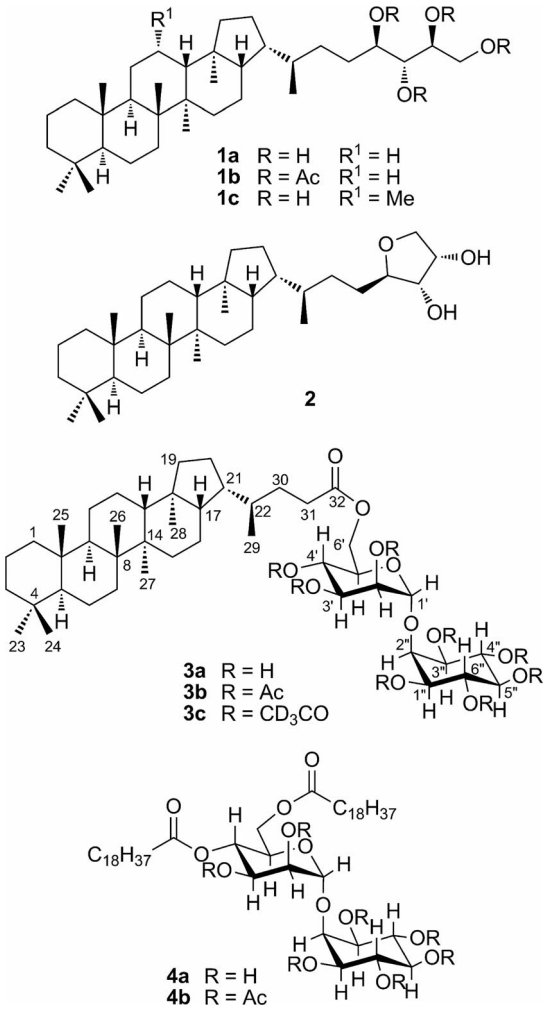
phase HPLC, and it turned out to be very similar to the glycolipid fraction of *P. simplex* from the Caribbean Sea, which has been extensively studied by our group. ^{1}H NMR spectroscopic analysis of the collected HPLC fractions showed the presence of the unusual metabolites previously reported from *P. simplex*, i.e., plakoside A and B,[5] simplexides,[6] 12-methylbacteriohopanetetrol,[7] and crasserides.[8] In addition, the HPLC separation provided the new hopanoid derivative, plakohopanoid (**3a**) as its peracetyl derivative **3b** (Scheme 1). Compound **3b** could not be deacetylated to give the natural hopanoid (i.e., **3a**), because **3b** is itself an ester. Nor were we able to isolate pure **3a** from the crude glycolipid fraction, because of the well-known low solubility of biohopanoids in most organic solvents.[9] However, the presence of natural **3a** in the glycolipid fraction could be demonstrated by an LC–HRMS experiment (see below).



Scheme 1.

The ESI HRMS spectrum of compound **3b** showed a pseudomolecular $[M + Na]^+$ ion peak at m/z = 1153.5885, indicating the molecular formula $C_{60}H_{90}O_{20}$. Analysis of the ^{1}H NMR spectrum of **3b** ($CDCl_3$) showed the molecule to be composed of a terpenoid moiety (6 methyl singlets at δ = 0.94, 0.93, 0.84, 0.80, 0.78, and 0.69 ppm, and a methyl doublet at δ = 0.92 ppm) and an acetylated carbohydrate moiety (13 carbinol protons between δ = 5.55 and 4.05 ppm, and 8 acetyl singlets between δ = 2.14 and 2.00 ppm).

Examination of the correlation peaks of the methyl protons in the HMBC spectrum allowed us to assign most of the carbon atoms in the terpene skeleton of **3b** and to sketch the partial structure depicted in Figure 1. This fitted well with a hopane skeleton, which, according to the ^{13}C chemical shifts, should be devoid of any functional groups. Indeed, a comparison of the ^{13}C spectra of **3b** and bacteriohopanetetrol tetraacetate (**1b**) showed that for all the signals of C-1–C-30 in the ^{13}C spectrum of bacteriohopanetetrol tetraacetate, there was a corresponding signal within 0.2 ppm in the spectrum of **3b**. This implied that the terpenoid skeletons of **1b** and **3b** had the same structure and relative configuration, i.e., that of an extended hopane. The configuration at C-22, which does not significantly affect ^{13}C chemical shifts,[10] was determined on the basis of the ^{1}H chemical shift of CH_3-29 (δ = 0.92 ppm), which is diagnostic for the 22*R* configuration.[10]

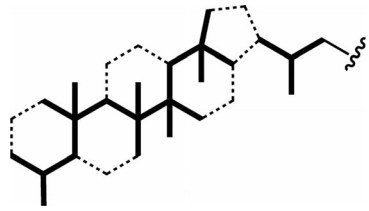


Figure 1. Partial structure of plakohopanoid peracetate **3b** as determined by long-range proton–carbon couplings of methyl protons (bold lines).

