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THE STEREOCHEMISTRY OF CRASSERIDES

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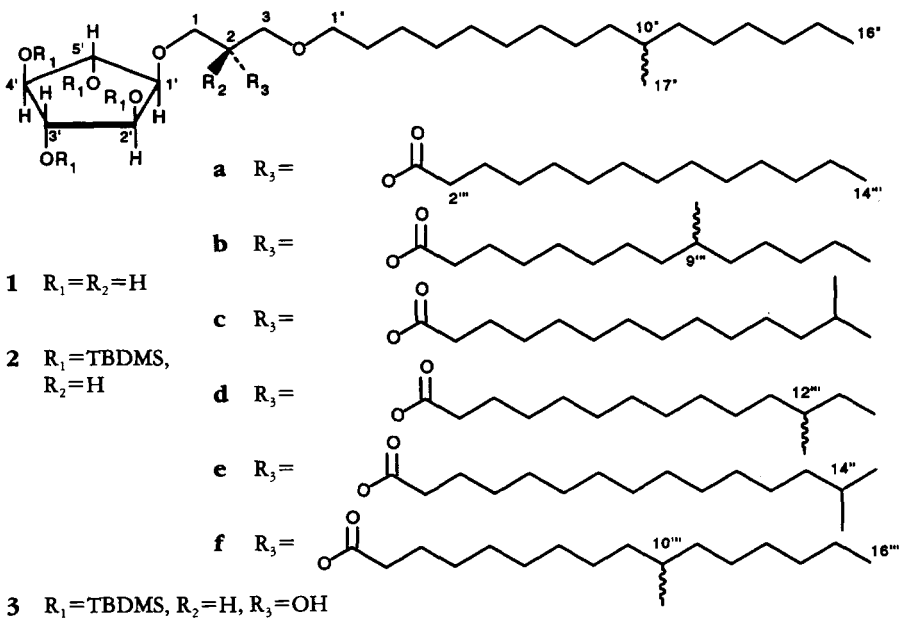
ABSTRACT.—Reported herein is the determination by the Mosher method of the absolute stereochemistry of the crasserides, which are glycolipids present in the marine sponge *Pseudoceratina crassa*, in which the sugar moiety is replaced by a five-membered cyclitol. In addition, the relative stereochemistry of the cyclitol ring was further substantiated by chemical evidence.

We have recently reported the isolation from the Caribbean sponge *Pseudoceratina crassa* and the structural elucidation of the crasserides (**1a–1f**), unprecedented analogues of glycolipids, in which the sugar moiety is replaced by an unique five-membered cyclitol (1,2). The determination of the structure and relative stereochemistry of compounds **1a–1f**, which differ only in the acyl chain, was based on spectral data and chemical evidence. At the same time that the paper on the structure elucidation of crasserides appeared, Kobayashi *et al.* published the isolation of the kerufferides from a *Luffariella*

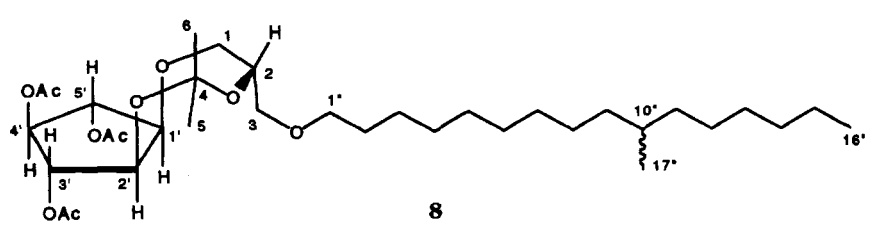


1 $R_1=R_2=H$

2 R_1=TBDMS, $R_2=H$

a $R_3=$ **b** $R_3=$ **c** $R_3=$ **d** $R_3=$ **e** $R_3=$ **f** $R_3=$

3 R_1=TBDMS, $R_2=H$, $R_3=OH$
4 R_1=TBDMS, $R_2=H$, R_3=O-(*R*)-MTPA
5 R_1=TBDMS, $R_2=H$, R_3=O-(*S*)-MTPA
6 $R_1=R_2=H$, $R_3=OH$
7 R_1=TBDMS, $R_2,R_3==O$



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species (3). Their structures were identical to those of the crasserides apart from the stereochemistry of the cyclitol ring, which was reported to be all-cis, although the spectral data of these two groups of compounds were almost identical. In a subsequent paper, Kobayashi proposed revised structures for kerufferides, which are now considered identical to those of crasserides (4).

