Glycolipids from Sponges. 11.[1] Isocrasserides, Novel Glycolipids with a Five-Membered Cyclitol Widely Distributed in Marine Sponges

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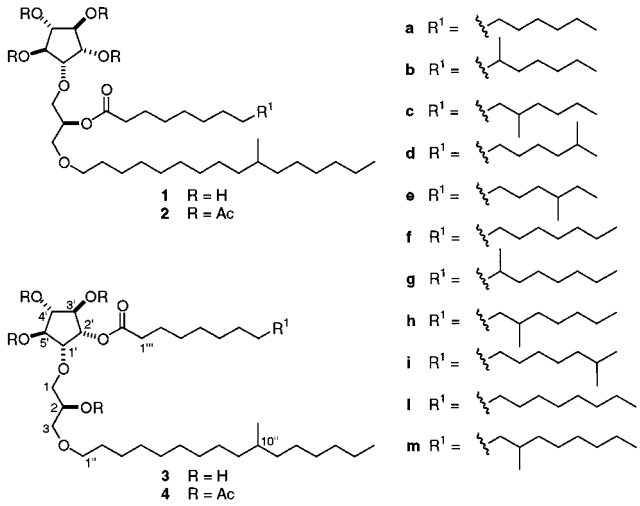
In addition to the previously reported crasserides (**1a**−**m**), marine sponges contain smaller amounts of the isomeric isocrasserides (**3a**−**m**). The structures of these new glycoglycerolipid analogues were determined by NMR analysis and chemical degradation. Crasserides and isocrasserides were present in all the species of marine sponges we analyzed and therefore appear to be characteristic of the phylum Porifera.

Crasserides (**1a**−**m**) are unique glycolipids first isolated from the Caribbean sponge *Pseudoceratina crassa* Hyatt (family Dysideidae). Their structures resemble common glycoglycerolipids such as MGDG (monogalactosyldiacylglycerol), but are different in that an *O*-1-alkyl group is present instead of the usual *O*-1-acyl group and, above all, the sugar unit is replaced by an unprecedented five-membered cyclitol, linked to *O*-3 with an ether bond.[2,3] Since their first isolation, crasserides have been shown to be present in all the species of sponges whose glycolipids have been studied by our group, namely, *Verongula gigantea* Hyatt (family Dysideidae), *Aplysina fulva* Pallas (family Aplysinidae), *Aplysina cauliformis* Carter (family Aplysinidae), *Neofibularia nolitangere* Duchassaing and Michelotti (family Mycalidae), *Agelas clathrodes* Schmidt (family Agelasidae), *Agelas dispar* Duchassaing and Michelotti (family Agelasidae), *Agelas conifera* Schmidt (family Agelasidae), *Agelas longissima* Pulitzer-Finali (family Agelasidae), *Plakortis simplex* Schultze (family Plakinidae), *Ectyoplasia ferox* Duchassaing and Michelotti (family Raspailiidae), and *Siphonodictyon coralliphagum* Rützler (family Niphatidae), so that they appear to be metabolites characteristic of the phylum Porifera. We wish to report here that crasserides are associated with minor amounts of related glycolipids, never described before, that we have named isocrasserides (**3a**−**m**). The isolation and structure determination of isocrasserides is the subject of this paper.

Results and Discussion

Isocrasserides were isolated as their peracetyl derivatives **4a**−**m** from the Caribbean sponge *Plakortis simplex*. Although the procedure of isolation as described here refers to this organism, it was applied to every species we have analyzed, with a few or no modifications. Specimens of *P. simplex* (64 g of dry weight after extraction) were collected in the summer of 1998 along the coast of Berry Island (Bahamas) and extracted in sequence with methanol and chloroform. The extract was partitioned between *n*-BuOH and water, and the *n*-BuOH phase was subjected to reversed-phase chromatography followed by normal-phase chromatography, yielding a crude glycolipid fraction. This was acetylated with Ac_2O in pyridine and repeatedly subjected to HPLC on a SiO_2 column using *n*-hexane/EtOAc (6:4) and *n*-hexane/EtOAc (8:2) as eluents.