The chemical shifts of the protons in the hopane part of the molecule were then identified using an HSQC (heteronuclear single-quantum correlation) experiment (Table 1). All the correlation peaks that were present in the COSY, TOCSY, and HMBC spectra were consistent with a hopane skeleton and with the assignment reported in Table 1. The structure of the remaining C_2 fragment of the side-chain that completed the structure of the hopanoid skeleton was deduced from the correlations of the methylene protons at C-31 (δ = 2.37 and 2.25 ppm) with the methylene protons at C-30 in the COSY spectrum, and with the ester carbonyl carbon atom at δ = 173.9 ppm in the HMBC spectrum.

For structural elucidation of the carbohydrate moiety of **3b**, a second set of one- and two-dimensional NMR experiments was recorded using C_6D_6 as the solvent, because a better signal dispersion was observed in the mid-field region of the ^{1}H NMR spectrum using this solvent. The ^{13}C NMR spectrum showed the presence of only one anomeric carbon (δ = 100.0 ppm), which was correlated in the HSQC spectrum with the ^{1}H doublet at δ = 4.98 ppm (J = 2.0 Hz). Using this resonance as the starting point, analysis of COSY correlation peaks allowed us to identify a sequence of four methine protons and a pair of methylene protons at δ = 5.65 (2′-H), 5.77 (3′-H), 5.85 (4′-H), 4.52 (5′-H), and 4.57/4.44 ppm (6′-H_2), accounting for a hexose. That this was in the pyranose form was deduced from the relatively

Table 1. NMR spectroscopic data for compound **3b**.

