Tetrahedron Vol. 49, No. 13, pp. 2711-2716, 1993
Printed in Great Britain

0040-4020/93 $6.00+
Pergamon Press Ltd

An Unusual Ether Glycolipid from the Senegalese Sponge *Trikentrion loeve* Carter

Valeria Costantino, Ernesto Fattorusso*, Alfonso Mangoni
Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy

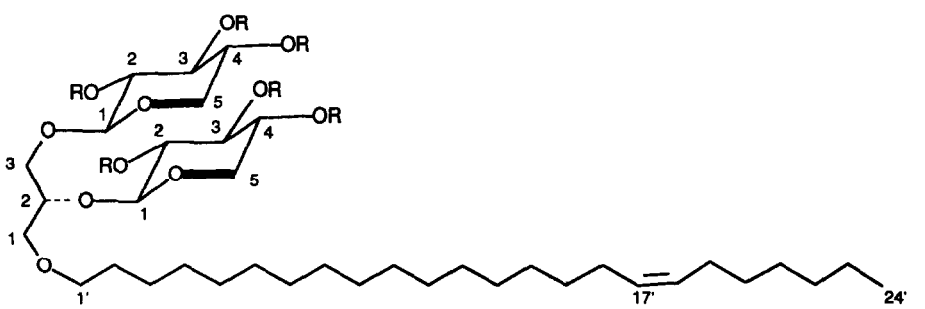
Maurice Aknin, Aliou Fall, Abdoulaye Samb
Departement de Chimie, Faculté des Sciences, Université Cheikh Anta Diop, Dakar, Senegal

Joseph Miralles
Departement de Biologie Végétale, Faculté des Sciences, U.C.A.D., Dakar, Senegal

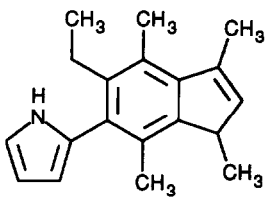
(*Received in UK* 16 *December* 1992)

Abstract: A unique ether glycolipid, **1**, characterized by the glycosylation of two glycerol hydroxy groups, has been isolated from the Senegalese sponge *Trikentrion loeve* Carter, and its structure determined by spectroscopic and chemical analysis.

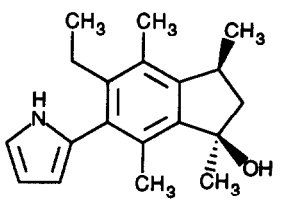
Ether glycolipids, which are mainly distributed among bacteria and mammalians, are characterized by the presence of 1 or 2 alkyl chains, linked by ether bonds to position 1, 2 or 3, and a carbohydrate moiety (most often galactose) linked glycosidically to one of the hydroxy groups of glycerol.[1] From *Trikentrion loeve* Carter (Axinellide, family Euryponidae), a sponge collected along the coast of Senegal, we have now isolated a new ether glycolipid, **1**, which is quite different from known compounds of this kind for the nature of the sugars and for the number of hydroxy groups involved in the glycosidic linkages. Two unusual nitrogen metabolites, trikentramine (**2**) and hydroxytrikentramine (**3**), have been recently isolated from the lipophilic extract of the same sponge.[2,3]



1 R = H **4** R = Ac



2



3

Air dried sponges were extracted at room temperature with $CHCl_3$/MeOH (2:1) and the crude extract (26.5 g) submitted to silica gel column chromatography with solvent of increasing polarity. HPLC separation (silica gel column, eluent EtOAc) of a fraction eluted with MeOH (524 mg) led to 246 mg of pure compound **1**.

