Keruffaride, a New All-*cis* Cyclopentanepentol-containing Metabolite from the Okinawan Marine Sponge *Luffariella* sp.

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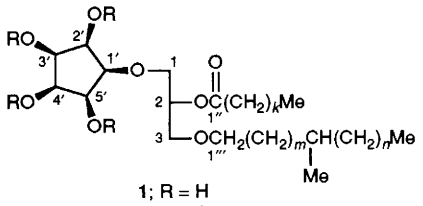
A new lipid containing the all-*cis* cyclopentanepentol moiety, keruffaride, has been isolated from the Okinawan marine sponge *Luffariella* sp. and its structure elucidated on the basis of spectral and chemical means; this is the first isolation of an all-*cis* cyclopentanepentol-containing metabolite from natural sources.

During our continuing studies on bioactive substances from Okinawan marine organisms,[1] we investigated the constituents of sponges of the genus *Luffariella* and reported isolation of manoalide-related sesterterpenes luffariolides A ~ E with cytotoxic activity.[2] Further investigation on extracts of another *Luffariella* sponge has now led to the isolation of a new all-*cis* cyclopentanepentol-containing metabolite, keruffaride **1**, which comprises a substituted glycerol and an unusual all-*cis* substituted cyclopentanepentol unit. Keruffaride **1** is the first example of a compound containing the all-*cis* substituted cyclopentanepentol moiety obtained from natural sources. Here, we describe the isolation and structure elucidation of **1**.

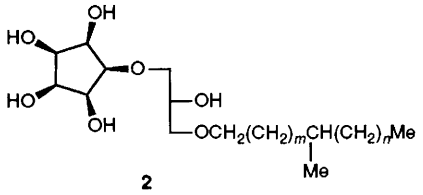
The sponge *Luffariella* sp. was collected off the Kerama Islands, Okinawa and kept frozen until used. The methanolic extract of the sponge was partitioned between ethyl acetate and water. The ethyl acetate-soluble fraction was subjected to silica gel flash column chromatography eluted with 10% methanol in chloroform followed by gel filtration on a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1) to give keruffaride (**1**, 0.0005%, wet weight).

Keruffaride **1** was obtained as a colourless oil and shown to



1; R = H
4; R = Ac

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



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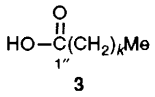

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be a mixture of homologous components† by its FABMS analysis, in which quasi-molecular ions $(M + Na)^+$ were observed at *m/z* 723, 709, 695 and others.‡ The molecular formula of the most abundant component was determined to be $C_{41}H_{80}O_8$ from the HRFABMS data [*m/z* 723.5736 $(M + Na)^+$, $C_{41}H_{80}O_8Na$, $\Delta - 1.5$ mmu]. The IR spectrum of **1** was indicative of the presence of ester carbonyl (ν_{max} 1720 cm^{-1}) and hydroxy (ν_{max} 3400 cm^{-1}) groups. The ^{13}C NMR spectrum of **1** showed signals due to an ester carbonyl, three oxymethylenes, six oxymethines, one sp^3 methine, three methyls and many sp^3 methylenes. Assignments of ^{13}C signals for protonated carbons were derived from the HSQC[3] spectrum of **1** (Table 1). Since one of two unsaturations was characterized by the carbonyl, keruffaride **1** was inferred to be monocyclic. The ^1H–^1H COSY spectrum of **1** revealed the presence of a glycerol unit (cross-peaks: H-1a/H-2, H-1b/H-2, H-2/H-3a and H-2/H-3b) and a cyclopentanepentol portion (cross-peaks: H-1′/H-2′, H-2′/H-3′, H-3′/H-4′, H-4′/H-5′ and H-5′/H-1′). The H-2 of the glycerol unit resonated at δ_H 5.19 and this chemical shift implied that the C-2 glycerol position was acylated. This fact was further supported by the ^1H–^{13}C long-range connectivity between the H-2 of the glycerol unit and the ester carbonyl (δ_C 174.0, C-1″), which was observed in the HMBC[4] spectrum of **1**. The C-1″ showed the HMBC cross-peak with methylene protons at δ_H 2.35 (2H, t, *J* 6.6 Hz; H_2-2″), which in turn was coupled with the methylene protons at δ_H 1.65 (H_2-3″) in the ^1H–^1H COSY spectrum of **1**. From these observations it was deduced that a fatty acid was connected to C-2 of the glycerol through an ester bond. The H-1′ (δ_H 3.69) of the cyclopentanepentol showed the HMBC correlation with one (δ_C 69.9, C-1) of two oxymethylene carbons of the glycerol unit. The other oxymethylene carbon (δ_C 69.0, C-3) of the glycerol unit showd the HMBC correlations to oxymethylene protons resonating at δ_H 3.41 and 3.39 (each 1H, t, *J* 6.6 Hz; H_2-1‴), which also showed the HMBC cross-peaks with an sp^3 methylene carbon at δ_C 29.5 (C-2‴). These findings suggested that an alkyl chain was attached to the C-3 of the glycerol unit through an ether linkage. Alkaline hydrolysis of **1** afforded two products, **2** and **3**. The ^1H NMR spectrum of **2** revealed that it contained the glycerol unit, and that the cyclopentanepentol moiety and alkyl chain were attached at C-1 and C-3 positions, respectively. The ^1H NMR spectrum of **2** showed a signal due to a secondary methyl group at δ_H 0.85 (3H, d, *J* 6.6 Hz), which was, therefore, located in the alkyl chain attached to C-3. The position of the secondary methyl group on that chain, however, remained undefined. The FABMS of **2** showed $(M + H)^+$ ions at *m/z* 463 and 449 (intensity ratio, 100:25). The carbon number of the alkyl chain at C-3 of the major component was, therefore, deduced to be 17 with no unsaturation ($C_{17}H_{35}$) and a $C_{16}H_{33}$ homologue for the alkyl chain at C-3 was contained as a minor component in **2**. The ^1H NMR spectrum of **3** showed that it was a mixture of saturated fatty acids with no branched methyl group. The EIMS analysis of **3** indicated the molecular ions at *m/z* 284, 270, 256 and 242, corresponding to $C_{18:0}$, $C_{17:0}$, $C_{16:0}$ and $C_{15:0}$ fatty acids with the intensity ratio of 12:38:100:31. This result was coincident with the FABMS data of **1**, which showed fragment ion peaks at *m/z* 459, 445, 431 and 417 with a similar intensity ratio. These ions were assignable to the C-2-side fragments generated by fission of the C-2/C-3 bond of the glycerol unit of **1**. The stereochemistry of the cyclopentanepentol moiety was deduced on the basis of the NOESY spectrum of the tetraacetate **4** in C_6D_6, which clearly showed the following cross-peaks: H-1′/H2′, H-2′/H-4′, H-4′/H-5′, H-3′/H-5′, H-5′/H-1′ and H-1′/H-3′. From these NOE correlations, the cyclopentanepentol moiety was indicated to be all-*cis* substituted. The structure of keruffaride was, therefore, concluded to be **1**.§

