Isolation of Five-Membered Cyclitol Glycolipids, Crasserides: Unique Glycerides from the Sponge *Pseudoceratina crassa*

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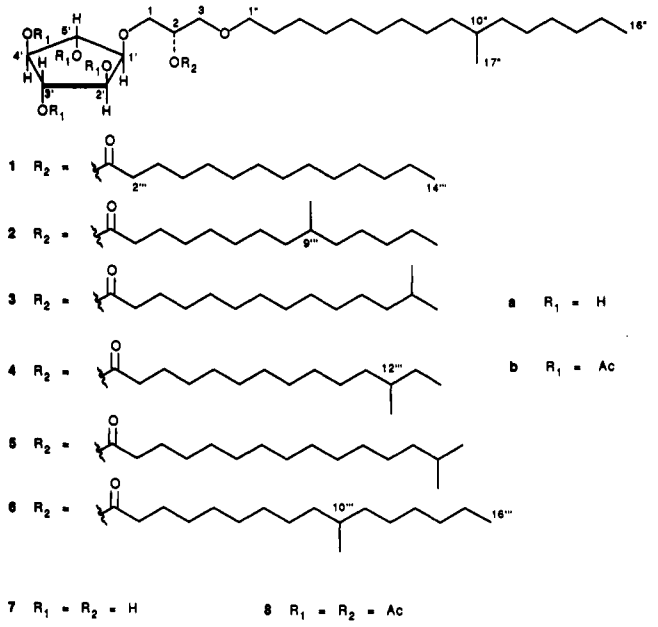
Crasserides 1a–6a, six new glycolipid analogues in which the sugar moiety is replaced by an unprecedented five-membered cyclitol, were isolated as a mixture from the sponge *Pseudoceratina crassa* (Hyatt), and their structures were determined by spectroscopic and chemical analysis. Compounds 1a–6a showed a high antifeedant activity on the fish *Carassius auratus*, which suggests their potential role as natural feeding deterrents.

Cyclitols[1] are polyhydroxycycloalkanes, present in plants and animals, which serve as precursors of more complex molecules such as phospholipids and phytates, which are components of cellular membrane structures. The most widely distributed cyclitol is *myo*-inositol, which appears to be present, both free and combined, in the tissue of virtually all living species and, on account of its structural features, is considered a carbocyclic analogue of pyranoside monosaccharides.

In the course of our investigation on bioactive compounds from marine invertebrates, we have now isolated crasserides (1a–6a), a mixture of six unique amphophilic



glycerides, from the Caribbean sponge *Pseudoceratina crassa* (Hyatt). They are close analogues of glycolipids, in which the sugar moiety is replaced by a five-membered cyclitol, attached to the glycerol molecule through an ether linkage. Simple cyclopentanepentols have been synthesized[2] but are unprecedented as naturally occurring compounds, even if some more complex five-membered cyclitols, such as funiculosin,[3] pactamycin,[4] and bacteriohopanetetrol ether,[5] have been isolated from natural sources. Due to their structural similarity to furanoside sugars, some five-membered cyclitols have also been utilized as intermediates in the preparation of carbocyclic analogues of nucleosides, which showed interesting antitumor, antimicrobial, and antiviral properties.[6]

P. crassa (synonyms: *Aiolochroia crassa*, *Ianthella ardis*) is a Keratospongia belonging to the suborder Verongida. It is a massive sponge, with knob-shaped conules, about 10 cm in diameter; the color in life, which was yellow for the collected specimens, is extremely variable within the species and turns soon to dark purple out of water. Specimens of *P. crassa* (131-g dry weight), collected along the coast of San Salvador Island during the summer of 1990 and stored frozen, were extracted first with MeOH/toluene (3:1) and then with $CHCl_3$. The EtOAc-soluble material from the extracts (11 g) was subjected to medium-pressure liquid chromatography (MPLC) on silica gel. Fractions eluted with EtOAc/MeOH (9:1) were rechromatographed by HPLC to give 44 mg of a viscous oil ($[\alpha]_D$ +10.0°), apparently pure by TLC and HPLC.

The mass spectrum, performed on its tetraacetate, showed a prominent molecular ion peak at m/z 854 and less intense peaks at m/z 840, 868, and 882, suggesting the presence of a mixture of homologues, which were not further separated. The presence of hydroxyl and ester groups was indicated by intense bands in the IR spectrum at ν_{max} 3448 and 1732 cm^{-1}, respectively. Preliminary structural elucidation, based on the analysis of the ^1H, ^{13}C, and DEPT NMR spectra, in combination with COSY and direct ^1H–^{13}C correlation (HETCOSY) NMR experiments, pointed to a complex glyceride. The presence of long aliphatic hydrocarbon chains was indicated by a large band at δ 1.26 in the ^1H NMR spectrum ($CDCl_3$), as well as by a cluster of methylene signals around δ 29.7 in the ^{13}C NMR spectrum. The latter also indicated the presence of a series of oxygen-bearing carbons, i.e. three oxymethylene groups at δ 71.9 (C-1″), 69.5 (C-1), and 69.2 (C-3), and six oxymethine groups at δ 81.9 (C-1′), 80.3 (C-3′), 79.0 (C-4′), 78.7 (C-5′), 73.2 (C-2′), and 71.5 (C-2).