Since our first paper, we have been able to isolate crasserides from several other species, namely, *Verongula gigantea, Aplysina fistularis fulva, Aplysina cauliformis*, and *Neofibularia nolitangere*, while Kobayashi *et al.* have also identified two additional producing species. Therefore, the crasserides appear to be widely distributed in sponges, and it was considered worthwhile to characterize them as thoroughly as possible. In this paper we report the absolute configuration of **1a–1f**, established through the application of the Mosher method performed on an appropriate derivative.

The application of the Mosher method required a structural modification in order to set free the secondary OH group of the glycerol moiety, which was judged the most suitable site for the reaction, after protection of the hydroxyl groups on the cyclitol ring. This was accomplished through the following sequence of reactions. The mixture of the crasserides was treated with *t*-butyldimethylchlorosilane (TBDMS-Cl) in DMF solution in the presence of imidazole at 60° for 12 h. In the mass spectrum, the obtained tetra-TBDMS derivatives **2a–2f** showed the highest fragment ion peaks at *m/z* 1113 ($[M-t\text{-butyl}]^+$ for **2f**), 1099 ($[M-t\text{-butyl}]^+$ for **2e**), 1085 ($[M-t\text{-butyl}]^+$ for **2b**, **2c**, and **2d**), and *m/z* 1071 ($[M-t\text{-butyl}]^+$ for **2a**), and four distinct singlets at δ 0.897, 0.893, 0.886, and 0.879 in the ^{1}H-nmr spectrum, due to the four *t*-butyl groups. Methanolysis of compounds **2a–2f** was performed with K_2CO_3/MeOH at room temperature for 24 h to give the tetra-TBDMS-crasseride alcohol **3**. Two aliquots of alcohol **3** were separately treated with *R*-(−)- and *S*-(+)-methoxy(trifluoromethyl)phenylacetyl (MTPA) chloride in pyridine solution at 70° for 12 h, thus obtaining the two epimer esters **4** and **5**, respectively. Even though the ^{1}H-nmr spectrum of esters **4** and **5** was rather crowded between δ 3 and 4, the methylene protons at C-1 and C-3 could be easily distinguished from the cyclitol protons and from the protons at C-1″ through cross-peaks in the COSY nmr spectra arising from their coupling with H-2, whose resonance was readily recognized as a quintet at δ 5.40 in **4** and δ 5.38 in **5**. The COSY spectrum also permitted the identification of all the cyclitol resonances, but not their assignment to the respective protons.

Discrimination between the resonances of H_2-1 and H_2-3, which is essential for the proper application of the Mosher method, was obtained through ROESY nmr experiments. The ROESY spectrum of the (*R*)-MTPA ester **4** showed a distinct correlation peak between the cyclitol proton at δ 3.91 and the methylene proton at δ 3.77, which is therefore linked to C-1. Likewise, in the spectrum obtained from the (*S*)-MTPA ester **5**, correlation peaks of the methylene proton at δ 3.47 with two cyclitol protons at δ 3.88 and 3.71 allowed the assignment of the relevant methylene as H_2-1.

The measured chemical shift values, which are summarized in Table 1, are in excellent agreement with the Mosher model (5), as modified by Ohtani *et al.* (6), which postulates a preferred conformation of the MTPA esters as the α-CF_3 and the carbonyl group of the MTPA in an eclipsed arrangement, with the phenyl group shielding and the methoxy group deshielding the neighboring protons. In the (*R*)-MTPA ester **4**, the protons at C-1 and all the cyclitol protons were shifted upfield relative to the (*S*)-MTPA ester **5**, while the protons at C-3 and at C-1″ were shifted downfield. Therefore, the stereochemistry at C-2 can be assigned as *S*.

