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KERUFFARIDE: STRUCTURE REVISION AND ISOLATION FROM PLURAL GENERA OF OKINAWAN MARINE SPONGES

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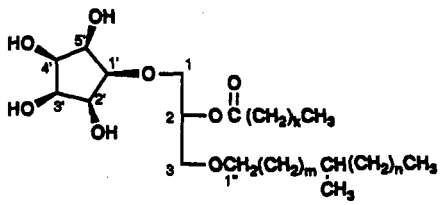
ABSTRACT.—Reexamination of the spectral data of keruffaride, first isolated from an Okinawan marine sponge *Luffariella* sp., revealed that the structure of its cyclopentanepentol moiety has to be revised to the same one as that contained in crasserides recently isolated from a Caribbean sponge *Pseudoceratina crassa*.

During our studies on bioactive compounds from Okinawan marine organisms (1,2) we isolated keruffaride, a glycolipid containing a cyclopentanepentol moiety, from the Okinawan marine sponge *Luffariella* sp. (order Dictyoceratida; family Thorectidae) (3). Almost at the same time, an Italian group reported the isolation of five-membered cyclitol glycolipids, the crasserides, from the Caribbean sponge *Pseudoceratina crassa* (4). We proposed that keruffaride possesses an all-cis cyclopentanepentol moiety, while crasserides were described as containing a different five-membered cyclitol structure. The close resemblance of the proposed structure **1** of keruffaride to crasseride [**2**] prompted us to reexamine our data of keruffaride, leading to the conclusion that our proposed structure for keruffaride has to be revised to **3**. This communication deals with the structure revision of keruffaride as well as our recent results concerning this cyclitol glycolipid; that is, we have isolated keruffaride from extracts of other Okinawan marine sponges of different genera, one of which also contained a deacyl derivative **4** of keruffaride as a natural product. In addition, keruffaride [**3**] was revealed to possess stimulation activity of nerve growth factor (NGF) synthesis.

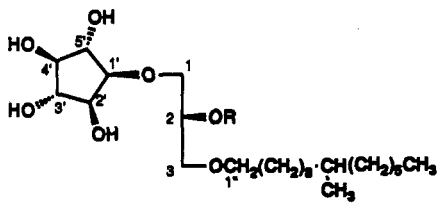
To resolve whether the structure of the cyclopentanepentol moiety of keruffaride is truly different from that of crasseride, the ^{1}H- and ^{13}C-nmr spectral data of keruffaride were thoroughly compared with those of crasseride described in the literature (4) to reveal that the spectral data corresponded well to each other. The ^{1}H-nmr data of the tetraacetate **5** of keruffaride [**3**] were also parallel to those of the tetraacetate of crasseride (4). Furthermore, a monoacetonide triacetate **6** was prepared from keruffaride through three steps (hydrolysis of the C-2 acyl group, treatment with 2,2-dimethoxypropane in the presence of PPTS, and acetylation), and the ^{1}H-nmr data for the cyclopentanepentol moiety of compound **6** were revealed to be completely identical with those of the monoacetonide triacetate derived from crasseride (Table 1). Compound **6** is a 1:4 mixture of homologues, while the monoacetonide triacetate derived from crasseride was reported to be a single compound. Comparison of the ^{1}H-nmr spectra, however, was possible because the signals due to the cyclopentanepentol moiety of **6** were singly observed. From these results, we concluded that keruffaride possesses the same cyclopentanepentol moiety as crasseride, and the structure of keruffaride, therefore, has to be revised to **3**. Our assignment of the stereochemistry of the cyclopentanepentol moiety had been based on NOESY correlations of the tetraacetate in C_6D_6 (3) for H-1′/H-2′, H-1′/H-4′, H-1′/H-5′, H-2′/H-3′, H-2′/H-4′, and H-3′/H-5′. These results indicate that NOESY correlations can be observed between trans-vicinal protons (H-1′/H-5′ and H-2′/H-3′) in five-membered rings.