The latter separation led to 36 mg of a mixture of peracetylated crasserides (**2a**−**m**), identified (except for the length and branching of the alkyl chains, see below) by comparison of its ^1H and ^{13}C NMR spectra with those of an authentic sample. In addition, 5.9 mg of a mixture of the novel peracetylated isocrasserides (**4a**−**m**) was also obtained.

Examination of the ^1H NMR spectrum in $CDCl_3$ of the mixture **4a**−**m** showed it to be very similar to that of the mixture of crasseride acetates **2a**−**m** because the spectra were almost identical, the most significant difference being the chemical shift of H_2-2″ at δ 2.37 instead of δ 2.31. Like crasserides, isocrasserides were isolated as a mixture of homologues with different branching at the alkyl chains, as shown by the complex methyl signal around δ 0.85, arising from the superimposition of the triplet at δ 0.88 of linear chains, the doublet at δ 0.85 of the iso chains, and the doublet at δ 0.83 of the anteiso and internally branched chains.[2]

The high-resolution EI mass spectrum of **4a**−**m** showed a series of molecular ion peaks at *m*/*z* 882.6498, 868.6339, and 854.6159, in agreement with the molecular formula $C_{48}H_{86}O_{12} + n CH_2$ ($n = 0-2$), identical to that of peracetylated crasserides **2a**−**m**.[2] The subsequent NMR experiments were performed using C_6D_6 as the solvent because in $CDCl_3$ the resonances of three cyclitol protons were overlapped. With the aid of the COSY spectrum we could readily identify the $CH_2-CH-CH_2$ system of the glycerol, the five cyclically arranged protons of the cyclitol, and the

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Table 1. ^{1}H and ^{13}C NMR Data of Isocrasserides **3a**−**m** (CD_3OD)[a] and Their Peracetyl Derivatives **4a**−**m** (C_6D_6)[b]

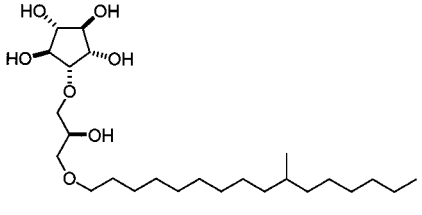
		3a−**m**		**4a**−**m**	
position		δ_H [mult., *J* (Hz)]	δ_C [mult.]	δ_H [mult., *J* (Hz)]	δ_C [mult.]
1	a	3.60 (dd, 10.0, 4.5)	73.1 (CH_2)	3.84 (m)	69.6 (CH_2)
	b	3.56 (dd, 10.0, 5.0)		3.81 (m)	
2		3.83[c]	70.4 (CH)	5.33 (quintet, 5.0)	71.5 (CH)
3	a	3.51[c]	73.0 (CH_2)	3.53 (dd, 10.5, 5.4)	68.9 (CH_2)
	b	3.44 (dd, 10.0, 6.3)		3.50 (dd, 10.5, 4.8)	
1′		3.72 (t, 6.3)[d]	82.5 (CH)	4.10 (t, 4.8)[d]	80.3 (CH)
2′		4.91 (t, 5.8)[d]	76.6 (CH)	5.45 (t, 5.5)[d]	73.6 (CH)
3′		3.89 (t, 5.9)[d]	79.1 (CH)	5.80 (t, 5.7)[d]	78.1 (CH)
4′		3.63 (t, 6.7)[d]	80.7 (CH)	5.51 (t, 4.7)[d]	78.0 (CH)
5′		3.84 (t, 6.9)[d]	80.1 (CH)	5.57 (t, 4.5)[d]	78.2 (CH)
1″	a	3.49[c]	72.4 (CH_2)	3.33 (ddd, 9.0, 6.6, 6.6)	71.4 (CH)
	b	3.49[c]		3.27 (ddd, 9.0, 6.6, 6.6)	
2″		1.60 (m)	30.5 (CH_2)	1.55 (m)	29.9 (CH_2)
3″		1.37[c]	26.2 (CH_2)	1.32[c]	26.4 (CH_2)
1‴					172.5 (C)
2‴		2.41 (m)	34.8 (CH_2)	2.25 (m)	33.7 (CH_2)
3‴		1.67 (m)	25.8 (CH_2)	1.62[c]	25.0 (CH_2)