The FAB mass spectrum (negative ion mode) of compound **1** showed a quasi-molecular ion at *m/z* 689 ([M^+–H]), corresponding to $C_{37}H_{70}O_{11}$. The ^1H NMR spectrum of **1** in CD_3OD displayed a large band at δ 1.32, a series of overlapping signals between δ 3 and 4, and two doublets at δ 4.45 (J = 7.0 Hz) and 4.28 (J = 7.0 Hz), attributable to two anomeric protons. These features suggested the glycolipidic nature of compound **1**. A better proton dispersion was obtained in the proton spectrum of the peracetylated derivative **4**, obtained by treatment of **1** with Ac_2O/Py at room temperature. This derivative was used for all the subsequent NMR structural studies. The ^1H NMR spectrum of **4** in $CDCl_3$ revealed six distinct methyl singlets between δ 1.99 and 2.03, corresponding to six acetoxy groups, while the corresponding six carbonyl carbon atoms resonated between δ 169.3 and 170.0 in the ^{13}C NMR spectrum. The region of the proton NMR spectrum between δ 3.3 and 5.4 contained a number of well resolved, although still partially overlapped, multiplets. A two-dimensional HOHAHA NMR spectrum performed on compound **4** clearly indicated that most of the above signals belonged to three separate spin systems; a COSY NMR experiment confirmed the connectivities, and allowed us to establish the sequence. Thus, a methine proton coupled with

Table 1. ^1H and ^{13}C NMR data of compound **4**.

Pos.		δ_H (mult., J in Hz)	δ_C (proton count)	Pos.		δ_H (mult., J in Hz)	δ_C (proton count)
1	a	3.47 (dd, 10 5, 3 7)	70.92 (CH_2)	5"	eq	4.07 (dd, 11 8, 3 9)	62.02 (CH_2)
	b	3.39 (dd, 10 5, 6 7)			ax	3.33 (dd, 11 8, 9 3)	
2		3.89 (quintet, 4 3)	76.89 (CH)	1"'		3.35 (t, 6 0)	71.76 (CH_2)
3	a	3.82 (dd, 11 0, 4 4)	68.79 (CH_2)	2"'		1.50 (quintet, 6 8)	28.9-29 7 (CH_2)
	b	3.61 (dd, 11 0,4 8)		3"'-15"'		1.22 (m)	28.9-29 7 (CH_2)
1'		4.53 (d, 7 0)	100.63 (CH)	16"'		1.98 (m)	26.09 (CH_2)
2'		4.85 (dd, 8 9, 6 2)	70.68 (CH)	17"'		5.31(t,4 5)	129.82 (CH)
3'		5.17 (t, 8 9)	71.47 (CH)	18"'		5.31 (t, 4 5)	129.82 (CH)
4'		4.90 (dt, 9 0, 9 0, 3 9)	68.91 (CH)	19"'		1.98 (m)	26.07 (CH_2)
5'	eq	4.05 (dd, 11 8, 3 9)	62.01 (CH_2)	20"'-21"'		1.22 (m)	28.9-29 7 (CH_2)
	ax	3.35 (dd, 11 8, 9 3)		22"'		1.22 (m)	31.71 (CH_2)
1"		4.69 (d, 7 0)	100.27 (CH)	23"'		1.22 (m)	22.59 (CH_2)
2"		4.85 (dd, 8 9, 6 9)	70.68 (CH)	24"'		0.85 (t, 7 0)	14.05 (CH_3)
3"		5.12 (t, 8 9)	71.47 (CH)	$\underline{CH_3}CO$		1.99-2 03	20.61-20 68 (CH_3)
4"		4.90 (dt, 9 0, 9 0, 3 9)	68.91 (CH)	$CH_3\underline{CO}$			169.33-170 02 (C)