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Table I. NMR Spectral Data of Compounds 1a–6a, 8, and 13[a]

pos		1a–6a ($CDCl_3$)[b] δ_H (mult, J (Hz))	1a–6a ($CDCl_3$)[b] δ_C	8 (C_6D_6)[c] δ_H (mult, J (Hz))	8 (C_6D_6)[c] δ_C	13 ($CDCl_3$)[d] δ_H (mult, J (Hz))
1	a	3.77 (dd, 10.9, 4.4)	69.5	3.81 (dd, 10.8, 4.4)	69.9	4.04 (dd, 13.0, 1.9)
	b	3.70 (dd, 10.9, 5.7)		3.77 (dd, 10.8, 5.8)		3.31 (dd, 13.0, 9.8)
2		5.16 (quintet, 5.1)	71.5	5.32 (quintet, 4.8)	71.8	4.17 (m)
3	a	3.57 (11.1, 4.1)	69.2	3.48 (d, 10.5, 5.4)	69.2	3.30 (dd, 10.3, 5.6)
	b	3.53 (11.1, 4.7)		3.45 (dd, 10.5, 4.7)		3.25 (dd, 10.3, 6.4)
5						1.43 (s)
6						1.40 (s)
1′		3.64 (t, 6.1)	81.9	4.08 (t, 4.5)	80.4	3.96 (dd, 5.2, 2.7)
2′		3.91 (t, 6.1)	73.2	5.37 (t, 5.4)	74.0	4.10 (dd, 8.5, 5.2)
3′		3.80 (t, 6.4)	80.3	5.79 (t, 5.7)	78.1	5.36 (dd, 8.5, 6.6)
4′		3.66 (t, 6.4)	79.0	5.50 (t, 4.7)	78.0	5.09 (dd, 6.6, 4.5)
5′		3.88 (t, 6.1)	78.7	5.53 (t, 4.1)	78.3	4.98 (dd, 4.5, 2.7)
1″	a	3.45 (dt, 9.2, 6.4)	71.9	3.29 (dt, 9.1, 6.6)	71.7	3.38 (t, 6.7)
	b	3.40 (dt, 9.2, 7.1)		3.24 (dt, 9.1, 6.5)		3.38 (t, 6.7)
2″		1.53 (m)	29.4	1.53 (quintet, 7.5)	30.1	1.51 (m)
3″		1.26[e]	26.0	1.30[e]	26.5	1.51 (m)
4″–7″		1.26[e]	29.2–30.1	1.30[e]	29.9–30.5	1.26[e]
8″		1.26[e]	27.2	1.30[e]	27.6	1.26[e]
9″	a	1.26[e]	37.2	1.30[e]	37.5	1.26[e]
	b	1.07 (m)		1.16 (m)		1.08 (m)
10″		1.26[e]	32.8	1.30[e]	33.2	1.26[e]
11″	a	1.26[e]	37.2	1.30[e]	37.5	1.26[e]
	b	1.07 (m)		1.16 (m)		1.08 (m)
12″		1.26[e]	27.2	1.30[e]	27.6	1.26[e]
13″		1.26[e]	29.2–30.1	1.30[e]	29.9–30.5	1.26[e]
14″		1.26[e]	31.9	1.30[e]	32.3	1.26[e]
15″		1.26[e]	22.7	1.30[e]	23.1	1.26[e]
16″		0.87 (t, 7.0)	14.1	0.91 (t, 7.1)	14.3	0.88 (t, 7.0)
17″		0.83 (d, 6.5)	19.7	0.93 (d, 6.4)	19.9	0.84 (d, 6.5)

[a] Assignment based on COSY, HETCOSY, and NOESY 2D NMR experiments and DEPT and NOE difference 1D NMR spectra. [b] Acyl chain resonances: δ_H 2.33 (2 H, t, J = 7 Hz, H_2-2″), 1.60 (2 H, m, H_2-3‴), 0.87 (t, J = 7 Hz, ω-CH_3), 0.85 (t, J = 0.85 (t, J = 6.5 Hz, isopropyl methyl groups of **3a** and **5a**), 0.83 (t, J = 7 Hz, branch methyl groups of **2a**, **4a**, and **6a**); ester CO resonance δ_C 174.1. [c] Acetoxy group resonances: δ_H 1.78 (3 H, s), 1.72 (3 H, s), 1.67 (3 H, s), 1.62 (6 H, s); δ_C 20.7, 20.2 (4 C), 169.86, 169.83, 169.75, 169.43, 169.40. [d] Acetoxy group resonances: δ_H 2.08 (3 H, s), 2.06 (3 H, s), 2.05 (3 H, s). [e] Submerged by other signals.

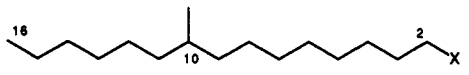
A HETCOSY NMR experiment allowed us to associate these carbons with the attached protons; they were, respectively, three further coupled AB systems [δ 3.45 and 3.40 (H_2-1″), δ 3.77 and 3.70 (H_2-1), and δ 3.57 and 3.53 (H_2-3)], five partly overlapped 1 H triplets [δ 3.64 (H-1′), 3.80 (H-3′), 3.66 (H-4′), 3.88 (H-5′), and 3.91 (H-2′)] and one 1 H quintet at δ 5.16 (H-2).