[a] Additional ^{1}H signals: δ 1.55 (1H, m, isopropyl methine group), 1.32 (large signal, alkyl chain methylene groups), 0.93 (3H, d, J = 6.5 Hz, ω-methyl group), 0.91 (3H, d, J = 6.7 Hz, isopropyl methyl groups), 0.89 (3H, d, J = 6.7 Hz, branch methyl group); additional ^{13}C signals: δ 32.8 (CH_2, ω-2), 30.0−29.1 (alkyl chain methylene carbon atoms), 23.3 (CH_2, ω-1), 22.8 (CH_3, isopropyl methyl groups), 19.9 (CH_3, branch methyl group), 14.1 (CH_2, ω methyl groups). [b] Additional ^{1}H signals: δ 1.79 (3H, s, Ac), 1.68 (3H, s, Ac), 1.64 (6H, s, Ac), 1.32 (large signal, alkyl chain methylene groups), 0.93 (3H, d, J = 7.0 Hz, branch methyl group), 0.91 (3H, d, J = 6.5 Hz, ω-methyl group), 0.90 (3H, d, J = 6.7 Hz, isopropyl methyl groups); additional ^{13}C signals: δ 169.7−169.3 (acetyl CO), 32.8 (CH_2, ω-2), 30.8−29.2 (alkyl chain methylene carbon atoms), 23.0 (CH_2, ω-1), 22.6 (CH_3, isopropyl methyl groups), 19.8 (CH_3, branch methyl group), 20.6−20.2 (acetyl CH_3), 14.2 (CH_2, ω methyl groups). [c] Submerged by other signals. [d] All cyclitol protons appeared as triplets in the ^{1}H NMR spectrum, even though the coupling constants with the vicinal protons are slightly different; combined analysis of all measured values allowed to determine the actual coupling constants as follows. Compounds **3a**−**m**: $J_{1',2'}$ = 6.0 Hz, $J_{2',3'}$ = 5.6 Hz, $J_{3',4'}$ = 6.2 Hz, $J_{4',5'}$ = 7.2 Hz, $J_{5',1'}$ = 6.6 Hz. Compounds **4a**−**m**: $J_{1',2'}$ = 4.9 Hz, $J_{2',3'}$ = 6.1 Hz, $J_{3',4'}$ = 5.1 Hz, $J_{4',5'}$ = 4.3 Hz, $J_{5',1'}$ = 4.7 Hz.

signals of one O-alkyl chain and one O-acyl chain, while the HMQC spectrum allowed the assignment of the pertinent carbon atoms (see Table 1). The HMBC spectrum confirmed the ether linkages between C-1 and C-1′ (couplings of H-1′ with C-1 and of H_2-1 with C-1′) and between C-3 and C-1″ (coupling of H_2-1″ with C-3).

In addition, the HMBC allowed us to identify the structural difference between crasserides and isocrasserides, i.e., the position of the fatty acyl chain. In fact, the carbonyl carbon atom at δ 172.5 (C-1‴), identified on the basis of its correlation peak with H_2-2‴, was also long-range coupled with the cyclitol proton at δ 5.45, which on the basis of COSY spectrum was vicinal to H-1′. These data demonstrated that the fatty acyl chain of isocrasserides is linked to a cyclitol hydroxyl group, either O-2′ or O-5′, rather than to a glycerol one.