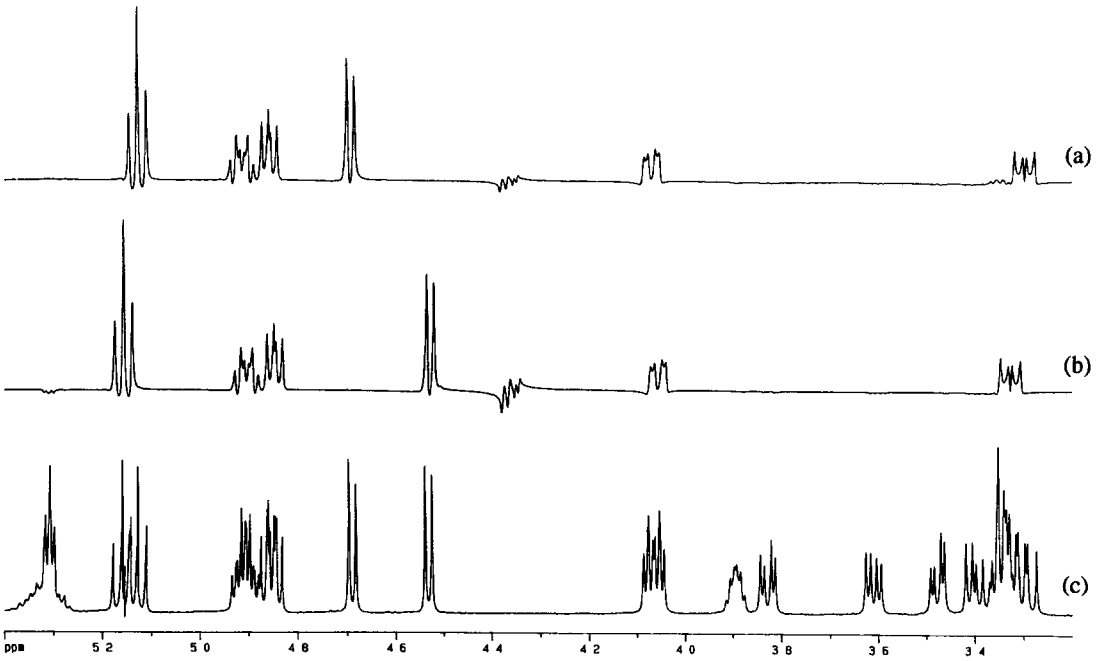
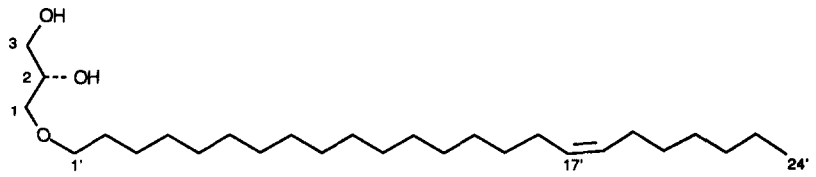


Figure 1. Sections of the HOHAHA spectrum of **4** at δ 4.69 (a) and δ 4.53 (b), in comparison with the low field region of the 1D proton NMR spectrum (c).

two pairs of methylene protons was evidenced, indicative of a glycerol moiety; the sequences of connectivities originating from either anomeric proton were shown to comprise, in sequence, three oxymethine and a couple of oxymethylene protons, suggesting two pentose units to be present in structure **4**. Short- and long-range shift correlated 2D ^{13}C-^{1}H NMR experiments were carried out. The short-range experiment (HETCOSY) permitted unambiguous association of the signals of protons whit the signals of the carbons to which they were attached (see Table 1). The long range experiment (COLOC) revealed connectivities between protons and carbons separated by two or three σ-bonds. This was particularly useful in revealing the linkage of the two sugar moieties with the glycerol fragment. Thus the proton resonating at δ 4.69 (H-1") showed a correlation peak to the carbon signal at δ 76.89 (C-2), while both H-3_a and H-3_b were coupled with the anomeric carbon (δ 100.63, C-1') of the remaining sugar moiety.

Further analysis of the ^{1}H NMR experiments established the nature of the two pentose groups, which resulted to be identical, as suggested by the sections of the HOHAHA spectrum in correspondence with the well-separated anomeric protons (see Figure 1). The subspectra appeared very similar, showing signals identical in the molteplicities and only slightly different in the chemical shift. The two pairs of oxymethylene protons resonated at relatively high field (δ 4.05, H-$5'_{eq}$;δ 3.35, H-$5'_{ax}$; δ 4.07, H-$5''_{eq}$; δ 3.33, H-$5''_{ax}$), thus indicating that the relevant oxygen atoms were part of an acetal rather than an ester group, and therefore that the two sugars were in pyranose form. This was confirmed by the long range coupling of H-$5'_{eq}$ with



5

C-1'and of H-5"$_{eq}$ with C-1" in the COLOC spectrum of **4**. Furthermore, the axial stereochemistry of all the sugar oxymethine protons (including the anomeric proton) was revealed by the values of their coupling constants, as determined from the HOHAHA subspectra (see Table 1). Thus, two β-xylopyranosyl groups were proven to be linked at position 2 and 3 of the glycerol unit.