Both keruffaride [**3**] and crasseride [**2**] are mixtures of homologous compo-



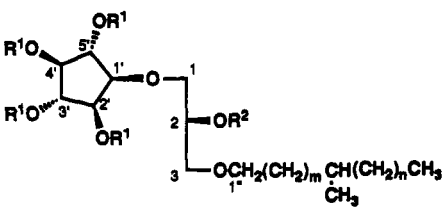
k=13, 14, 15, and 16 (31:100:38:12)
m+n=12 and 13 (25:100)

1

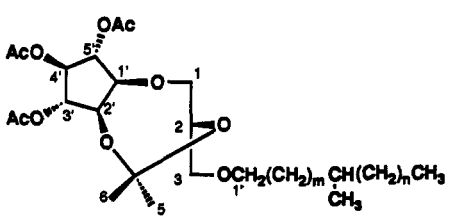


R=$CO(CH_2)_{12}Me$
$CO(CH_2)_7CH(Me)(CH_2)_4Me$
$CO(CH_2)_{11}CHMe_2$
$CO(CH_2)_{10}CH(Me)CH_2Me$
$CO(CH_2)_{12}CHMe_2$
$CO(CH_2)_8CH(Me)(CH_2)_5Me$

2



3 $R^1=H$, $R^2=CO(CH_2)_kMe$
4 $R^1=R^2=H$
5 $R^1=Ac$, $R^2=CO(CH_2)_kMe$



6

nents. Fatty acids with a branched methyl group are attached at the C-2 position of the glycerol unit of crasseride [**2**], whereas keruffaride [**3**] contains only unbranched fatty acids at this position. The relative stereochemistry at the C-2 position of crasseride [**2**] was assigned (4) on the basis of difference nOe experiments on the monoacetonide triacetate derived from **2**. We failed, however, in difference nOe experiments of the monoacetonide triacetate **6** derived from keruffaride [**3**] in $CDCl_3$ since the methyl protons (H_3-5 and H_3-6) as well as H-2 and H-2′ resonated very closely to each other (Table 1), and selective irradiation of these proton signals was unsuccessful. To obtain information on the relative stereochemistry of the C-2 position, a NOESY experiment on compound **6** was applied in C_6D_6 on a 600 MHz spectrometer (Figure 1). As a result, the nOe correlations were observed for H_3-5/H-2′ and H_3-6/H-2. The assignments of these

TABLE 1. ^{1}H-nmr Data Comparison of the Monoacetonide Triacetate Derived from Keruffaride and Crasseride in $CDCl_3$ (400 MHz).

Proton	From keruffaride		From crasseride[a]	
	δ_H	J in Hz	δ_H	J in Hz
H_a-1	4.04 dd	14.0, 1.6	4.04 dd	13.0, 1.9
H_b-1	3.24–3.31 m		3.31 dd	13.0, 9.8
H-2	4.17 m		4.17 m	
H_a-3	3.24–3.31 m		3.30 dd	10.3, 5.6
H_b-3	3.24–3.31 m		3.25 dd	10.3, 6.4
H-5 (3H)	1.43 s		1.43 s	
H-6 (3H)	1.40 s		1.40 s	
H-1′	3.96 dd	5.1, 2.6	3.96 dd	5.2, 2.7
H-2′	4.10 dd	8.7, 5.1	4.10 dd	8.5, 5.2
H-3′	5.36 dd	8.7, 6.6	5.36 dd	8.5, 6.6
H-4′	5.09 dd	6.6, 5.1	5.09 dd	6.6, 4.5
H-5′	4.98 dd	5.1, 2.6	4.98 dd	4.5, 2.7
H-1″ (2H)	3.38 t	6.6	3.38 t	6.7
CH_3CO	2.08 s		2.08 s	
CH_3CO	2.07 s		2.06 s	
CH_3CO	2.06 s		2.05 s	

[a]Data are from Costantino *et al.* (4).