The COSY spectrum indicated that the proton at δ 5.16 was coupled with two pairs of methylene protons (δ 3.77, 3.70 and δ 3.57, 3.53, respectively), thus suggesting the presence of a glycerol moiety, acylated in position 2 on account of the low-field chemical shift of the relevant proton. The coupling between both protons of the AB system at δ 3.45, 3.41 (H_2-1″) and a 2 H multiplet at δ 1.53 (H_2-2″), which was in turn coupled with a signal resonating in the large band at δ 1.26 (H_2-3″), was indicative of a long *O*-alkyl chain, while the coupling of a 2 H triplet at δ 2.33 (H_2-2‴) with a 2 H multiplet at δ 1.60 (H_2-3‴) coupled in turn with a signal at δ 1.26 (H_2-4‴), together to the ester CO resonance at δ 174.1 in the ^{13}C NMR spectrum, indicated the presence of a long-chain acyl group. Finally, the COSY spectrum allowed us to establish that the remaining five low-field protons were part of the same spin system and that they were cyclically arranged: starting for example from the signal at δ 3.64, we could trace the correlation pathway in sequence to protons at δ 3.91, 3.80, 3.66, 3.88, and finally, again to the proton at δ 3.64. The above data, together with the chemical shift values of the protons involved, are very suggestive of a cyclopentanepentol part structure. This fragment is connected to the rest of the molecule by an ether linkage, as determined by acetylation of compounds **1a–6a** with Ac_2O in pyridine at room temperature. The resulting tetraacetate **1b–6b** showed in the ^1H NMR spectrum acyl shifts for four out of the five cyclopentane oxymethine protons, while the resonance of the fifth oxymethine proton and those of the three oxymethylene groups were practically unaffected (see the Experimental Section). These results, establishing two ether bridges between C-1 and C-1′ and between C-3 and C-1″, unequivocally defined the broad features of compounds **1a–6a** as a glycerol molecule O-substituted with a long-chain alkyl group in position 3, a long-chain acyl group in position 2, and a tetrahydroxycyclopentyl group in position 1, the only uncertainty being the nature of the alkyl and acyl chains. In the high-field region of the ^1H NMR spectrum of compounds **1a–6a** the following signals were present: a triplet at δ 0.87 and two doublets at δ 0.85 and 0.83, indicative of methyl groups attached to a methylene and to two different methine groups, respectively. However integration on these resonances did not provide values appropriate for an integral number of methyl groups. This implies that crasserides **1a–6a** were not simply homologues, as suggested by the mass spectrum, but differed also in the branching of the long-chain acyl and/or alkyl groups.

To gain information on the nature of alkyl and acyl chains, compounds **1a–6a** were therefore subjected to methanolysis by refluxing in MeOH/MeONa for 20 min. TLC of the crude reaction mixture afforded the alcohol **7** as a single pure substance as judged by MS (pentaacetate) and ^1H NMR and a mixture of fatty acid methyl esters. By GLC–MS analysis this mixture was shown to comprise six different methyl esters, identified on the basis of their GLC retention times and mass fragmentation: methyl tetradecanoate (10.1%), methyl 9-methyltetradecanoate (33.5%), methyl 13-methyltetradecanoate (36.6%), methyl

12-methyltetradecanoate (10.2%), methyl 14-methylpentadecanoate (6.1%), and methyl 10-methylhexadecanoate (3.4%). Unbranched, iso, and anteiso compounds showed GLC retention times and mass spectra identical to those of authentic samples. The position of branching at C-9 of methyl 9-methyltetradecanoate (M^+ = m/z 256) was indicated by the mass spectral fragmentation pattern.[7] The spectrum showed two relatively intense peaks separated by 28 amu at m/z 157 ($C_9H_{17}O_2$) and 185 ($C_{11}H_{21}O_2$), originating from α-cleavages with respect to the tertiary carbon atoms carrying the methyl branch, and the absence of a peak at m/z 171, corresponding to the ion $C_{10}H_{19}O_2$, which could only be formed by double cleavage and rearrangement. Furthermore, diagnostic peaks at m/z 153 ($C_{11}H_{21}O_2$ – MeOH) and 135 ($C_{11}H_{21}O_2$ – MeOH – H_2O) were present. Analogously, the methyl branching of methyl 10-methylhexadecanoate was indicated by large peaks at m/z 171 ($C_{10}H_{19}O_2$), 199 ($C_{12}H_{23}O_2$), 167 ($C_{12}H_{23}O_2$ – MeOH), and 149 ($C_{12}H_{23}O_2$ – MeOH – H_2O) and by the very low intensity of the peak at m/z 185 ($C_{11}H_{21}O_2$).

