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Note

Complete ^{1}H and ^{13}C spectral assignment of floridoside

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Abstract

Floridoside (2-*O*-α-D-galactopyranosylglycerol) was extracted from the red marine alga *Rhodymenia palmata*, and purified by ion-exchange chromatography: 1D and 2D NMR spectroscopy experiments were used to unambiguously assign the complete ^{1}H and ^{13}C spectra. © 2002 Elsevier Science Ltd. All rights reserved.

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Floridoside (2-*O*-α-D-galactopyranosylglycerol) was first isolated in 1930 by Colin and Guéguen[1] from the Rhodophyceae *Rhodymenia palmata* and its molecular structure was established by Putman and Hassid[2] in 1954. Floridoside is considered to be the main photosynthetic reserve product in most red algae,[3–6] where it is also believed to function as an intracellular osmotic regulator.[7,8]

A number of reports have dealt with the NMR spectroscopy characterization of floridoside[6,8–11] and the HR-MAS NMR technique was even used to detect it in intact plant material.[12,13] However, some data are still incomplete and controversial. The present study reports on the isolation of floridoside from the red alga *R. palmata* and its subsequent complete NMR spectral assignment.

Extraction of low-molecular weight components from *R. palmata* and subsequent purification by ion-exchange chromatography led to the isolation of pure floridoside in the neutral fraction with an overall yield of 1.7% on a dry weight basis, consistent with concentrations previously reported.[14–16]

The ^{13}C and ^{1}H resonances of floridoside, assigned using COSY, TOCSY, HMQC and HMBC experiments, are reported in Table 1.

The ^{13}C NMR assignments reported here for floridoside are in good fitting with the data given by Meng et al.[17], with however a shift difference of 1.7 ppm probably due to some variations in temperature or pH. On the other hand, the results are quite different from the ones reported by Karsten et al.[8] and Abreu et al.[9] While it was thought that C-1′ was at lower field than C-6 and C-4 at upper field than C-3, it is obvious from the 2D NMR correlations that these assignments can be interchanged.

1. Experimental

Material.—*R. palmata* (Lamouroux) Silva was collected from the mi-sublittoral zone at Plouzané, in the Bay of Brest, France. Fresh material was then briefly washed with water and frozen in liquid nitrogen.

Floridoside isolation.—Plant material (200 g fresh weight) was ground in liquid nitrogen and then extracted with 12:5:3 MeOH–CHCl$_3$–water (400 mL) for

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Table 1
^1H–^{13}C chemical shifts and correlations observed in the HMQC, HMBC, COSY and TOCSY experiments of a solution (99.9% D_2O) of floridoside

Atoms	δ (ppm)		$J_{H\text{–}C}$ correlations		$J_{H\text{–}H}$ correlations		
	^{13}C	^1H	$^2J_{H\text{–}C}$	$^3J_{H\text{–}C}$	$^3J_{H\text{–}H}$	$^4J_{H\text{–}H}$	$^5J_{H\text{–}H}$
1	100.98	5.14 *d* ($J_{H\text{–}H}$ 4 Hz)	71.3	72.18, 73.92, 81.6	3.82	3.81, 3.9, 4.09	3.99
2	71.3	3.82 *m*	72.18, 100.98		3.9, 5.14	3.99	
3	72.18	3.90 *m*	71.3, 72.12	100.98	3.82, 3.99	5.14	
4	72.12	3.99 *d*	72.18, 73.92	71.3, 63.97	3.9, 4.09	3.82	5.14
5	73.92	4.09 *t*	63.97, 72.12	100.98	3.74, 3.99	5.14	
6	63.97	3.74 *m*	73.92	72.12	4.09		
1′	64.24	3.76 *m*	81.6	63.18			
2′	81.6	3.81 *m*	63.18, 64.24	100.98			
3′	63.18	3.78 *m*	81.6	64.24			

The primed atoms refer to the glycerol moiety.

1 h at rt. The hydroalcoholic phase was concentrated in a rotary evaporator, and then purified by passing successively through columns of AG 50 (200 mL, 20–50 mesh, X8, H^+, Biorad) and AG 1 (200 mL, 20–50 mesh, X8, OH^-, Biorad) finally washed with distilled water (500 mL). The neutral effluent was evaporated to dryness and redissolved in hot EtOH. White floridoside crystals (500 mg, 1.7%) were obtained after partial evaporation of the alcoholic fraction; mp 129 °C, lit.[2] 128.5 °C, lit.[18] 127–127.5 °C, lit.[19] 132–134 °C; $[\alpha]_D$ $+164°$ (*c* 0.97, water), lit.[2] $[\alpha]_D$ $+165°$ (*c* 3.35, water), lit.[18] $[\alpha]_D$ $+172°$ (*c* 0.7, water), lit.[19] $[\alpha]_D$ $+162°$ (*c* 1, water).

NMR spectroscopy.—^1H and ^{13}C NMR experiments were performed at 298 K on a Bruker DRX 500 spectrometer from solutions of pure floridoside in 99.9% D_2O. Chemical shifts are expressed in ppm relative to 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propanoic acid sodium salt. ^1H–^1H double-quantum-filtered correlated spectroscopy COSY-DQF, ^1H–^1H total correlation spectroscopy TOCSY with a mixing time of 100 ms, ^1H–^{13}C heteronuclear multiple quantum coherence HMQC, ^1H–^{13}C heteronuclear multiple bond correlation HMBC, and *J*-modulated spin echo experiments were employed to assign signals and were performed according to the standard pulse sequences provided by Bruker.

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