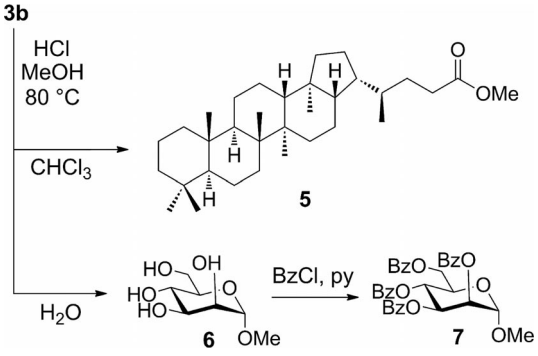
Pos.		700 MHz, $CDCl_3$ δ_H [ppm] (J [Hz])[a]	175 MHz, $CDCl_3$ δ_C, type	500 MHz, C_6D_6 δ_H [ppm] (J [Hz])[a]	125 MHz, C_6D_6 δ_C, type
1		1.64, 0.76	40.1, CH_2	1.65, 0.77	40.6, CH_2
2		1.56, 1.34	18.7, CH_2	1.60, 1.38	19.1, CH_2
3		1.34, 1.12	42.1, CH_2	1.38, 1.16	42.4, CH_2
4		–	33.4, C	–	33.4, C
5		0.70	56.1, CH	0.75	56.5, CH
6		1.48, 1.31	18.6, CH_2	1.53, 1.34	19.1, CH_2
7		1.45, 1.21	33.2, CH_2	1.49, 1.23	33.7, CH_2
8		–	41.8, C	–	42.0, C
9		1.24	50.4, CH	1.28	50.8, CH
10		–	37.4, C	–	37.6, C
11		1.52, 1.29	20.9, CH_2	1.53, 1.27	21.3, CH_2
12		1.44, 1.39	23.9, CH_2	1.42, 1.42	24.3, CH_2
13		1.31	49.2, CH	1.30	49.6, CH
14		–	41.6, C	–	41.9, C
15		1.34, 1.23	33.6, CH_2	1.31, 1.22	34.0, CH_2
16		1.71, 1.52	22.8, CH_2	1.66, 1.50	23.1, CH_2
17		1.28	54.4, CH	1.18	54.6, CH
18		–	44.3, C	–	44.5, C
19		1.52, 0.90	41.5, CH_2	1.54, 0.88	41.8, CH_2
20		1.81, 1.54	27.5, CH_2	1.81, 1.64	27.8, CH_2
21		1.73	45.8, CH	1.68	46.3, CH
22		1.51	36.2, CH	1.56	36.6, CH
23		0.84 (s)	33.3, CH_3	0.912 (s)	33.6, CH_3
24		0.78 (s)	21.6, CH_3	0.863 (s)	21.8, CH_3
25		0.80 (s)	15.9, CH_3	0.857 (s)	16.2, CH_3
26		0.93 (s)	16.5, CH_3	0.976 (s)	16.9, CH_3
27		0.94 (s)	16.6, CH_3	0.960 (s)	16.7, CH_3
28		0.69 (s)	15.8, CH_3	0.720 (s)	16.0, CH_3
29		0.92 (d, 7.6)	19.7, CH_3	0.954 (d, 6.5)	19.9, CH_3
30		1.81, 1.29	30.5, CH_2	2.04, 1.43	31.1, CH_2
31	a	2.37 (ddd, 15.9, 10.6, 5.0)	30.9, CH_2	2.45 (ddd, 15.9, 9.9, 5.5)	31.3, CH_2
	b	2.25 (ddd, 15.9, 10.0, 6.0)		2.41 (ddd, 15.9, 9.1, 6.8)	
32		–	173.9, C	–	173.5, C
1′		4.95 (d, 1.7)	99.4, CH	4.98 (d, 2.0)	100.0, CH
2′		5.36 (dd, 3.1, 1.7)	69.6, CH	5.65 (dd, 3.2, 2.0)	70.4, CH
3′		5.39	65.5, CH	5.77 (dd, 10.0, 3.2)	69.3, CH
4′		5.40	68.6, CH	5.85 (t, 10.0)	66.0, CH
5′		4.18 (m)	69.4, CH	4.52 (ddd, 10.0, 3.9, 2.0)	70.5, CH
6′	a	4.26 (dd, 12.5, 4.3)	61.7, CH_2	4.57 (dd, 12.3, 3.9)	62.0, CH_2
	b	4.09 (dd, 12.5, 2.2)		4.44 (dd, 12.3, 2.0)	
1′′		5.00 (dd, 10.5, 2.5)	69.6, CH	4.88(dd, 10.6, 2.4)	70.3, CH
2′′		4.29 (t, 2.5)	76.4, CH	4.20 (t, 2.4)	77.3, CH
3′′		5.08 (dd, 10.5, 2.5)	70.3, CH	5.01 (dd, 10.4, 2.4)	70.7, CH
4′′		5.53 (dd, 10.5, 9.9)	69.5, CH	5.902(dd, 10.4, 9.7)	70.6, CH
5′′		5.19 (t, 9.9)	70.6, CH	5.33 (t, 9.7)	71.3, CH
6′′		5.49 (dd, 10.5, 9.9)	69.3, CH	5.897 (dd, 10.4, 9.7)	70.0, CH
Ac		2.14, 2.09, 2.07, 2.06, 2.04, 2.03, 2.01, 2.00 (8 singlets)	20.9–20.5, CH_3	1.935, 1.880, 1.672, 1.672, 1.665, 1.625, 1.599, 1.599 (8 singlets)	20.4–20.1, CH_3
			169.9–169.4, C		169.6–169.1, C

[a] Multiplicity not shown for overlapping signals; chemical shifts for these signals were determined by 2D NMR spectroscopy.