The structure of the *O*-alkyl chain in compounds **1a–6a** was recognized on the basis of the ^1H NMR spectrum of the methanolysis product **7** and by the mass spectrum of its pentaacetate **8**. Specifically, the high-resolution mass spectrum of the pentaacetate **8**, which lacked a molecular ion peak, allowed us to determine the molecular formula $C_{35}H_{60}O_{12}$ from the first fragment peak at m/z 612.3741 ($C_{33}H_{56}O_{10}$, M^+ – AcOH), which implies a saturated C_{17} alkyl chain. Furthermore, the ^1H NMR spectrum (CD_3OD) of the alcohol **7** displayed, together with the large methylene signal at δ 1.32, two methyl signals at δ 0.93 (3 H, t) and 0.89 (3 H, d), consistent with the presence of a methyl-branched alkyl chain. The following series of reactions provided valuable information to establish the position of the methyl branching. Compound **7** was heated in refluxing 57% aqueous HI for 12 h; the resulting iodide **9** was treated with silver acetate in AcOH under reflux for



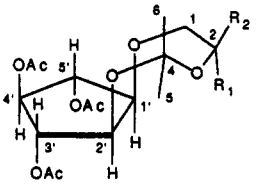
9 X = CH_2I
10 X = CH_2OAc
11 X = CH_2OH
12 X = COOH

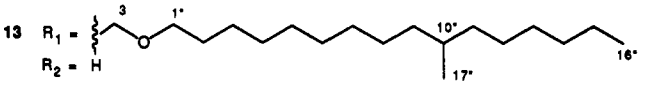
24 h to give the acetate **10**. The corresponding free alcohol **11**, obtained by alkaline methanolysis of acetate **10**, was oxidized with CrO_3 in AcOH to the carboxylic acid **12**. Finally, diazomethane methylation of the acid **12** gave a methyl ester which turned out to be identical to methyl 10-methylhexadecanoate, obtained by methanolysis of **1a–6a** as described above. Thus the structure of crasserides, apart from the stereochemistry, was completely established.

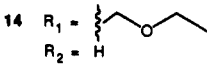
The relative stereochemistry of the cyclopentane ring carbon atoms was delineated by a series of NOE measurements. The NOE experiments were performed on the pentaacetate **8**, for the favorable features of its ^1H NMR spectrum in $CDCl_3/C_6D_6$ (1:1), which is characterized by a good proton dispersion. Furthermore, all the cyclopentane protons could be confidently assigned through the COSY spectrum starting from the signal of H-1′, which resonates at considerably higher field than the remaining oxymethine protons (see Table I). The observed NOE enhancements (see Table II) agreed with a trans relationship between all pairs of adjacent protons, except H-1′ and H-2′. The most diagnostic enhancements were observed for H-3′ upon irradiation of H-5′ (and vice versa), which illustrated their cis relationship, and for H-1′ and H-2′ on irradiation of H-4′, consistent with these three protons being on the same face of the molecule. The cis relationship between H-1′ and H-2′ was confirmed by the very large enhancement (11.6%) experienced by the latter while saturating the former.

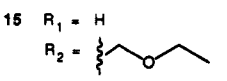
Attempts to deduce the relative configuration at C-2 from the NOE's measured for compound **8** were unsuccessful. Different enhancements of H2, H-1′, H-2′, and H-5′ were observed by irradiation of either proton (δ 3.82 and 3.77) of the methylene at C-1; however, these results could not be related with a particular configuration at C-2, since the rotation about bonds C-1/O, C-1′/O, and C-1/C-2 is relatively unconstrained, and a molecular modeling analysis showed that a large number of conformations consistent with the experimental data exist for each of the two epimers.

In order to overcome these difficulties, we prepared the monoacetonide **13** for treatment of the alcohol **7** with 2,2′-dimethoxypropane in the presence of an acidic ion-exchange resin, followed by acetylation of the resulting acetonide. Information on the structure of compound **13** was obtained from the molecular ion peak at m/z 628.4175 in the high-resolution mass spectrum, consistent with the molecular formula $C_{34}H_{60}O_{10}$, that indicates that **13** is a triacetyl monoacetonide derivative of compound **7**. This was confirmed by the ^1H NMR spectrum, which showed three acetoxymethine multiplets at δ 5.36, 5.09, and 4.98, three acetyl methyl singlets at δ 2.08, 2.06 and 2.05, and two acetonide methyl signals at δ 1.43 and 1.40. A COSY spectrum and some NOE difference measurements allowed us to locate these substituents in **13**. The COSY spectrum









indicated that the three low-field acetoxymethine protons were consecutive and therefore were to be located either at C-3′, C-4′, and C-5′ or at C-2′, C-3′, and C-4′. As a consequence, the hydroxyl groups involved in the formation of the ketal function must be either C-2 and C-2′ or C-2 and C-5′, respectively. The latter possibility, which requires the two high-field protons (δ 3.96 and 4.10) of the cyclopentane ring to be trans, was excluded by the NOE's measured between these two protons [4% enhancement of signal at δ 3.96 (H-1′) on irradiation at δ 4.10 (H-2′) and 2% enhancement for the reverse experiment, compared to 1% enhancement of signal at δ 3.96 (H-1′) on irradiation at δ 4.98 (H-5′) and 2% enhancement for the reverse experiment], that point to their cis relationship.

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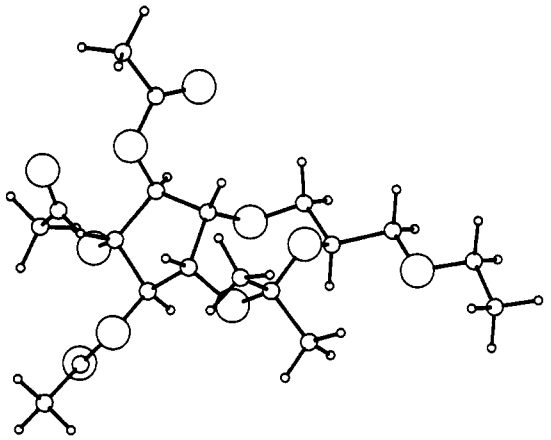

Figure 1. CHARMm lowest energy conformation of model compound **14**.