Since the two possible locations of the fatty acyl chain differed only in the stereochemistry, to complete the structure elucidation it was first necessary to prove that crasserides and isocrasserides have the same stereochemistry. For this purpose, 2 mg of the mixture **4a**−**m** was subjected to methanolysis with 1 M HCl in 92% MeOH, followed by SiO_2 HPLC. This yielded a mixture of fatty acid methyl esters and a pure compound, which showed optical rotation and ^{1}H and ^{13}C NMR spectra identical to those reported for deacylcrasseride **5**.[2]



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At this point, a diagnostic NOE measurement was used to determine whether the acyl chain was linked to the hydroxyl group *cis* (i.e., at C-2) or *trans* (i.e., at C-5) to the glycerol unit. Saturation of H-1′ led to an increase of the intensity of both vicinal protons, resonating at δ 5.45 and 5.57, respectively. However, for the signal at δ 5.45 (the same signal giving rise to a correlation peak with the fatty acyl chain carbonyl in the HMBC spectrum) the enhancement was much larger (5.9% compared to 2.0%). Consequently, this signal was attributed to H-2′, and the acyl chain was located at O-2′, thus completing the structure elucidation of isocrasserides except for the length and branching of the long alkyl and acyl chains.

The length and branching of the alkyl/acyl chains could be determined only by chemical degradation, in the same way as we did with crasserides. The mixture of fatty acid methyl esters obtained by methanolysis of isocrasserides was subjected to GLC-MS analysis, and 11 different compounds were identified, as reported in Table 2. Identification of unbranched, iso, and anteiso fatty acid methyl esters was based on comparison of their retention times and mass spectra with those of authentic samples; as for the 9-methyl and 10-methyl esters, the methyl branching was located on the basis of the characteristic fragmentation pattern of these compounds[2,4] (for example, the relatively intense fragment peaks at *m*/*z* 185 and 157 of methyl 9-methyltetradecanoate originate from the favored α-cleavage with respect to the tertiary carbon atom; the latter fragment ion is accompanied by ions arising from rearrangement of one and two protons to give diagnostic peaks at *m*/*z* 158 and 159, respectively, while subsequent loss of methanol and water from the fragment at *m*/*z* 185 gives rise to characteristic peaks at *m*/*z* 153 and 135). As for the alkyl chain at O-3, we repeated the degradation procedure described in ref 2 (ether cleavage with HI, nucleophilic substitution on the resulting iodide with silver acetate, hydrolysis, CrO_3 oxidation, and diazomethane methylation) using compound **5** and obtained the same fatty acid methyl ester, namely, 10-methylhexadecanoate. The same degradation procedures were repeated using peracetylated cras-

Table 2. Fatty Acid Methyl Esters Obtained from Crasserides and Isocrasserides from Different Species of Sponges

fatty acid methyl ester	suffix letter in **1**−**4**	t_R (min)	*P. simplex*		*A. clathrodes*		*S. coralliphagum*		*P. crassa*[a]
			1	**3**	**1**	**3**	**1**	**3**	**1**
tetradecanoate[b]	**a**	12.24	1.8%	2.2%	12.8%	5.5%	4.7%	6.6%	7.8%
9-methyltetradecanoate[c]	**b**	13.76	9.2%	18.2%	28.2%	18.0%	17.2%	21.2%	25.8%
10-methyltetradecanoate[d]	**c**	13.86		3.7%	6.1%	3.9%	1.4%	2.6%	
13-methyltetradecanoate[b]	**d**	14.25	23.7%	29.5%	13.4%	10.5%	36.3%	33.8%	28.2%
12-methyltetradecanoate[b]	**e**	14.47	3.3%	1.5%	2.3%	2.2%	8.6%	6.6%	7.8%
pentadecanoate[b]	**f**	15.37	2.9%	2.5%	2.2%	2.7%	2.2%	2.0%	
9-methylpentadecanoate[e]	**g**	16.82	14.4%	9.4%	9.5%	11.9%	7.6%	6.7%	
10-methylpentadecanoate[f]	**h**	16.90	4.0%	3.0%	7.6%	13.0%	3.2%	2.4%	
14-methylpentadecanoate[b]	**i**	17.51	8.6%	6.4%	5.0%	7.1%	8.2%	5.8%	4.7%
hexadecanoate[b]	**l**	18.55	9.0%	6.5%	1.4%	4.4%	3.4%	2.5%	
10-methylhexadecanoate[g]	**m**	19.98	23.2%	17.1%	11.5%	20.7%	7.2%	9.8%	25.8%