shielded chemical shift of 5′-H and the coupling between 5′-H and C-1′ in the HMBC spectrum. The large couplings (10.0 Hz) of 4′-H with both 3′-H and 5′-H were indicative of the axial orientation of these three protons, while the small coupling (3.3 Hz) of 3′-H with 2′-H showed that the latter proton was equatorial. Therefore, the hexopyranose unit was a mannopyranose unit. The α anomeric configuration of the mannose unit was deduced from the 173 Hz coupling constant between 1′-H and C-1′ (which we could measure from the residual 1′-H,C-1′ one-bond correlation observed in the HMBC spectrum), because it is known from the literature[11] that $^1J_{C,H}$ coupling constants are around 158–162 Hz for axial anomeric protons, and around 169–171 Hz for equatorial anomeric protons. The remaining six oxymethine protons observed in the mid-field region of the proton NMR spectrum were shown from COSY data to be cyclically arranged, and therefore were indicative of an inositol unit. The *myo* configuration of the inositol was deduced by coupling constant analysis (Table 1), which showed all the inositol protons to be axial except for 2′′-H (δ = 4.20 ppm). The shielded chemical shift of 2′′-H and the HMBC correlation between 2′′-H and C-1′ showed that the *myo*-inositol was glycosylated at O-2′′ by the α-mannopyranose unit. Finally, the linkage between the terpene and

carbohydrate moieties of plakohopanoid was identified as an ester bond between the carbonyl C-32 and the mannose 6′-oxymethylene group, on the basis of the correlation peaks in the HMBC spectrum between both C-6′ protons and C-32.

The carbohydrate moiety of plakohopanoid peracetate (**3b**) is very similar to that of discoside peracetate (**4b**), previously isolated by our group from *Discodermia dissoluta*,[12] but also present in Caribbean specimens of *Plakortis* (unpublished data) as well as in the Indonesian specimen studied in this paper. In **4a**, there are two fatty acid residues ester-linked to the mannosylinositol unit at O-4′ and O-6′. In plakohopanoid (**3a**) they are replaced by a single C_{32} hopanoyl unit linked at O-6′. A comparison between the proton NMR spectra of **3b** and **4b** revealed that all the carbohydrate signals show almost identical chemical shifts and coupling constants in the two compounds, further confirming structure **3b**. The absolute configuration of plakohopanoid was established by micro-scale chemical degradation (Scheme 2). Acidic methanolysis of **3b** with 1 M HCl in 92% MeOH produced a mixture of hopanoic acid methyl ester (**5**) and methyl mannopyranoside (**6**). After partitioning between $CHCl_3$ and H_2O, these two compounds were found, respectively, in the $CHCl_3$ and H_2O layers.



Scheme 2. Degradation procedure of plakohopanoid peracetate (**3b**).

Hopanoic ester **5** was purified by HPLC (SiO_2, *n*-hexane/EtOAc, 95:5), and its ^1H NMR spectrum matched that reported in the literature.[13] In addition, the positive optical rotation of **5** ($[\alpha]_D$ = +83) confirmed the absolute configuration of the hopane skeleton. Methyl mannopyranoside (**6**) was benzoylated and purified by HPLC as described[12] to give tetrabenzoate **7**. The ^1H NMR and CD spectra of compound **7** were identical to those reported for synthetic methyl α-D-mannopyranoside perbenzoate.[12] This completed the elucidation of the structure and stereochemistry of plakohopanoid peracetate **3b**.

As reported above, we were not able to obtain natural **3a** in pure form. The possible pre-occurrence of an acetyl group in the natural compound was therefore excluded by acetylating a small portion of the crude glycolipid mixture from *P. simplex* with deuterated acetic anhydride. The resulting octakis(trideuteroacetate)-protected plakohopanoid (i.e., **3c**) was isolated, and it showed a proton NMR spectrum identical to that of **3b**, except for the absence of the eight acetyl singlets.

A further confirmation of the presence of natural plakohopanoid **3a** in *P.* cf. *lita* came from high-resolution HPLC–ESI-MS analysis of the crude glycolipid mixture (before