Table II. Nuclear Overhauser Effects Detected on Compound 8

saturated signal	enhanced signals (% enhancement)
H-1a	H-2 (8.3), H-5′ (4.4), H-1′ (5.1)
H-1b	H-2 (8.5), H-1′ (4.9), H-5′ (1.6)
H-2	H-1a (2.6), H-1b (2.5), H-1′ (2.3), H_2-3 (2.1)
H_2-3	H-2 (15.8)
H-1′	H-2′ (11.6), H-5′ (4.5), H-4′ (3.4), H-1b (2.0), H2 (1.5), H-1a (1.3)
H-2′	H-1′ (7.7), H-3′ (4.1), H-4′ (0.7)
H-3′	H-2′ (1.7), H-4′ (1.0), H-5′ (0.7), H-1′ (0.4)
H-4′	H-3′ (4.2), H-1′ (2.3), H-2′ (0.4)
H-5′	H-1′ (4.6), H-3′ (2.4), H-1a (1.5), H-1b (1.0)

NOE experiments performed on the acetonide **13** also provided decisive information on the relative stereochemistry at C-2. Upon irradiation of the methyl resonating at δ 1.40 (H_3-6), enhancement of H-2 was observed (8%), while saturation of the methyl signal at δ 1.43 (H_3-5) caused the intensity of H-2′ to increase (11%). Since eight-membered rings are quite flexible, an unambiguous interpretation of the NOE results required some knowledge about the conformational behavior of the two possible epimers. This was achieved by molecular mechanics analysis on model compounds **14** and **15** in the CHARMm force field. A set of 500 conformations for each epimer was generated by a high temperature (1500 K) molecular dynamics (HTMD) simulation (see the Experimental Section), which has been shown to be a suitable method for sampling conformational space of highly connected, flexible molecules;[8] the resulting structures were optimized by molecular mechanics. The energy of the structures generated in this way spread over a range of 19.5 kcal/mol for compound **14** and 19.7 kcal/mol for compound **15**. In the majority of the conformations generated for epimer **14** (469 out of 500) the distance H-2′/H_3-5 (the average position of the three methyl protons is considered) ranged between 2.58 and 2.93 Å, and the distance H-2/H_3-6 between 2.62 and 2.93 Å; besides, the energy of the 31 structures kept out was much higher (ΔE = 8.2 kcal/mol or more) than that of the lowest energy conformer. Likewise, in 452 out of 500 conformations of compound **15** the distance H-2′/H_3-5 was in the range 2.50–2.81 Å, but the distance H-2/H_3-6 was much longer, between 4.13 and 4.58 Å; once again, the energy of the 48 excluded structures was high (ΔE > 8.0 kcal/mol). In the light of the above results, the strong NOE enhancements detected between H-2 and H_3-6 and between H-2′ and H_3-5 can only be accounted for by assuming for **13** (and therefore for the natural crasserides **1a–6a**) the relative stereochemistry of model compound **14**.

Molecular modeling analysis also accounted for the relatively small nuclear Overhauser effects observed between H-1′ and H-2′ of compound **13** in comparison with the large ones measured between the same protons in compound **8**. In fact, H-1′ and H-2′ lie spatially very close to other protons, which provide them alternative relaxation pathways, thus decreasing the NOE effects. Particularly, the proximity of H-2′ to H_3-5 has been discussed above, while the distance of H-1′ from one of the two protons at C-2 resulted to be at least as short as 2.35 Å in 411 of the 500 conformations generated for **14** and in all the conformations at less than 5.6 kcal/mol.

Therefore, crasserides can be formulated as (2*S*,2′*S*,3′*R*,4′*R*,5′*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-2-*O*-tetradecanoyl-3-*O*-(10″-methylhexadecyl)glycerol (**1a**), (2*S*,2′*S*,3′*R*,4′*R*,5′*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-2-*O*-(9‴-methyltetradecanoyl)-3-*O*-(10″-methylhexadecyl)glycerol (**2a**), (2*S*,2′*S*,3′*R*,4′*R*,5′*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-2-*O*-(13‴-methyltetradecanoyl)-3-*O*-(10″-methylhexadecyl)glycerol (**3a**), (2*S*,2′*S*,3′*R*,4′*R*,5′*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-2-*O*-(12‴-methyltetradecanoyl)-3-*O*-(10″-methylhexadecyl)glycerol (**4a**), (2*S*,2′*S*,3′*R*,4′*R*,5′*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-2-*O*-(14‴-methylpentadecanoyl)-3-*O*-(10″-methylhexadecyl)glycerol (**5a**), (2*S*,2′*S*,3′*R*,4′*R*,5′*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-2-*O*-(10‴-methylhexadecanoyl)-3-*O*-(10″-methylhexadecyl)glycerol (**6a**), or their respective enantiomers.

Antifeedant assays[9] on the fish *Carassius auratus* showed that compounds **1a–6a** possess a high feeding deterrence at a concentraion as low as 30 μg/cm^2 of food pellets. The biological activity exhibited by crasserides **1a–6a** points to a potential role of these compounds as natural feeding deterrents.