[a] From ref 2. [b] Identified by comparison of retention time and mass spectrum with those of an authentic sample. [c] Diagnostic MS fragmentation peaks at *m/z* 256 (M^+, 19), 185 (13), 159 (3), 158 (7), 157 (64), 153 (11), 135 (10). [d] Diagnostic MS fragmentation peaks at *m/z* 256 (M^+, 32), 199 (25), 173 (3), 172 (2), 171 (9), 167 (10), 149 (14). [e] Diagnostic MS fragmentation peaks at *m/z* 270 (M^+, 25), 185 (16), 159 (2), 158 (5), 157 (19), 153 (13), 135 (10). [f] Diagnostic MS fragmentation peaks at *m/z* 270 (M^+, 36), 199 (24), 173 (4), 172 (5), 171 (15), 167 (9), 149 (12). [g] Diagnostic MS fragmentation peaks at *m/z* 284 (M^+, 44), 199 (31), 173 (4), 172 (6), 171 (14), 167 (11), 149 (13).

serides from *P. simplex* as starting material, and the results were similar.

The alkyl chain of crasserides and isocrasserides from *P. simplex* is the same as that of crasserides from *Pseudoceratina crassa*,[2] while there are differences in the kind and relative amounts of the acyl chains. To determine whether the acyl chains of crasserides and isocrasserides are related to the species from which the compounds have been isolated, we analyzed the fatty acid methyl esters obtained by methanolysis of crasserides and isocrasserides isolated from two more species, *Agelas clathrodes* and *Siphonodictyon coralliphagum*. As reported in Table 2, we found that all the samples contained more or less the same fatty acid methyl esters. However, differences in the amounts of each compound were observed, especially between samples from different species, while fatty acid methyl esters of crasserides and isocrasserides from the same organism were quite similar. As usual in sponges, the percentage of methyl-branched fatty acids, as well as that of odd-carbon fatty acids, was remarkably high.

An important problem arising from the isolation of isocrasserides as peracetyl derivatives is their possible formation from crasserides by acyl migration during the acetylation reaction. To rule out this possibility, we needed to isolate nonacetylated isocrasserides from the glycolipid fraction of *P. simplex* without using acetylation. The isolation procedure was more complex, and the yield was low (we often observe low recovery after a direct-phase chromatography of nonacetylated glycolipids, probably due to some irreversible adsorption). Nevertheless, we succeeded in isolating 1.3 mg of isocrasserides **3a**−**m**. Their characterization was based on MS and ^{1}H, COSY, and HMQC NMR spectra, and all data (including the relative intensity of molecular ion peaks in the mass spectrum) were in accordance with structures **3a**−**m** (see Experimental Section). In particular, the deshielded chemical shift of the cyclitol proton H-2′ (δ 4.91) was clear proof of the location of the acyl chain. Conclusive evidence of the structure of compounds **3a**−**m** came from their acetylation, which gave a mixture of compounds indistinguishable from peracetylated isocrasserides **4a**−**m** by MS and NMR.

Because acyl migration is also known to occur during chromatography on SiO_2, another possibility to consider was isomerization of crasserides in the course of the column chromatography separation yielding the crude glycolipid fraction from which isocrasserides were obtained. Therefore, we subjected 10 mg of crasserides **1a**−**m** to column chromatography on silica gel in the same conditions as those used for the isolation of isocrasserides (the same eluents were used, and the duration of the chromatography was the same). Crasserides were eluted with EtOAc/MeOH (9:1), and their ^{1}H NMR spectrum showed no evidence of the presence of isocrasserides, nor were isocrasserides found in the previous or in the subsequent fractions.