Several highly functionalized cyclopentanes possessing useful biological activities have been isolated from terrestrial microorganisms.[5] Keruffaride **1** is, however, the first compound containing the all-*cis* substituted cyclopentanepentol moiety from natural sources.

Table 1 ^1H and ^{13}C NMR spectral data of keruffaride **1**

Position	^1H, δ		*J*/Hz	^{13}C, δ	
1 (a)	3.79	dd	9.9, 4.6	67.0	t
(b)	3.77	dd	9.9, 5.0		
2	5.19	m		71.1	d
3 (a)	3.56	dd	10.5, 5.6	69.0	t
(b)	3.54	dd	10.5, 5.2		
1′	3.69	dd	4.0, 4.8	82.5	d
2′	3.93	dd	4.8, 4.4	79.3	d
3′	3.72	dd	4.4, 5.1	79.3	d
4′	3.87	dd	5.1, 4.4	80.7	d
5′	3.96	dd	4.4, 4.0	73.5	d
1″				174.0	s
2″	2.35 (2H)	t	6.6	34.4	t
1‴ (a)	3.41	dt	9.7, 6.4	72.0	t
(b)	3.39	dt	9.7, 6.7		
2‴	1.55 (2H)	m		29.5	t

† HPLC analysis of **1** using reversed-phase column (ODS) eluted with 75% methanol showed one broad peak (RI detection) with small shoulders. Further purification was not attempted because only a small amount had been isolated.

‡ Keruffaride **1** is a mixture of eight congeners possessing five discrete molecular weights (672, 686, 700, 714 and 728). The calculated ratio of the molecular weights were 7:51:100:37:11, based on the intensity ratio of (quasi-)molecular ions of the hydrolysis products (**2** and **3**) observed in their FAB and EIMS, respectively. This calculated ratio was almost consistent with the observed intensity ratio of the FABMS fragment ions of the parent molecule **1** at *m/z* 509, 523, 537, 551 and 565 in the ratio of 5:33:100:40:11. These fragment ions were ascribable to the C-2-side fragments $[M - CH_2OC_5H_5(OH)_4]^+$ generated by fission of the C-1/C-2 bond of the glycerol unit of **1**. The ratio of the $[M + Na]^+$ ions of **1** (*m/z* 695, 709, 723, 737 and 751) was, however, *ca.* 34:100:43:16:6, which was incompatible with the calculated ratio probably due to the fact that the intensities of the $[M + Na]^+$ ions were quite small, compared with those of the fragment ions of $[M - CH_2OC_5H_5(OH)_4]^+$.

§ The all-*cis* cyclopentanepentol moiety is achiral and the absolute configuration of the chiral centre at C-2 remains unassigned.

We thank Mr Z. Nagahama for his help with sponge collection and Dr J. Fromont, James Cook University of North Queensland, for identification of the sponge. This work was partly supportd by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Received, 24th July 1992; Com. 2/03971K

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