Experimental Section

General Methods. Mass spectra were obtained by EI at 70 eV. ^{1}H and ^{13}C NMR spectra were determined at 500 and 125 MHz, respectively, using the solvent signal as internal standard. Methyl, methylene, and methyne carbons were distinguished by DEPT experiments. Homonuclear ^{1}H connectivities were determined by using the COSY experiment. One bond heteronuclear ^{1}H–^{13}C connectivities were determined with a HETCOSY pulse sequence optimized for $^1J_{CH}$ of 135 Hz. Two and three bond ^{1}H–^{13}C connectivities were determined by a COLOC experiment, optimized for $^{2,3}J_{CH}$ of 8 Hz.

Extraction and Isolation. Specimens of *P. crassa* were collected (depth 15 m) in the summer of 1990 along the coast of San Salvador Island (Bahamas) and identified by Dr. M. Pansini (University of Genoa). They were stored frozen at −20 °C when still alive and dispatched to the laboratory. Reference specimens are deposited at the Dipartimento di Zoologia, University of Genoa. The collected animals (131-g dry wt after extraction) were homogenized and successively extracted with MeOH–toluene, 3:1 (5 × 1 L), and with $CHCl_3$ (3 × 1 L). After evaporation of the solvent, the methanolic extracts were partitioned between EtOAc (4 × 500 mL) and water (500 mL). The combined EtOAc and $CHCl_3$ extracts were dried (Na_2SO_4) and concentrated in vacuo to afford 11 g of a dark brown oil, which was chromatographed by MPLC on an SiO_2 column using a solvent gradient system from *n*-hexane to EtOAc and then to MeOH. Fractions eluted with EtOAc/MeOH (9:1) afforded a mixture containing

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(9) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *Comp. Biochem. Physiol.* **1982**, *73B*, 471.

compounds **1a–6a** (188 mg). HPLC chromatography on a Hibar LiChrospher Si60 (10- × 250-mm) column with a mobile phase of EtOAc yielded 44 mg of an inseparable mixture of **1a–6a**, judged otherwise pure by ^{1}H NMR.

Crasserides 1a–6a: $[\alpha]^{25}_D$ ($CHCl_3$) + 10.0°; IR (neat) ν_{max} 3348 and 1732 cm^{-1}; ^{1}H and ^{13}C NMR data are reported in Table I.

General Acetylation Procedure. The alcohol was allowed to stay overnight with 200 μL of Ac_2O in 0.5 mL of anhydrous pyridine. MeOH was added, and the mixture was evaporated and treated with toluene in order to remove traces of pyridine.

Crasseride tetraacetates 1b–6b: MS *m/z* (assignment, relative intensities) 882 (**6b**, M$^+$, 0.2), 868 (**5b**, M$^+$, 0.7), 854 (**2b–4b**, M$^+$, 2.3), 840 (**1b**, M$^+$, 0.8), 821 (**6b**, M$^+$ – AcO, 0.6), 807 (**5b**, M$^+$ – AcO, 1.7), 793 (**2b–4b**, M$^+$ – AcO, 5.8), 779 (**1b**, M$^+$ – AcO, 1.8), 6.27 (8), 613 (28), 599 (88), 585 (8), 569 (25), 551 (21), 537 (62), 523 (16), 375 (18), 373 (30), 331 (24), 311 (32), 301 (100), 259 (98), 242 (90); ^{1}H NMR δ 5.33 (1 H, t, *J* = 5.6 Hz), 5.13 (1 H, m), 5.12 (1 H, m), 5.10 (1 H, m), 5.05 (1 H, q, *J* = 5.1 Hz, H-2), 3.95 (1 H, t, *J* = 4.7 Hz, H-1′), 3.73 (1 H, dd, *J* = 10.7 and 4.6 Hz, H-1a), 3.64 (1 H, dd, *J* = 10.7 and 5.8 Hz, H-1b), 3.52 (1 H, dd, *J* = 10.8 and 5.3 Hz, H-3a), 3.50 (1 H, dd, *J* = 10.8 and 4.9 Hz, H-3b), 3.44 (1 H, dd, *J* = 9.3 and 6.7 Hz, H-1″a), 3.39 (1 H, dd, *J* = 9.3 and 7.0 Hz, H-1″b), 2.31 (2 H, t, *J* = 6.5 Hz), 2.12 (3 H, s), 2.09 (3 H, s), 2.07 (6 H, s), 1.61 (2 H, q, *J* = 7 Hz), 1.54 (2 H, m), 1.26 (bs), 1.07 (2 H, m), 0.88 (t, *J* = 7.0 Hz), 0.85 (t, *J* = 6.5 Hz), 0.83 (t, *J* = 6.5 Hz).

Methanolysis of Crasserides 1a–6a. Compounds **1a–6a** (40 mg) were refluxed with 0.5 M NaOMe in MeOH (5 mL) for 15 min. H_2O was added, and the reaction mixture was extracted with $CHCl_3$ (3 × 5 mL). The extract was washed with H_2O, dried over Na_2SO_4, and evaporated in vacuo. The crude reaction mixture was subjected to TLC [SiO_2, $CHCl_3$/MeOH (9:1)], thus obtaining a mixture of fatty acid methyl esters (12 mg) and the pure alcohol **7** (23 mg); 5.0 mg of **7** were acetylated to give 7.2 mg of the pentaacetate **8**.