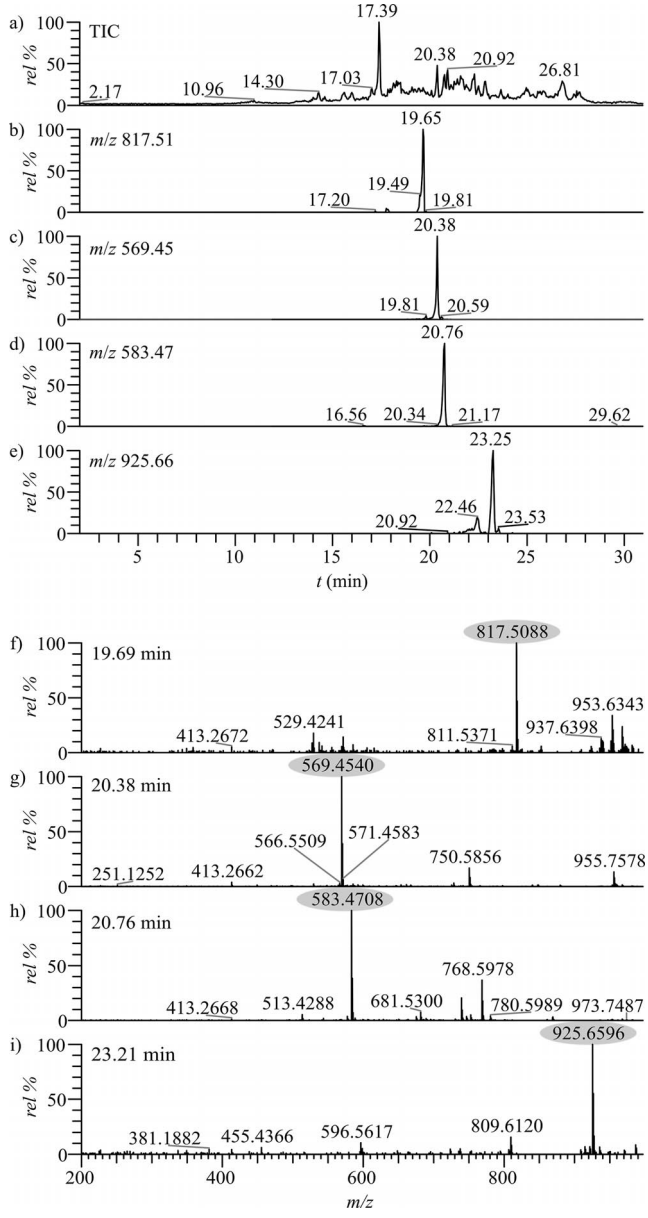


Figure 2. HPLC–ESI-MS analysis of the crude glycolipid fraction of *P.* cf. *lita* and extracted-ion chromatograms of the $[M + Na]^+$ ions of several metabolites of the sponge. From top to bottom: (a) total ion chromatogram; (b) extracted ion chromatogram of mass *m/z* = 817.51 for plakohopanoid (**3a**); (c) extracted ion chromatogram of mass *m/z* = 569.45 for bacteriohopanetetrol (**1a**); (d) extracted ion chromatogram of mass *m/z* = 583.47 for 12-methylbacteriohopanetetrol (**1c**); (e) extracted-ion chromatogram of mass *m/z* = 925.66 for the major homologue of discoside (**4a**); (f–i) mass spectra at retention times 19.69, 20.38, 20.76, and 23.21 min, showing peaks for **3a** (calcd. 817.5072), **1a** (calcd. 569.4540), **1c** (calcd. 583.4697), and **4a** (calcd. 925.6587), respectively.

acetylation). Extracted-ion chromatograms were produced at mass values corresponding to plakohopanoid (**3a**) and to three compounds previously isolated from *P. simplex*, namely bacteriohopanetetrol (**1a**), 12-methylbacteriohopanetetrol (**1c**),[7] and discoside (**4a**).[12] The chromatograms showed peaks for all these compounds, with an elution order that matched their expected lipophilicity (Figure 2). In addition, each of the full mass spectra recorded at the elution time of the individual compounds showed a prominent peak, whose exact mass value was in very good agreement with the calculated mass.

Conclusions

The structure of plakohopanoid (**3a**) was determined by a combination of spectroscopic analysis and micro-scale chemical degradation. Plakohopanoid represents a new type of natural hopanoid derivative, composed of a C_{32} hopanoid acid ester linked to a carbohydrate part. Even though **3a** was isolated from a sponge, its constituent parts, hopanoic acid and mannosyl-*myo*-inositol, are clearly of bacterial origin. Therefore, its bacterial biosynthesis is very likely, especially if one considers that sponge-associated bacteria are extremely abundant in *Plakortis* species (they account for over 50% of the sponge weight), and that there is evidence[3] that several of the many metabolites isolated from this sponge are actually produced by the bacterial symbionts.

Until now, C_{32} hopanoic acids have been found in sediments and other geological formations, and have been considered to be diagenetic products, formed through abiotic degradation of the biohopanoids present in bacteria. Diagenetic transformation from biohopanoids to geohopanoids can take place quickly after the death of bacteria, and is well documented. However, the presence of plakohopanoid in a marine living organism shows that there is a biosynthetic pathway to C_{32} hopanoic acids, and these substances should therefore no longer with certainty be considered to be geohopanoids.