Finally, the possibility that acyl migration occurred during the extraction process was considered. Crasserides **1a**−**m** were left for 48 h in MeOH solution and for 48 h in $CHCl_3$ solution at 40 °C. The solvent was removed, and the ^{1}H NMR spectrum of the sample was recorded. The spectrum was identical to that of crasserides, and there was no evidence of formation of even small amounts of isocrasserides, thus confirming that isocrasserides are not artifacts, but genuine metabolites from *P. simplex*.

Experimental Section

General Experimental Procedures. FABMS spectra were performed in a glycerol matrix on a VG Prospec-Autospec (Fisons) mass spectrometer. Optical rotations were measured at 589 nm on a Perkin-Elmer 192 polarimeter using a 10 cm microcell. ^{1}H and ^{13}C NMR spectra were determined on a Bruker AMX-500 spectrometer at 500.13 and 125.77 MHz, respectively; chemical shifts were referenced to the residual solvent signal (C_6D_6: δ_H = 7.15, δ_C = 128.0; CD_3OD: δ_H = 3.34, δ_C = 49.0). Homonuclear ^{1}H connectivities were determined by the COSY experiment. The reverse multiple-quantum heteronuclear correlation (HMQC) spectra were recorded by using a pulse sequence with a BIRD pulse 0.5 s before each scan to suppress the signal originating from protons not directly bound to ^{13}C; the interpulse delays were adjusted for an average $^1J_{CH}$ of 142 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiment was optimized for a $^3J_{CH}$ of 8.3 Hz. The steady-state one-dimensional NOE difference experiments were acquired on a sample prepared under argon, using a period of presaturation of 2 s; for the quantification of NOE enhancements, the saturated peak in the difference spectrum was used as internal reference. GC−MS spectra were performed on a Hewlett-Packard 5890 gas chromatograph with a MSD HP 5970 MS mass selective detector, a split/splitless injector, and a fused-silica column, 25 m × 0.20 mm HP-5 (cross-linked 25% Ph Me silicone, 0.33 mm film thickness); the temperature of the column was varied, after a delay of 5 min from the injection, from 150 °C to 300 °C with a slope of 3 °C min^{-1}; quantitative determination was based on the area of the GLC peaks. High-performance liquid chromatographies (HPLC) were achieved on a Varian 2510 apparatus equipped with a Varian Star 9040 refractive index detector.

Source of Biological Materials. *P. simplex* was collected in the summer of 1998 near the coast of Berry Island

(Bahamas); *A. clathrodes* was collected in the summer of 1998 near the coast of Grand Bahama Island (Bahamas), and *S. coralliphagum* was collected in the summer of 2000 near the coast of Little San Salvador Island (Bahamas). All the sponges were identified by Prof. M. Pansini (University of Genoa, Italy). Reference specimens were deposited at the Istituto di Zoologia, University of Genoa, Italy (*P. simplex*, Ref. No. 98-36; *A. clathrodes*, Ref. No. 98-04; *S. coralliphagum*, Ref. No. 27-00).