TABLE 1. Selected ^{1}H-Nmr Data of Crasseride (*R*)-MTPA [**4**] and (*S*)-MTPA [**5**] Esters.

	Compound	
	4	**5**
H_2-1	3.77, 3.61–3.53[a]	3.61, 3.47
H_2-3	3.61–3.53[b]	3.75, 3.64
H_2-1″	3.34, 3.29	3.46, 3.38
Cyclitol protons	4.09, 3.91, 3.74, 3.74, 3.61–3.53[c]	4.05, 3.88, 3.71, 3.71, 3.50

[a–c]The chemical shift of these overlapping signals lies in the reported range.

Because the relationship between the configuration of C-2 and those of the cyclitol carbon atoms is known from the previous paper (2), this fully defined the absolute configuration of the whole molecule (apart from the configuration at C-10″).

The availability of the tetra-TBDMS-crasseride alcohol **3** provided a simple way to further support the relative stereochemistry of the cyclitol ring, which was assigned in the previous paper (2) as follows. The nOe enhancements measured for the acetylated crasserides showed H-1′, H-2′, and H-4′ to be on the same side of the molecule; by analogy, H-3′ and H-5′ were also shown to be cis-oriented. These data are in accordance only with structures **1a–1f** or with an all-cis stereochemistry. The latter stereochemistry was ruled out by the chemical behavior of the crasserides, which by treatment of the pentahydroxy compound **6** with 2,2-dimethoxypropane and subsequent acetylation produced the eight-membered cyclic acetonide **8** with a 50% yield, while no acetonide involving two cyclitol hydroxy groups could be isolated (2).

This negative evidence has now been substantiated by oxidation of the secondary hydroxyl group of the tetra-TBDMS-crasseride alcohol **3**, which resulted in the destruction of the chiral center at C-2. In the case of an all-cis stereochemistry, the resulting ketone would behave like a meso compound in the cyclitol part of the molecule, which is not affected by the very distant asymmetric C-10″; therefore, at most three distinct signals from the cyclitol protons would appear in the ^{1}H-nmr spectrum. The oxidation, performed by treatment of compound **3** with 10% CrO_3 in pyridine at 50° overnight, yielded a product whose ^{1}H-nmr spectrum (interpreted with the aid of a COSY spectrum) is in full agreement with structure **7**, but not with an all-cis stereochemistry. Four distinct signals from the cyclitol protons (two protons are fortuitously coincident) were present in the ^{1}H-nmr spectrum, and they were almost coincident with those of the parent alcohol, **3**. Moreover, as a further demonstration of the asymmetric structure of the cyclitol ring, the two protons at C-1 are diastereotopic, and appeared as an AB system at δ 4.19 and 4.14.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Eims spectra were recorded on a Kratos MS80 mass spectrometer. ^{1}H-Nmr spectra were determined at 500 MHz on a Bruker AMX 500 spectrometer, using $CDCl_3$ as solvent and as internal standard (δ 7.26). The ROESY spectra were performed using a mixing time of 450 msec and a 2000 Hz spin-lock field.

ANIMAL MATERIAL.—As described previously (1,2).

EXTRACTION AND ISOLATION.—As described previously (2).

SILYLATION OF CRASSERIDES.—To 11.5 mg of compounds **1a–1f** in 200 ml of DMF, 50 mg (4 equivalents) of *t*-butyldimethylsilyl chloride (TBDMS-Cl) and 50 mg (8 equivalents) of imidazole were added. The reaction was allowed to proceed at 60° for 12 h, and then quenched with MeOH (1 ml). The reaction mixture was brought to dryness, and partitioned between *n*-hexane and MeOH. After removal of the solvent, the *n*-hexane layer gave an oil (19.5 mg) which was

subjected to hplc on a Si gel column with a mobile phase of *n*-hexane-EtOAc (95:5), thus yielding a mixture of the tetra-TBDMS derivatives **2a–2f** (8.5 mg), judged otherwise pure by tlc and ^{1}H-nmr spectroscopy.

