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Note

X-ray structure of a sodium salt of digeneaside isolated from red alga *Ceramium botryocarpum*

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ABSTRACT

X-ray diffraction analysis has been recently used to determine the crystal structure of the floridoside (2-*O*-α-D-galactopyranosylglycerol) isolated from red alga *Palmaria palmata* and *Dilsea carnosa*, respectively [Simon-Colin, C.; Michaud, F.; Léger, J.-M.; Deslandes, E. *Carbohydr. Res.* **2003**, *338*, 2413–2416; Vonthron-Senechau, C.; Sopkova-de Oliveira Santos. J.; Mussio, I.; Rusig, A. M. *Carbohydr. Res.* **2008**, *343*, 2697–2698]. In this present study, a similar analysis was performed on another compound belonging to the glycopyranosyl–glycerols family present in red algae, digeneaside. The crystal structure of a hydrated sodium salt of digeneaside (sodium 2-*O*-α-D-mannopyranosyl-D-glycerate monohydrate) was determined by single-crystal X-ray diffraction analysis at 110 ± 3 K. The space group is *C*2 with $Z = 4$, $a = 17.9315(12)$, $b = 6.2693(4)$, $c = 10.7805(7)$ Å, beta = 90.746(7)°.

During photosynthesis, marine red seaweeds biosynthesize different types of low-molecular-weight carbohydrates (LMWCs) such as floridoside (2-*O*-α-D-galactopyranosylglycerol)[1,2], digeneaside (2-*O*-α-D-mannopyranosyl-D-glyceric acid),[3,4] and D- and L-isofloridoside (1-*O*-α-D/L-galactopyranosyl-L-glycerol).[5,6] In the past, digeneaside and isofloridoside have been regarded as useful chemotaxonomic tools for *Ceramiales* and *Bangiales*, respectively. However, recent papers reported some exceptions that challenged this rule[7–9] and which highlighted the biochemical diversity of LMWCs in Florideophyceae. A study by Karsten and co-workers in 2007[10] clearly demonstrated that only isofloridoside can be considered as a chemotaxonomic marker, in contrast to digeneaside and floridoside.

The chemical structures of glycopyranosyl–glycerols from marine red seaweeds were fully studied by NMR spectroscopy.[11–13] However, only floridoside was subjected to crystallographic analyses.[14,15] In this Letter, the crystal structure of natural digeneaside in salt form, purified from the red alga *Ceramium botryocarpum*, was investigated by a single-crystal X-ray diffraction analysis. The results reported herein continue the crystallographic study of the glycopyranosyl–glycerols series present in red algae, started by Simon-Colin and co-workers.[14]

Thin, planar transparent crystals have been analyzed by X-ray diffraction at room temperature, 170 K and 110 K. No structural change was noticed in this temperature range. The results reported hereafter are related to the studies carried out at 110 ± 3 K. Crystal and structure refinement data are summarized in Table 1. The use of low temperature was aimed at fixing the $CH(CH_2OH)(COO^-)$ group, which nevertheless could not be refined without assuming some disorder. A rather satisfactory fitting model implies three slightly different conformations with statistical occupancy factors 0.45, 0.35, and 0.20. Comparison of displacement parameters, bond lengths, and angles found for the three conformations of this group is given in Table 2. These lengths and angles agree approximately with the literature[17] while those found for the ordered part are quite consistent: C–C lengths range from 1.511(4) Å [C(5)–C(6)] to 1.525(4) Å [C(4)–C(5)] and C–O lengths range from 1.408(3) Å [C(1)–O(8)] to 1.439(3) Å [C(5)–O(7)] while angles range from 105.8(2)° [O(7)–C(5)–C(6)] to 114.5(2)° [C(1)–O(7)–C(5)].

The resulting asymmetric unit is shown in Figure 1 with the most frequent conformation of the $CH(CH_2OH)(COO^-)$ group. The chair conformation is stabilized by interactions of oxygen atoms with sodium ions. The absolute configuration could not be determined from anomalous dispersion effects. It has been chosen by analogy with other biological glycerol derivatives (i.e., floridoside). The chirality of the asymmetric carbon atoms follows: C(1) R, C(2) S, C(3) S, C(4) R, C(5) R, and C(10) R.