1-*O*-(2′,3′,4′,5′-Tetrahydroxycyclopentyl)-3-*O*-(10″-methylhexadecyl)glycerol (7): $[\alpha]^{25}_D$ (MeOH) –4.5°; ^{1}H NMR (CD_3OD) δ 3.95 (1 H, m, H-2), 3.88 (1 H, m, H-1′), 3.85 (1 H, m, H-2′), δ 3.77 (1 H, m, H-3′), 3.76 (1 H, m, H-1a), 3.58 (1 H, m, H-4′), 3.56 (1 H, m, H-5′), 3.56 (1 H, dd, H-1b), 3.51 (1 H, m, H-3a), 3.48 (1 H, m, H-3b), 3.33 (2 H, m, H_2-1″), 1.60 (2 H, t, *J* = 7 Hz, H_2-2″), 1.32 (bs, aliphatic chain), 1.13 (2 H, m, H-9″b and H-11″b), 0.93 (3 H, t, *J* = 7 Hz, H_3-16″), 0.89 (3 H, t, *J* = 6.5 Hz, H_3-17″).

1-*O*-(2′,3′,4′,5′-Tetraacetoxycyclopentyl)-2-*O*-acetyl-3-*O*-(10″-methylhexadecyl)glycerol (8): $[\alpha]^{25}_D$ ($CHCl_3$) + 16°; HRMS *m/z* (assignment, relative intensities) 612.3741 ($C_{33}H_{56}O_{10}$, M$^+$ – AcOH, 1.0), 569.3706 ($C_{33}H_{56}O_{10}$, M$^+$ – AcOH – Ac, 18), 417.1400 ($C_{18}H_{25}O_{12}$, fragment A, 30), 355.3332 ($C_{22}H_{43}O_3$, fragment B, 37), 301.0923 ($C_{13}H_{17}O_8$, fragment C, 100), 259.0812 ($C_{11}H_{15}O_7$, fragment C – C_2H_2O, 38), 242.0787 ($C_{11}H_{14}O_6$, fragment C – AcO, 38), 183.0652 ($C_9H_{11}O_4$, 25.0), 140.0463 ($C_7H_8O_3$, 30.8); ^{1}H and ^{13}C NMR data are reported in Table I.

Analysis of the Methyl Ester Mixture. The identification of the fatty acid methyl esters obtained from methanolysis of crasserides **1a–6a** was based upon their GLC retention times and GLC–MS spectra. A fused-silica column, 25-m × 0.20-mm HP-5 (cross-linked 25% Ph Me silicone, 0.33-μm film thickness) was used. The temperature of the column was varied, after a delay of 5 min from the injection, from 100 to 250 °C with a slope of 5 °C/min. Quantitation is based on the area of the GLC peak.

Methyl tetradecanoate: 10.1%; t_R = 23.934 min; t_R and MS spectrum were identical to those of an authentic sample.

Methyl 9-methyltetradecanoate: 33.5%; t_R = 25.120; MS *m/z* (relative intensity) 256 (13), 213 (20), 199 (3), 185 (13), 157 (55), 153 (14), 143 (37), 135 (13), 129 (17), 115 (12), 111 (12), 101 (16), 97 (17), 87 (76), 84 (20), 74 (100), 69 (37), 57 (49), 55 (48), 43 (54).

Methyl 13-methyltetradecanoate: 36.6%; t_R = 25.493 min; t_R and MS spectrum were identical to those of an authentic sample.

Methyl 12-methyltetradecanoate: 10.2%; t_R = 25.622 min; t_R and MS spectrum were identical to those of an authentic sample.

Methyl 14-methylpentadecanoate: 6.1%; t_R = 27.575 min; t_R and MS spectrum were identical to those of an authentic sample.

Methyl 10-methylhexadecanoate: 33.5%; t_R = 29.247; MS *m/z* (relative intensity) 284 (22), 241 (24), 199 (26), 185 (2), 171 (10), 167 (9), 153 (12), 149 (13), 143 (53), 130 (15), 129 (20), 115 (7), 111 (12), 101 (10), 97 (22), 87 (78), 84 (33), 74 (100), 69 (31), 57 (38), 55 (51), 43 (50).

Cleavage of Alcohol 7 with Hydroiodic Acid. Alcohol **7** (10 mg) was dissolved in 57% HI (1 mL) in a sealed tube, and the mixture was kept at 130 °C for 12 h. After cooling the reaction mixture was extracted with Et_2O (3 × 5 mL), and the extracts were washed in succession with water, a saturated solution of K_2CO_3, and 40% $Na_2S_2O_3$. The ether solution, dried over Na_2SO_4 and evaporated in vacuo, yielded 7.7 mg of 10-methylhexadecyl iodide **9**.

10-Methylhexadecyl iodide (9): MS *m/z* (relative intensity) 239 (10), 197 (3), 183 (5), 169 (6), 155 (9), 141 (8), 127 (9), 113 (12.8), 99 (50), 71 (80), 57 (100), 43 (58); ^{1}H NMR ($CDCl_3$) δ 3.19 (2 H, t, *J* = 7 Hz), 1.83 (2 H, q, *J* = 7 Hz), 1.26 (bs), 0.87 (3 H, t, *J* = 7 Hz), 0.83 (3 H, d, *J* = 6.5 Hz).