What remained for the final determination of structure **1** was the identification of the substituent linked to the oxygen atom at C-1, which, on the basis of the molecular ion peak observed in the negative FAB-MS spectrum of **1**, could be a $C_{24}H_{47}$ fragment. It was identified ad a (Z)-tetracos-17-enyl group on the basis of the following evidence. All the resonances expected for this substituent were present in the ^{1}H NMR spectrum of **4** (δ 3.35, 2H, t, J = 6.0 Hz, H_2-1'''; δ 1.50, 2H, quintet, J = 6.8 Hz, H_2-2'''; δ 5.31, 2H, t, J = 4.5 Hz, H-17''' and H-18'''; δ 1.98, m, H_2-16''' and H_2-19''', δ 0.85, t, J = 7.0 Hz, H_3-24'''), and all the required ^{1}H-^{1}H couplings were evident in the COSY spectrum. The corresponding ^{13}C NMR signals were identified from the HETCOSY experiment; the high field chemical shift of the allylic methylene groups defined the *Z* stereochemistry of the double bond.[4] The location of the double bond along the linear carbon chain was determined from chemical evidence. Compound **1** was subjected to acidic methanolysis to give the alkylglycerol **5**, which was oxidized with $KMnO_4/NaIO_4$. GC-MS analysis of the oxidization mixture, led to the identification of heptanoic acid, thus indicating that the double bond must link C-17''' and C-18''' in **3**.

Finally, the configuration at C-2 was established from the positive optical rotation exhibited by the methanolysis product **5**, which is a general feature of all the long-chain 1-alkyl-*sn*-glycerols.[5]

EXPERIMENTAL SECTION

General methods. FAB-MS spectra were obtained in a glycerol matrix on a VG ZAB mass spectrometer (Xe atoms of energy of 2-6 kV). FT-IR spectra were recorded on a Bruker IFS-48 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 192 polarimeter in methanol solution, using a sodium lamp operating at 589 nm and a 10-cm microcell.

^{1}H and ^{13}C NMR spectra were determined on a Bruker AMX-500 spectrometer. Methyl, methylene and methine carbons were distinguished by a DEPT experiment. One-bond heteronuclear ^{1}H-^{13}C connectivities were determined with an XHCORR experiment, optimized for an average C-H coupling of 135 Hz. Two-

and three-bond heteronuclear ^{1}H-^{13}C connectivities were determined by a COLOC experiment, optimized for $^{2,3}J_{CH}$ of 8 Hz.

The 2D HOHAHA experiment was performed in the phase-sensitive mode (TPPI) using a MLEV-17 sequence for mixing.[6] The spectral width was 1139 Hz; 128 experiments (mixing time 100 ms) were aquired in 4K data points. For processing, a 45°-shifted sine bell function was applied in both dimensions before transformation. The resulting digital resolution in F2 was 0.28 Hz/pt.

High performance liquid chromatographies (HPLC) were performed on a Varian 2050 apparatus equipped with an RI-3 refractive index detector, using Hibar columns.

Collection and extraction. Specimens of *T loeve* were collected by hand (SCUBA) at Thiouri-ba, Senegal (depth 40-45 m). Reference specimens are deposited at the Centre de Oceanologie de Marseille, Station Marine d'Endoume. The animals (531.4 g, dry weight) were dried to the air and extracted with $CHCl_3$/MeOH 2:1 (500 ml × 6) at room temperature. The extracts were evaporated *in vacuo* to give an oily residue (26.5 g), which was chromatographed on an SiO_2 column with solvents of increasing polarity.

Isolation of 1. Evaporation of fractions eluted with MeOH afforded a mixture (524 mg) containing compound **1**. The mixture was purified by HPLC using a Hibar LiChrosorb Si60 (10 × 250 mm) column with a mobile phase of $CHCl_3$/MeOH (9:1), giving 246 mg of pure compound **1**.

1-O-(Z-tetracos-17-enyl)-2,3-di-O-(β-D-xylopyranosyl)-sn-glycerol (1): mp 98-100 °C; $[\alpha]_D^{25}$ –26.9°; FABMS, negative ion mode *m/z* 690 ($[M-H]^+$); ^{1}H and ^{13}C NMR data are reported in Table 1.