The interactions with sodium involve all oxygen atoms of the mannose ring and only them. As can be seen in Figure 2, each digeneaside anion is bound to three sodium cations and vice versa.

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Table 1
Crystal and structure refinement data

Empirical formula	$C_9H_{17}NaO_{10}$
Formula weight (g/mol)	308.22
Temperature (K)	110(3)
Wavelength (Å)	0.71073
Crystal system, space group	Monoclinic, *C*2
Unit cell dimensions	
a (Å)	17.9315(12)
b (Å)	6.2693(4)
c (Å)	10.7805(7)
β (°)	90.746(7)
Volume (Å^3)	1211.82(14)
Z, calculated density (Mg/m^3)	4, 1.689
Absorption coefficient (mm^{-1})	0.183
Max. and min. transmission (calcd)	0.9927 and 0.9713
F(000)	648
Crystal size (mm)	0.16 × 0.15 × 0.04
Crystal description	Colorless plate, main faces {001}
Theta range for data collection	3.44–30.51°
Limiting indices	$-25 \leqslant h \leqslant 20$, $-8 \leqslant k \leqslant 8$, $-15 \leqslant l \leqslant 15$
Reflections collected/unique	6184/1985 [*R*(int) = 0.0462]
Completeness to theta = 30.51°	99.0%
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1985/30/189
Goodness-of-fit on F^2	0.975
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0486$, $wR_2 = 0.0881$
R indices (all data)	$R_1 = 0.0702$, $wR_2 = 0.1049$
Largest diff. peak and hole (e Å^{-3})	0.304 and −0.310

Table 2
Disordered terminal group of the digeneaside molecule. Isotropic displacement parameters U (Å^2), bond distances (Å), and bond angles (°) of the three superimposed conformations A, B, and C

	Conf. A [0.45]	Conf. B [0.35]	Conf. C [0.20]
O(8)	0.020(1)	0.020(1)	0.020(1)
C(9)	0.015(1)	0.015(1)	0.015(1)
O(90)	0.026(1)	0.026(1)	0.026(1)
C(10)	0.023(1)	0.023(1)	0.023(1)
C(11)	0.019(1)	0.019(1)	0.019(1)
O(12)	0.018(1)	0.018(1)	0.018(1)
O(13)	0.019(1)	0.019(1)	0.019(1)
C(9)–O(90)	1.43(2)	1.42(2)	1.40(3)
C(9)–C(10)	1.59(2)	1.51(2)	1.51(2)
C(10)–C(11)	1.52(2)	1.53(3)	1.54(3)
C(11)–O(12)	1.244(9)	1.263(13)	1.273(14)
C(11)–O(13)	1.25(3)	1.26(2)	1.28(3)
O(8)–C(10)–C(9)	99.7(4)	110.1(4)	106.6(6)
O(8)–C(10)–C(11)	109.3(5)	119.0(7)	115.4(9)
C(9)–C(10)–C(11)	106.5(6)	101.9(8)	128.9(10)
O(90)–C(9)–C(10)	107.5(5)	109.0(9)	103.3(13)
O(12)–C(11)–O(13)	125.7(6)	126.0(9)	122.6(12)
C(10)–C(11)–O(12)	122.2(6)	116.9(9)	117.9(11)
C(10)–C(11)–O(13)	121.1(16)	116.7(14)	119(2)

Atoms O(8) and C(10) have been refined anisotropically as belonging to the common skeleton, all other atoms in the table have been refined with a common isotropic displacement parameter for the three positions. Statistical occupancy factors are given in square brackets.

This gives rise to infinite chains generated by screw axes parallel to ***b***. Interatomic distances and angles within a chain are given in Table 3.

Several hydrogen bonds are expected in addition to ionic bonds. They appear to involve either the $CH(CH_2OH)(COO^-)$ group of digeneaside or the water molecule. The disorder makes this difficult to state unambiguously. Table 4 gathers only the reliable data in this respect. Figure 3 shows the molecular packing projected along ***b*** axis. It can be described as a close packing of parallel digeneaside–sodium chains, interacting through hydrogen bonds as well as van der Waals forces, which form layers parallel to (001). Interactions between layers are less dense. The only hydrogen bonds between layers imply water molecules, which is likely to display a disorder correlated to that of the ramifications of digeneaside.