Tetra-TBDMS-crasserides [**2a–2f**].—Eims (70 eV) *m/z* 1113 ([M−*t*-butyl]$^+$ for **2f**, 1), 1099 ([M−*t*-butyl]$^+$ for **2e**, 4), 1085 ([M−*t*-butyl]$^+$ for **2b**, **2c**, and **2d**, 20), and 1071 ([M−*t*-butyl]$^+$ for **2a**, 6), 611 (24), 537 (100), 457 (28), 301 (41), 299 (30); ^{1}H nmr ($CDCl_3$) δ 5.12 (1H, quintet, J=5.0 Hz, H-2), 4.11 (1H, dd, J=7.1 and 3.9 Hz, cyclitol proton), 3.88 (1H, m, cyclitol proton), 3.72 (1H, m, cyclitol proton), 3.69 (1H, m, cyclitol proton), 3.65 and 3.53 (2H, further coupled AB system, J_{AB}=10.0 Hz, J_{AX}=5.4 Hz, J_{BX}=4.8 Hz, H_2-1 or H_2-3), 3.58 (2H, d, J=5.1 Hz, H_2-3 or H_2-1), 3.51 (1H, m, cyclitol proton), 3.43 and 3.38 (2H, further coupled AB system, J_{AB}=9.3 Hz, H_2-1″), 2.30 (2H, t, J=7.6 Hz, H_2-2‴), 1.53 (2H, q, J=7.5 Hz, H_2-2″), 0.897 (3H, s), 0.893 (3H, s), 0.886 (3H, s), 0.879 (3H, s), 0.083–0.064 (24H).

METHANOLYSIS OF TETRA-TBDMS-CRASSERIDES.—Compounds **2a–2f** (8.5 mg) were dissolved in 1 ml of *n*-hexane, and 1 ml of saturated K_2CO_3 in MeOH was added. The mixture was allowed to react under stirring for 24 h at room temperature, then the *n*-hexane phase was recovered and taken to dryness. Hplc on Si gel of the residue (8.5 mg) with *n*-hexane-EtOAc (95:5) gave 5.5 mg of the pure alcohol **3**.

Tetra-TBDMS-crasseride alcohol [**3**].—Eims (70 eV) *m/z* 861 ([M−*t*-butyl]$^+$, 11), 729 (7), 597 (55), 457 (24), 375 (31), 343 (57), 301 (80), 285 (38), 187 (53), 147 (65), 131 (100); ^{1}H nmr ($CDCl_3$) δ 4.10 (1H, dd, J=6.8 and 3.4 Hz, cyclitol proton), 3.96 (1H, m, H-2), 3.93 (1H, m, cyclitol proton), 3.74 (2H, m, cyclitol protons), 3.55 (1H, dd, J=6.8 and 3.4 Hz, cyclitol proton), 3.51–3.42 (6H, m, H_2-1, H_2-3, and H_2-1″), 0.899 (3H, s), 0.894 (3H, s), 0.888 (3H, s), 0.881 (3H, s), 0.086–0.070 (24H).

SYNTHESIS OF THE (*R*)- AND (*S*)-MTPA ESTERS OF ALCOHOL **3**.—To compound **3** (1.5 mg) in 200 ml of anhydrous pyridine, 20 ml of (*R*)-MTPA chloride [MTPA=α-methoxy-α-(trifluoromethyl)-phenylacetyl] were added, and the mixture was heated in a sealed tube at 70° for 24 h. After cooling, 5 ml of H_2O and solid K_2CO_3 were added, and the solution was extracted with $CHCl_3$ (5 ml). The organic phase was evaporated to dryness, and partitioned between MeOH and *n*-hexane. The *n*-hexane phase, after evaporation of the solvent, yielded 1.5 mg of the (*R*)-MTPA ester **4**. The use of (*S*)-MTPA chloride in the same procedure led to 1.4 mg of the (*S*)-MTPA ester **5**.