Isocrasseride Tetraacetates (4a−m). *P. simplex* was frozen immediately after the collection and kept frozen until extraction. The specimens (110 g of dry weight after extraction) were homogenized and extracted with methanol (3 × 1 L) and then with chloroform (2 × 1 L); the combined extracts were partitioned between H_2O and *n*-BuOH. The organic layer was concentrated in vacuo and afforded 42.9 g of a dark brown oil, which was chromatographed on a column packed with RP-18 silica gel. A fraction eluted with $CHCl_3$ (10.5 g) was further chromatographed on a SiO_2 column with solvent of increasing polarity. The glycolipid fraction (960 mg) was eluted with EtOAc/MeOH (9:1) and divided into two portions. The first portion (500 mg) was acetylated with Ac_2O in pyridine for 12 h and separated by HPLC on a SiO_2 column [eluent: *n*-hexane/EtOAc (6:4)], thus affording a mixture (143 mg) containing peracetylated crasserides and isocrasserides together with other glycolipids. Further direct-phase HPLC purification [eluent: *n*-hexane/EtOAc (8:2)] gave 36 mg of peracetylated crasserides (**2a**−**m**), identified by comparison of their ^1H and ^{13}C NMR spectra with those of an authentic sample. In addition, 5.9 mg of peracetylated isocrasserides (**4a**−**m**) was obtained as a colorless oil: $[\alpha]^{25}_D$ +6.2 (*c* 0.6, $CHCl_3$); ^1H and ^{13}C NMR, Table 1; EIMS (assignment, relative intensities) *m*/*z* 882 (M^+ of **4m**, 2), 868 (M^+ of **4g**−**l**, 3), 854 (M^+ of **4b**−**f**, 5), 822 (M^+ of **4m** − AcOH, 1), 821 (M^+ of **4m** − AcOH − H, 1), 808 (M^+ of **4g**−**l** − AcOH, 1), 807 (M^+ of **4g**−**l** − AcOH − H, 2), 794 (M^+ of **4b**−**f** − AcOH, 2), 793 (M^+ of **4b**−**f** − AcOH − H, 4), 613 (16), 599 (21), 569 (89), 555 (31), 521 (12), 507 (17), 483 (48), 375 (42), 355 (100), 331 (47), 311 (35), 259 (81), 242 (52); HREIMS *m*/*z* 882.6470 (M^+ of **4m**, $C_{50}H_{90}O_{12}$ gives 882.6432), 868.6339 (M^+ of **4g**−**l**, $C_{49}H_{88}O_{12}$ gives 868.6276), 854.6159 (M^+ of **4b**−**f**, $C_{48}H_{86}O_{12}$ gives 854.6119).

Nonacetylated Crasserides (3a−m). The remaining portion (460 mg) of glycolipid fraction from the SiO_2 column was subjected to HPLC on a SiO_2 column, with *n*-hexane/*i*-PrOH (8:2) as eluent, giving 40.2 mg of a fraction composed of crasserides, isocrasserides, and other glycolipids. This fraction was further purified by HPLC on SiO_2 and EtOAc/*i*-PrOH (97:3) as eluent. This separation gave crasserides **1a**−**m** (9.3 mg) with no impurities and a fraction (4.1 mg) mainly composed of isocrasserides, but also containing crasserides. A further HPLC separation, under the same conditions, of the above fraction gave 1.5 mg of a mixture, entirely composed of isocrasserides **3a**−**m**, as an amorphous solid, $[\alpha]^{25}_D$ +3 (*c* 0.1, MeOH); ^1H and ^{13}C NMR, Table 1; HRFABMS (negative ion mode, triethanolamine matrix) *m*/*z* 713.5976 ([M of **3m** − H]$^-$, $C_{42}H_{81}O_8$ gives 713.5931), 699.5811 ([M of **3g**−**l** − H]$^-$, $C_{41}H_{79}O_8$ gives 699.5775), 685.5650 ([M of **3b**−**f** − H]$^-$, $C_{40}H_{77}O_8$ gives 685.5618).

Methanolysis of Isocrasserides. Isocrasserides (2 mg) were dissolved in 1 mL of 1 N HCl in 92% MeOH, and the obtained solution was kept for about 12 h at 40 °C in a sealed tube. The reaction mixture was dried under nitrogen and passed through a SiO_2 column. Elution with $CHCl_3$ gave a mixture of fatty acid methyl esters, which was analyzed by GLC-MS; subsequent elution with EtOAc/MeOH (9:1) afforded pure deacylcrasseride **5**, identified on the basis of its optical rotation and of its MS and NMR spectral data compared with those of an authentic sample.[2]

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