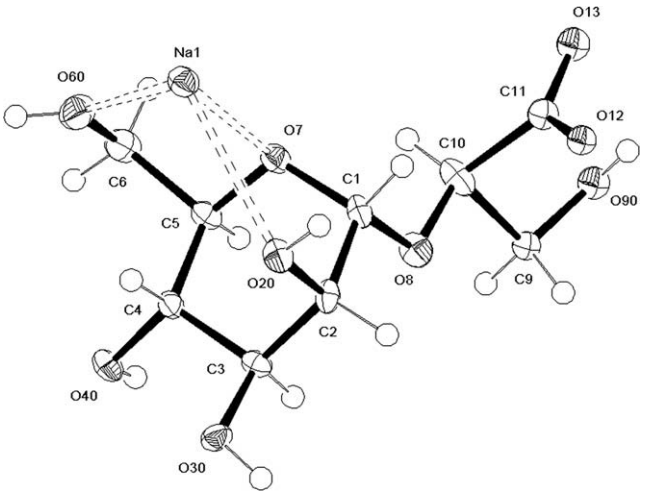

Figure 1. ORTEP view of asymmetric unit of monohydrated sodium digeneaside salt showing the labeling of non-hydrogen atoms. For the sake of legibility, the water molecule is not represented nor the two less occupied positions of the disordered terminal group. Thermal ellipsoids are drawn at 50% probability level.

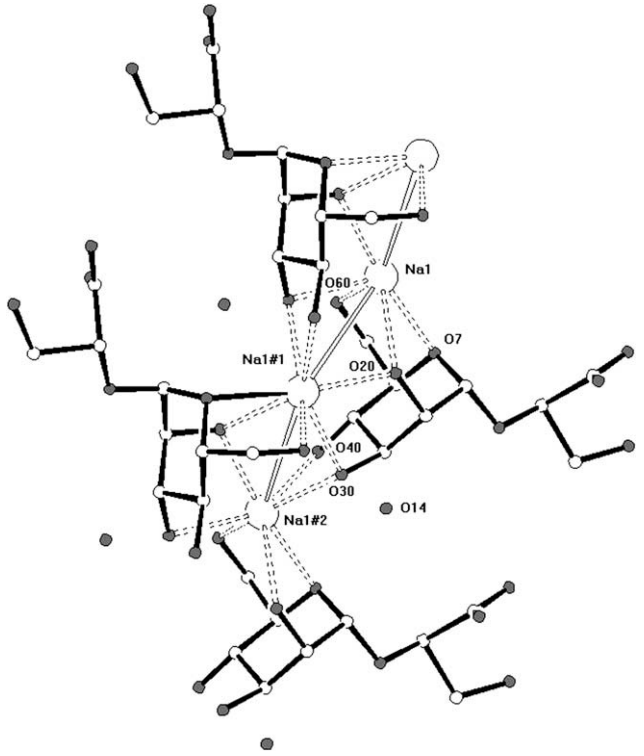

Figure 2. Molecular packing implying ionic bonds. Oxygen atoms are drawn in gray, including water.

This layer description is consistent with the thin flat morphology parallel to (001) exhibited by the crystals.

1. Experimental

1.1. Preparation of pure digeneaside

Digeneaside was extracted and isolated in the red alga *C. botryocarpum* Griffiths ex Harvey, supplied by the Laboratoire de Biotechnologie et Chimie marines (LBCM), Lorient, France. A freeze-dried powder of plant material (100 g) was extracted two