The occurrence of 12-methylbacteriohopanetetrol (**1c**) in the Indonesian specimen of *Plakortis* also deserves a comment. This compound was isolated by our research group in 2000 from a Caribbean specimen of *P. simplex*,[7] and it was the first example of a hopanoid methylated at C-12. Since then, no further 12-methylhopanoids have been found, which may be compared to the 19 naturally occurring 2-methylhopanoids and the 25 naturally occurring 3-methylhopanoids that have been described to date. Bacterial communities associated with many species of sponges are highly specific, phylogenetically very different from free-living bacteria in the surrounding water, and consistently conserved in specimens collected at different times and in different geographical areas,[14] suggesting vertical transmission within their hosts[15] and an independent evolutionary history. Therefore, it is very possible that a unique biosynthetic pathway to 12-methylhopanoids has been developed in some symbiotic bacteria of *Plakortis* sponges. This is further proof of the biosynthetic diversity and biotechnological potential of sponge-associated bacteria.

Experimental Section

General Methods: Optical rotations were measured at 589 nm with a Jasco P-2000 polarimeter using a 10 cm microcell. High Resolution ESI-MS and ESI-MS/MS spectra were performed with a Thermo LTQ Orbitrap XL mass spectrometer. The spectra were recorded by infusion into the ESI source using MeOH as the solvent. EI-MS spectra were performed using an Agilent 6850 II/5973 MSD GC–MS instrument and a HP-5MS capillary column (Agilent, 5% phenyl methyl siloxane; 30 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas, injection was in split mode, the program was as follows: hold at 150 °C for 15 min, heat to 300 °C at 5 °C min^{-1}, hold at 300 °C for 10 min. NMR spectra were determined with Varian Unity Inova spectrometers at 500 MHz and 700 MHz. Chemical shifts were referenced to the residual solvent signals ($CDCl_3$: δ_H = 7.26, δ_C = 77.0; C_6D_6: δ_H = 7.15, δ_C = 128.15). For an accurate measurement of the coupling constants, the one-dimensional ^{1}H NMR spectra were transformed at 64 K points (digital resolution: 0.09 Hz). Homonuclear ^{1}H connectivities were determined by COSY experiments. The HSQC and HMBC experiments were adjusted, respectively, for an average $^1J_{CH}$ of 142 Hz and a $^{2,3}J_{CH}$ of 8.3 Hz. HPLC was performed with a Varian Prostar 210 apparatus equipped with a Varian 350 refractive index detector.

Animal Material: A specimen of *Plakortis* cf. *lita* (order Homosclerophorida, family Plakinidae) was collected in January 2008 along the coasts of the Bunaken island in the Bunaken Marine Park of Manado. A voucher sample (No. MAN08–02) has been deposited at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II.

Extraction and Isolation: The sponge (380 mL by volume before extraction, and 50 g by dry weight after extraction) was cut into pieces and extracted with MeOH (3 × 1.5 L), MeOH/$CHCl_3$ (4 × 1.5 L) and $CHCl_3$ (2 × 1.5 L). The MeOH extracts were partitioned between H_2O and BuOH, and the organic layer was added to the $CHCl_3$ extracts to give 8.5 g of a dark brown oil, which was subjected to chromatography on a column packed with R-18 silica-gel. The fraction that was eluted with 100% $CHCl_3$ (1.8 g) was subjected to further chromatographic separation on a SiO_2 column with solvent of increasing polarity. The fraction that was eluted with EtOAc/MeOH (7:3) was mainly composed of glycolipids. This fraction (0.4 g) was acetylated by treatment with Ac_2O in pyridine overnight, and then subjected to HPLC separation on a SiO_2 column [10 μm, 250 × 10 mm; eluent: *n*-hexane/EtOAc (6:4); flow: 4 mL min^{-1}], affording crasserides (44 mg),[8] bacteriohopanetetrol **1a** and 12-methylbacteriohopanetetrol **1c** (as a mixture, 112 mg),[7] plakoside A and B and simplexides (as a mixture, 16 mg),[5,6] and discoside (5 mg),[12] all as their respective peracetylated derivatives, which were identified from their ^{1}H NMR spectra. In addition, the HPLC separation gave pure plakohopanoid as its peracetyl derivative **3b** (2.1 mg).