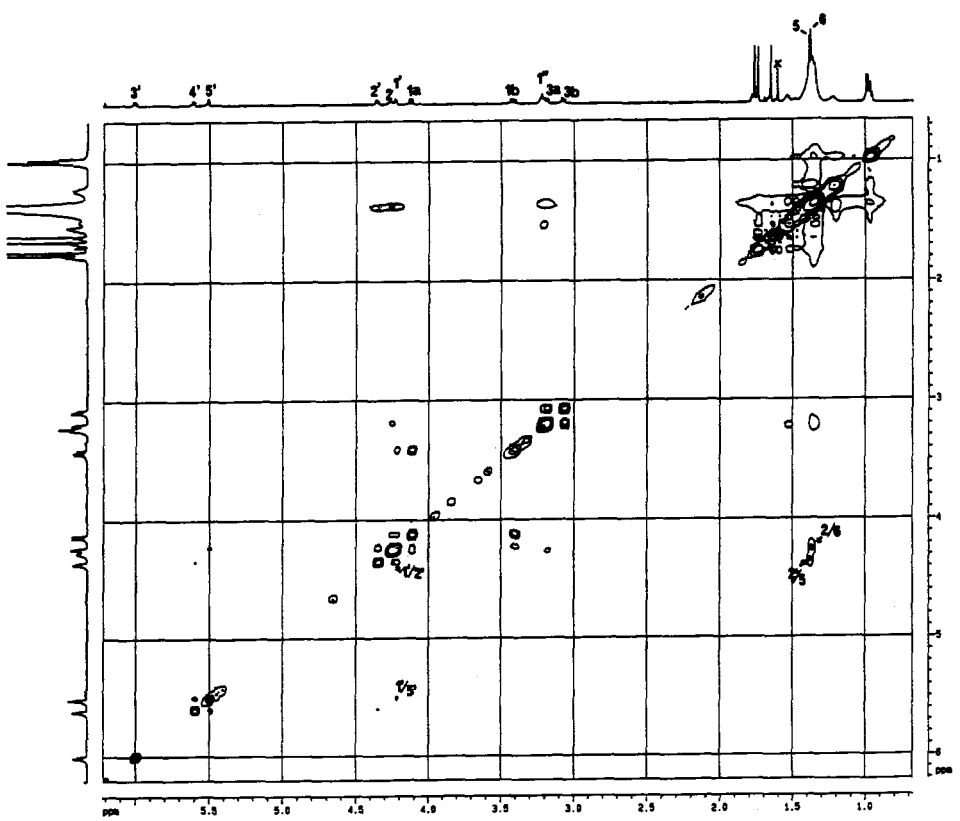


FIGURE 1. The NOESY spectrum of monoacetonide triacetate **6** in C_6D_6. The NOESY spectrum (mixing time 800 msec) was recorded in the phase-sensitive mode, and a total of 256 increments of 1K data points were collected.

proton signals were confirmed by a ^{1}H-^{1}H COSY spectrum and NOESY correlation data: H-1′ (δ_H 4.22) showed a distinct NOESY cross peak with H-2′ (δ_H 4.35) and showed a very weak one with H-5′ (δ_H 5.50), implying that the two high-field protons on the cyclopentane ring are cis and, therefore, the acetonide functionality is present on the C-2′ hydroxyl group. These observations fully corresponded to the nOe data of the monoacetonide triacetate derived from crasseride [**2**] described in the literature (4). The relative stereochemistry of the C-2 position of keruffaride [**3**] was concluded to be parallel to that of crasseride [**2**]. The position of the secondary methyl group on the ether chain of **3** (main component, m+n=13) was assumed to be on the C-10″ position, being also parallel to that of crasseride [**2**].

By further investigation on the chemical constituents of Okinawan marine sponges of various collections, we also isolated keruffaride [**3**] from two other sponges belonging to the genera *Biemna* (order Poecilosclerida; family Desmacellidae; Gray, 1867) and *Xestospongia* (order Petrosida; family Petrosiidae; de Laubenfels, 1932). From the former sponge, the deacyl derivative **4** of keruffaride, which was previously obtained by hydrolysis of keruffaride [**3**] (3), was isolated as a natural product for the first time.

Since keruffaride [**3**] is found (0.0004–0.001%, wet wt) in sponges of three different genera [*Luffariella* (3), *Biemna*, and *Xestospongia*], it might be a generally occurring metabolite of sponges and play some significant role in the life of sponges. Keruffaride [**3**] at 30–100 μg/ml was found to exhibit 3–4-fold stimulation activity of nerve growth factor (NGF) synthesis in cultured astroglial cells. Since NGF synthesis-enhancers are anticipated as drugs for peripheral or central nerve disorders (5), this cyclitol glycolipid might be a lead compound for such type drugs.

EXPERIMENTAL

GENERAL METHODS.—Optical rotations were recorded on a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers. The NOESY spectrum was recorded on a Bruker AMX-600 spectrometer. Fabms was obtained on a JEOL HX-110 spectrometer. Wako C-300 Si gel was used for cc, and tlc was carried out on Merck Si gel GF_{254}.

SPONGE MATERIALS.—The sponge *Luffariella* sp. (3) also contained luffariolides F and G (2), and the description of the sponge material has already been reported (2). The sponges of *Biemna* sp. and *Xestospongia* sp. were collected by netting at Unten Harbor, Okinawa Island, and kept frozen until used. The specimen of *Biemna* sp. was a very dark brown to purple black sponge when preserved with some foreign material in the mesohyl. Skeleton a loose unispicular or bispicular reticulation of styles without fiber development. Numerous large sigmas throughout the mesohyl. Styles 552–612×12–13 μm; sigmas 96 μm, rephides 210 μm long. The specimen of *Xestospongia* sp. was a compressible sponge, fawn in color with preservation. The skeleton was a reticulation of spicules with no obvious spongin fiber, skeleton not very dense or compact. Spicules strongyles and strongyloxeas were of large size range; thin forms occur. Main size range is 360–396×12 μm. Voucher specimens of *Biemna* sp. (SS-857) and *Xestospongia* sp. (SS-856) were deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