Table 3
Digeneaside–sodium interactions

Na(1)–Na(1)$^{\#1}$	3.9799(15)
O(7)–Na(1)	2.467(2)
O(20)–Na(1)	2.754(3)
O(60)–Na(1)	2.314(3)
O(20)–Na(1)$^{\#1}$	2.366(3)
O(30)–Na(1)$^{\#1}$	2.550(3)
O(30)–Na(1)$^{\#2}$	2.446(3)
O(40)–Na(1)$^{\#2}$	2.458(2)
Na(1)–Na(1)$^{\#1}$–Na(1)$^{\#2}$	103.93(5)
Na(1)–O(20)–Na(1)$^{\#1}$	101.79(8)
Na(1)$^{\#1}$–O(30)–Na(1)$^{\#2}$	105.58(8)

Bond lengths (Å) and main angles (°) within digeneaside-sodium chains.
Symmetry transformations used to generate equivalent atoms: (#1) $-x+1/2$, $y+1/2$, $-z$; (#2) x, $y+1$, z.

Table 4
Selected hydrogen bonds in sodium digeneaside salt layers (Å and °)

D–H···A	d(D–H)	d(H···A)	d(D···A)	Angle (DHA)
O(20)–H(20)···O(12A)$^{\#3}$	0.84	1.77	2.597(10)	167.1
O(30)–H(30)···O(12A)$^{\#4}$	0.84	1.90	2.692(10)	156.1
O(40)–H(40)···O(14)	0.84	1.90	2.733(3)	175.1

Interactions implying the disordered terminal group in its less occupied positions are not given.
Symmetry transformations used to generate equivalent atoms: (#3) $-x+1$, y, $-z$; (#4) $-x+1$, $y+1$, $-z$.

times with CH_3OH–$CHCl_3$–H_2O 12:5:3 (v/v/v) (10% w/v), under magnetic stirring, at room temperature. The aqueous methanolic phase resulting from this extraction was concentrated on a rotary evaporator and was freeze dried (13.5 g). Next, 1 g of the 13.5 g of the crude extract obtained was dissolved in H_2O, and was then applied to an ion exchange column (Cl- form, 1 X8, 100-200 mesh, 200 mL, Dowex-Fluka Chemika), converted to OH– form. The column was first washed with distilled H_2O (500 mL), and then eluted with solution of NaOH 1 M (300 mL). After neutralization, the sample was desalted by gel-filtration through a Sephadex G10 column (100 × 1.5 cm i.d.) eluted with distilled H_2O. Elution profiles were determined by the phenol–sulfuric acid method and by conductivity monitoring for sugar and salt detection, respectively. Sugar fractions were pooled and freeze dried giving 100 mg of pure digeneaside.

1.2. Preparation of crystals of digeneaside salt

Boiling ethanol (40 mL) was swiftly added to a solution of digeneaside (50 mg of lyophilized powder of digeneaside dissolved in 200 μL of distilled H_2O). Translucent flat crystals (10 mg, 2%) were obtained by slow evaporation of solvents at room temperature. The melting point was estimated to be 190–195 °C on a Kofler bench apparatus, in agreement with the literature (mp 190–192 °C).[16]

1.3. Spectroscopic analyses

^{1}H NMR (500 MHz, D_2O) δ 4.88 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.08 (dd, 1H, $J_{2,1}$ 1.5, $J_{2,3}$ 3.5 Hz, H-2), 3.93 (dd, 1H, $J_{3,2}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3), 3.67 (m, 1H, $J_{4,3}$ 10.0 Hz, H-4), 3.74 (m, 1H, H-5), 3.89 (m, 1H, H-6_a), 3.76 (m, 1H, H-6_b), 4.21 (dd, 1H, $J_{2',3'a}$ 3.0, $J_{2',3'b}$ 7.0 Hz H-2′), 3.84 (d, 2H, $J_{3'a,2'}$ 3.0 Hz, H-3′$_a$), 3,77 (d, 2H, $J_{3'b,2'}$ 6.5 Hz, H-3′$_b$), ^{13}C NMR (600 MHz, D_2O), δ 101.4 (C-1), 72.9 (C-2), 73.2 (C-3), 69.6 (C-4), 75.8 (C-5), 63.8 (C-6), 179.8 (C-1′), 80.8 (C-2′), 65.9 (C-3′). These chemical shifts are in agreement with those reported in Ascensio et al.,[12] except for the ^{13}C NMR assignments showing a shift difference of 2.9 ppm probably due to some variation of pH and calibration methods.