Conversion of Iodide 9 to Alcohol 11. Iodide **9** (7 mg) in 5 mL of glacial AcOH was stirred and heated under reflux with 80 mg of powdered silver acetate for 24 h. The mixture was diluted with 50 mL of Et_2O, freed from insoluble material by centrifugation, and washed successively with water, a saturated solution of NaCl, and a saturated solution of $NaHCO_3$ until neutral. The ether solution was dried over Na_2SO_4 and brought to dryness, thus affording 6.5 mg of the acetate **10**. It was subjected to methanolysis as described above; the crude reaction mixture was extracted with *n*-hexane (3 × 10 mL), and evaporation of the solvent gave 4 mg of alcohol **11**.

10-Methylhexadecyl acetate (10): MS *m/z* (relative intensity) 298 (0.2), 238 (1.0), 210 (1.2), 168 (8), 153 (15), 140 (8), 126 (13), 125 (11), 112 (15), 111 (31), 97 (42), 83 (40), 71 (35), 69 (40), 61 (26), 57 (56), 55 (48), 43 (100), 41 (36); ^{1}H NMR ($CDCl_3$) δ 4.16 (2 H, t, *J* = 7 Hz), 2.05 (3 H, s), 1.63 (2 H, m), 1.26 (bs), 0.88 (3 H, t, *J* = 7 Hz), 0.83 (3 H, d, *J* = 6.5 Hz).

10-Methyl-1-hexadecanol (11): MS *m/z* (relative intensity) 210 (2), 168 (4), 153 (10), 140 (6), 126 (12), 125 (15), 112 (21), 111 (42), 97 (59), 83 (60), 71 (63), 69 (65), 57 (100), 55 (83), 43 (81); ^{1}H NMR ($CDCl_3$) δ 3.62 (2 H, t, *J* = 7 Hz), 1.55 (2 H, m), 1.26 (bs), 0.87 (3 H, t, *J* = 7 Hz), 0.83 (3 H, d, *J* = 6.5 Hz).

Conversion of Alcohol 11 to Methyl 10-Methylhexadecanoate. To a solution of 3.5 mg of alcohol **11** in 2 mL of glacial AcOH at 40 °C was added dropwise with stirring a saturated solution of CrO_3 in glacial AcOH until a permanent brown color was obtained. After further stirring for 2 h at room temperature, water (2 mL) and solid $NaHSO_3$ were added to remove the excess oxidant. The solution was strongly acidified with 5 N H_2SO_4 and extracted with *n*-hexane, and the solvent was evaporated in vacuo, thus obtaining 3.5 mg of 10-methylhexadecanoic acid (**12**) as an oil. The product was dissolved in 1 mL of Et_2O, and an ether solution of CH_2N_2 was added dropwise until the yellow color persisted. Evaporation of the solvent led to 3.7 mg of a pure product that showed a GLC retention time and mass spectrum identical to those of methyl 10-methylhexadecanoate.

Synthesis of Acetonide 13. Alcohol **7** (2 mg) was dissolved in 0.5 mL of 2,2-dimethoxypropane and allowed to react with stirring for 12 h in the presence of 20 mg of acid ion-exchange resin DOWEX 50. The reaction mixture was freed from the resin by filtration and brought to dryness. The residual oil (2 mg), without any further purification, was acetylated as described above. HPLC chromatography of the acetylation product on a Hibar Superspher Si60 (4 × 250-mm) column with a mobile phase of *n*-hexane/EtOAc (7:3) yielded 0.7 mg of the acetonide **13**.

Crasseride acetonide (13): HRMS *m/z* 628.4175 ($C_{34}H_{60}O_{10}$, calcd 628.4186); MS *m/z* (assignment, relative intensities) 628 (M$^+$, 2), 613 (M$^+$ – CH_3, 5), 569 (M$^+$ – AcO, 10), 450 (6), 355 (53), 301 (34), 259 (32), 215 (28), 210 (100), 168 (37), 157 (34), 139 (36), 115 (33); ^{1}H and ^{13}C NMR data are reported in Table I.

Molecular Modeling. Molecular modeling studies were performed using the Quanta/CHARMm[10] 3.2 program on a

(10) Molecular Simulations Inc. 200 Fifth Avenue, Waltham, MA 02154.

Personal Iris 4D-35G computer. The effect of the solvent was approximated by using a dielectric constant of 4.806 (chloroform), and all the energy terms were calculated. Molecular dynamics simulations involved a heating period of 1.2 ps, followed by a 1.2-ps equilibration period and then 100 ps of dynamics simulation. The time step of integration was 1 fs. Bond lengths involving hydrogen atoms were kept fix using the SHAKE[11] algorithm. The coordinates produced by the simulation were saved every 0.2 ps, giving 500 structures. Each of them was subjected to energy minimization using the conjugated gradient protocol. All energies are relative to the lowest energy conformer (E = 6.40 kcal/mol for **14**; E = 8.17 kcal/mol for **15**).

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Supplementary Material Available: ^{1}H NMR spectra of compounds **1a–6a**, **1b–6b**, **7**, **8**, **9**, **10**, **11**, **12**, and **13** and mass spectra of methyl 9-methyltetradecanoate, methyl 10-methylhexadecanoate, and compounds **1b–6b**, **8**, **9**, **10**, **11**, and **13** (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from ACS; see any current masthead page for ordering information.

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