ISOLATION.—The MeOH extract of the sponge of *Biemna* sp. (1.7 kg, wet wt) was evaporated under reduced pressure and the residue (36 g) was partitoned between EtOAc (400 ml×3) and 1 M NaCl (400 ml). The EtOAc-soluble material (877 mg) was partially (432 mg) subjected to Si gel flash cc (50×3 cm) with gradient elution of EtOAc in hexane (0–100%) and MeOH in $CHCl_3$ (50–100%). The fraction (71 mg) eluted with 50% MeOH in $CHCl_3$ was separated by the second Si gel column eluted with 0–100% MeOH in $CHCl_3$. The fraction (15 mg) eluted with 8% MeOH in $CHCl_3$ was then subjected to a Sephadex LH-20 column [MeOH-$CHCl_3$ (1:1)] to give keruffaride [**3**] (6.1 mg, 0.0004% wet wt), and the fraction (9 mg) eluted with 10% MeOH in $CHCl_3$ was further purified by LH-20 column [MeOH-$CHCl_3$ (1:1)] to yield deacylkeruffaride [**4**] (3.1 mg, 0.002%). Keruffaride [**3**] (5.4 mg, 0.001% wet wt) was also isolated by similar procedures from the MeOH extract of the sponge of *Xestospongia* sp. (0.5 kg).

DEACYLKERUFFARIDE [**4**].—$[\alpha]^{19}_D$ −5.7° (*c*=0.21, MeOH); ^{1}H nmr (CD_3OD) δ_H 3.95 (1H, m, H-2), 3.88 (1H, m, H-1′), 3.85 (1H, m, H-2′),

3.77 (1H, m, H-3′), 3.76 (1H, m, H_a-1), 3.58 (1H, m, H-4′), 3.56 (1H, m, H-5′), 3.56 (1H, m, H_b-1), 3.51 (1H, m, H_a-3), 3.48 (1H, m, H_b-3), 3.48 (2H, m, H_2-1″), 1.60 (2H, m, H_2-2″), 1.32 (br s, aliphatic chain), 0.93 (3H, t, J=7 Hz), 0.89 (3H, d, J=6.5 Hz); ^{13}C nmr (CD_3OD) δ_C 73.3 (t, C-1), 70.9 (d, C-2), 73.1 (t, C-3), 75.1 (d, C-1′), 80.0 (d, C-2′), 82.0 (d, C-3′), 81.6 (d, C-4′), 84.5 (d, C-5′), 72.7 (t, C-1″), 30.6 (t, C-2″), 14.4 (q), 20.1 (q); fabms *m/z* 463, $[M+H]^+$ 449.

MONOACETONIDE TRIACETATE [**6**].—To a solution of alcohol **4** (1.8 mg) in CH_2Cl_2 (2 ml), PPTS (5 mg) and 2,2-dimethoxypropane (0.3 ml) were added and then stirred overnight. After evaporation of the solvent under reduced pressure, the residue was acetylated by the usual method. The purification of the acetylation product on a Si gel column eluted with hexane-EtOAc (7:3) yielded compound **6** (1.4 mg): ^{1}H nmr ($CDCl_3$ see Table 1); ^{1}H nmr (C_6D_6) δ_H 4.12 (d, J=12.7 Hz, H_a-1), 3.41 (dd, J=12.7 and 9.4 Hz, H_b-1), 4.28 (1H, m, H-2), 3.19 (1H, dd, J=9.8 and 5.6 Hz, H_a-3), 3.07 (1H, dd, J=9.8 and 6.3 Hz; H_b-3), 1.39 (3H, s), 1.37 (3H, s), 4.22 (1H, br s, H-1′), 4.35 (1H, dd, J=8.5 and 5.0 Hz, H-2′), 6.01 (1H, dd, J=8.5 and 6.4 Hz, H-3′), 5.61 (1H, dd, J=6.4 and 3.9 Hz, H-4′), 5.50 (1H, br s, H-5′), 3.22 (2H, m, H_2-1″), 1.77 (3H, s, CH_3CO), 1.74 (3H, s, CH_3CO), 1.65 (3H, s, CH_3CO), 0.98 (3H, d, J=6.5 Hz), 0.96 (3H, t, J=7.3 Hz).

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