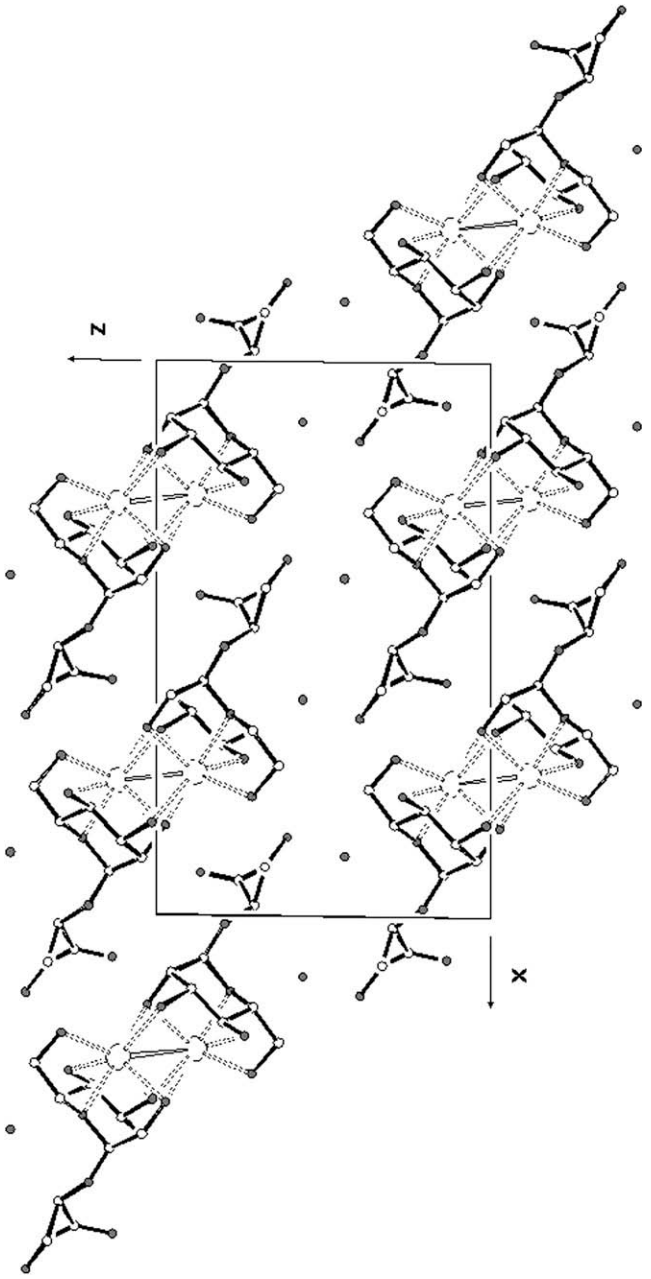

Figure 3. Projection along *y* axis (direction of the sodium-digeneaside chains) showing two layers of parallel chains (*xy* direction) with respect to the outlined unit cell. Oxygen atoms are drawn in gray.

HRESIMS analysis of the sodium salt of digeneaside (MW = 290) recorded in positive ion mode produced a main ion at *m/z* 267 attributed to $[M-Na]^-$. These results were in good agreement with those reported in Ascensio et al.[12]

1.4. X-ray studies

Crystals were grown at room temperature by slow evaporation of saturated ethanolic solution of the compound. X-ray diffraction data were collected on an Oxford Diffraction X-Calibur-2 four-circle diffractometer with Sapphire-2 CCD detector and Cryojet nitrogen stream cryostat, using graphite monochromatized Mo Kα radiation. The data were reduced by means of Crysalis software.[18] No absorption correction was needed owing to the small size of the crystal. The structure was solved by direct methods, using SHELXS97 program,[19] and refined on F^2 by weighted anisotropic full-matrix least-

square methods, using SHELXL97 program.[19] The disordered group was refined isotropically with a common displacement parameter for each atom in the three positions. Drawings and additional geometric analyses were carried out with ORTEP[20] and PARST[21] programs, respectively. All these programs were used within WINGX package.[22]

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