Crasseride (R)-*MTPA ester* [**4**].—Eims (70 eV) *m/z* 1077 ([M−*t*-butyl]$^+$, 4), 871 (8), 789 (9), 603 (55), 529 (74), 457 (55), 375 (28), 301 (85), 291 (50), 73 (100); ^{1}H nmr ($CDCl_3$) δ 7.58 (2H, m, aromatic protons), 7.40 (3H, m, aromatic protons), 5.40 (1H, dddd, J=5.8, 5.8, 5.8, and 4.0 Hz, H-2), 4.09 (1H, ddd, J=6.6, 3.3, and 1.5 Hz, cyclitol proton), 3.91 (1H, ddd, J=3.5, 3.0, and 1.5 Hz, cyclitol proton), 3.77 (1H, dd, J=10.0 and 5.9 Hz, H-1a), 3.74 (2H, m, cyclitol protons), 3.61–3.53 (4H, m, H-1b, H_2-3, and one cyclitol proton), 3.56 (3H, s, OMe), 3.34 and 3.29 (2H, further coupled AB system, J_{AB}=9.2 Hz, H_2-1″), 1.47 (2H, m, H_2-2″), 0.884 (3H, s), 0.881 (3H, s), 0.876 (3H, s), 0.872 (3H, s), 0.083–0.035 (24H).

Crasseride (S)-*MTPA ester* [**5**].—Eims (70 eV) *m/z* 1077 ([M−*t*-butyl]$^+$, 2), 871 (3), 789 (4), 603 (34), 529 (44), 457 (32), 375 (20), 301 (78), 291 (43), 73 (100); ^{1}H nmr ($CDCl_3$) δ 7.58 (2H, m, aromatic protons), 7.37 (3H, m, aromatic protons), 5.38 (1H, m, H-2), 4.05 (1H, ddd, J=6.6, 2.3, and 1.8 Hz, cyclitol proton), 3.88 (1H, m, cyclitol proton), 3.75 (1H, dd, J=11.1 and 2.7 Hz, H-3a), 3.71 (2H, m, cyclitol protons), 3.64 (1H, dd, J=11.1 and 7.4 Hz, H-3b), 3.61 (1H, dd, J=9.6 and 4.5 Hz, H-1a), 3.58 (3H, s, OMe), 3.50 (1H, m, cyclitol proton), 3.47 (1H, m, H-1b), 3.46 (1H, m, H-1″a), 3.38 (1H, ddd, J=9.3, 6.7, and 6.7 Hz, H-1″b), 1.53 (2H, m, H_2-2″), 0.891 (3H, s), 0.878 (3H, s), 0.870 (6H, s), 0.077–0.032 (24H).

OXIDATION OF ALCOHOL **3**.—A solution of **3** (2 mg) in 0.2 ml of pyridine and 0.5 ml of a 10% CrO_3-pyridine complex was allowed to stand overnight at 50°. After cooling, the mixture was diluted with EtOAc (5 ml) and washed with 10% AcOH, 5% $NaHCO_3$, and H_2O, and dried over anhydrous Na_2SO_4. Hplc purification [Si gel column, *n*-hexane-EtOAc (95:5)] gave 1.0 mg of the ketone **7**.

2-Oxocrasseride [**7**].—Eims (40 eV) *m/z* 859 ([M−*t*-butyl]$^+$, 1), 727 (5), 653 (2), 571 (10), 497 (7), 457 (8), 385 (8), 315 (9), 301 (28), 185 (14), 147 (29), 131 (78), 129 (100); ^{1}H nmr ($CDCl_3$) δ 4.35 (2H, s, H_2-3), 4.19 and 4.14 (2H, AB system, J=16.7 Hz, H_2-1), 4.14 (1H, m, cyclitol proton), 3.94 (1H, m, cyclitol proton), 3.73 (2H, m, cyclitol protons), 3.54 (1H, dd, J=6.9 and 3.0 Hz, cyclitol proton), 3.46 (2H, t, J=6.8 Hz, H_2-1″), 0.895 (3H, s), 0.890 (3H, s), 0.881 (6H, s), 0.104–0.050 (24H).

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