Plakohopanoid Peracetate (3b): Colorless amorphous solid. $[\alpha]_D^{25}$ = +57 (*c* = 0.1 in $CHCl_3$). ^{1}H NMR (700 MHz, $CDCl_3$ and 500 MHz, C_6D_6) and ^{13}C NMR (175 MHz, $CDCl_3$ and 125 MHz, C_6D_6): see Table 1. HRMS (ESI$^+$, MeOH): calcd. for $C_{60}H_{90}O_{20}Na$ $[M + Na]^+$ 1153.5918; found 1153.5885.

HPLC–ESI-MS Analysis: For LC-MS, the Orbitrap MS spectrometer was coupled to an Agilent model 1100 LC, which included a solvent reservoir, in-line degasser, binary pump, and refrigerated

autosampler. A 2.6 μm Kinetex C18 column (50 × 2.1 mm), maintained at room temperature, was used. It was eluted at 0.2 mL min^{-1} with H_2O and MeOH, using as a gradient elution 60–100% MeOH over 15 min and hold 15 min. A 2 mg mL^{-1} MeOH solution of the crude glycolipid fraction was prepared, and 5 μL of this solution was injected. The results are shown in Figure 2.

Degradation Analysis of Compound 3b: Compound **3b** (0.5 mg) was dissolved in 1 N HCl in 92% MeOH (500 μL), and the solution obtained was kept at 80 °C for about 12 h. The reaction mixture was dried under nitrogen and then partitioned between H_2O and $CHCl_3$. The H_2O layer contained methyl glycoside **6**, which was benzoylated with benzoyl chloride (50 μL) in pyridine (500 μL) at 25 °C for 16 h. The reaction was quenched with MeOH, and after 30 min, the mixture was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum with an oil pump for 24 h, and the residue was purified by HPLC [SiO_2 column, 5 μm, 250 × 4.6 mm; eluent: *n*-hexane/*i*PrOH (99:1); flow: 1 mL min^{-1}]. The chromatogram contained a peak (t_R = 9.5 min), which was collected and identified as methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**7**) by comparison of its ^{1}H NMR and CD spectra with those reported.[12] The $CHCl_3$ layer from the methanolysis reaction was concentrated and purified by HPLC [SiO_2 column, 5 μm, 250 × 4.6 mm; eluent: *n*-hexane/EtOAc (95:5); flow: 1 mL min^{-1}] to give pure compound **5** (0.2 mg).

Methyl (22*R*)-33,34,35-Trinorbacteriohopan-32-oate (5): $[\alpha]_D^{25}$ = +83 (*c* = 0.015 in $CHCl_3$); ref.[13] +58. ^{1}H NMR ($CDCl_3$): data matched that reported.[13] MS (EI): *m*/*z* (%) = 484 (4.6) [M$^+$], 469 (9.2), 369 (20.0), 263 (100), 231 (6.4), 191 (32.2).

Supporting Information (see footnote on the first page of this article): 1D and 2D NMR spectra of compound **3b**. EI-MS and ^{1}H NMR spectra of compound **5**.

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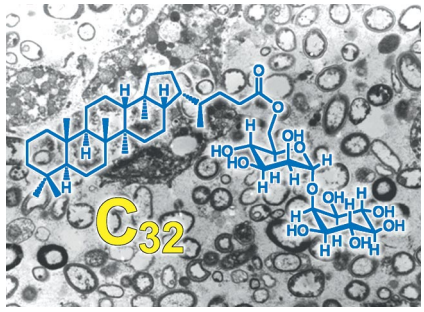
Natural Products

Plakohopanoid is a new type of hopanoid composed of a C_{32} hopanoic acid ester linked to a mannosyl-*myo*-inositol. It is probably produced by bacterial symbionts of *Plakortis* cf. *lita*. The existence of a biosynthetic pathway to the C_{32} hopanoic acid shows that this compound should not be necessarily considered to be a geohopanoid.



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Blurring the Boundary between Bio- and Geohopanoids: Plakohopanoid, a C_{32} Biohopanoid Ester from *Plakortis* cf. *lita*

Keywords: Natural products / Biosynthesis / Terpenoids / Glycolipids / Hopanoids