Acetylation of compound 3. Compound **3** (50 mg) was allowed to stay overnight with 200 μL of Ac_2O in 0.5 mL of anhydrous pyridine. MeOH was added, and the mixture evaporated to dryness to yield 65 mg of the hexaacetate **4.**

1-O-(Z-tetracos-17-enyl)-2,3-di-O-(β-D-xylopyranosyl)-sn-glycerol hexaacetate (4): $[\alpha]_D^{25}$ -25.3°;^{1}H and ^{13}C NMR data are reported in Table 1.

Methanolysis of compound 1: Compound **1** (100 mg) was kept overnight in anhydrous 2 M HCl-MeOH (1 mL) in a stoppered reaction vial at 70°C. After cooling, the reaction mixture was neutralized with solid $AgCO_3$, and the supernatant was freed from insoluble material by centrifugation, and then partitioned between water and EtOAc. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, thus obtaining the pure alkylglycerol **5** (49 mg)

1-O-(Z-tetracos-17-enyl)-sn-glycerol (5): $[\alpha]^{25}_D$ +4.5°; ^{1}H NMR (CD_3OD): δ 5.38 (2H, m, H-17 and H-18), δ 3.78 (1H, quintet, J = 6.5 Hz, H-2), δ 3.61 (1H, dd, J = 11.2 and 5.2 Hz, H-1_a), δ 3.54 (1H, dd, J = 11.2 and 5.8 Hz, H-1_b), δ 3.51 (1H, dd, J = 9.9 and 4.8 Hz, H-3_a), δ 3.44 (1H, dd, J = 9.9 and 6.2 Hz, H-3_b), δ 3.49 (2H, t, J = 6.5, H_2-1'), δ 2.07 (4H, m, H_2-16' and H_2-19'), δ 1.62 (2H, quintet, J = 6 Hz, H_2-2'), δ 0.94 (3H, t, J = 6.5 Hz, H_3-24'), .

Oxidation of 5: To **5** (2 mg) in tert-butyl alcohol (2 ml), K_2CO_3 0.04 M (0.3 ml) and an aqueous solution (1.8 ml) 0.023 M in $KMnO_4$ and 0.09 M in $NaIO_4$ were added. The reaction mixture was allowed to proceed at 37 °C for 18 h. After acidification with H_2SO_4 5 N, the solution was decolorized with aqueous $NaHSO_4$ 1 M and extracted with Et_2O (4 ml in 2 portions). After drying over $CaSO_4$, the combined ethereal extract were concentrated to 0.5 ml. The resulting solution, analyzed by GLC-MS, was found to contain *n*-heptanoic acid.

AKNOWLEDGEMENT

This work was supported by CNR, Progetto Finalizzato Chimica Fine II, and by M.U.R.S.T. (40% and 60%), Rome, Italy. Mass spectral data were provided by Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli. The assistance of the staff is greatly appreciated.

REFERENCES

1. Egge,H.: Ether Glycolipds. In *Ether Lipids*; Mangold, H.K.; Paltauf, F.Eds.; Academic Press, Inc.:New York, 1983; pp.141-156.
2. Aknin, M.; Miralles, J.; Kornprobst, J.M.; Faure, R.; Gaydou, E.M.; Boury-Esnault, N.; Kato,Y.; Clardy, J.; *Tetrahedron Letters*, **1990**, *31*, 2979-2982.
3. Aknin, M.; Miralles, J.; Kornprobst, J.M.; Faure, R.; Gaydou, E M..; Kato,Y.; Clardy, J. *Tetrahedron Letters*, **1992**, in press.
4. Gunstone, F. D.; Pollard, M. R.; Scrimgeour, C. M.; Vedanayagam, H. S. *Chem. Phys. Lipids* **1977**, *18*, 115.
5. Baer, E.; Fisher, H. O. L. *J. Biol. Chem.* **1941**, *140*, 407.
6. Bax, A.; Davis, D.G. *J. Mag. Res.*, **1986**, *67*, 565.