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NON-TERPENOID COMPOUNDS FROM *PLOCAMIUM CARTILAGINEUM*

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Key Word Index—*Plocamium cartilagineum*; Plocamiaceae; alga; biopolymers; glycosides; poly(β-hydroxybutyrate); 2-*O*-α-D-galactopyranosylglycerol; floridoside.

Abstract—The isolation and characterization of poly(β-hydroxybutyrate) (PHB) and 2-*O*-α-D-galactopyranosylglycerol (floridoside) from the red alga *Plocamium cartilagineum* are described. This is the first reported occurrence of PHB in a macroalga.

INTRODUCTION

The red alga *Plocamium cartilagineum* has been the subject of an extensive investigation for its halogenated monoterpenes [1]. In a previous communication, we have described the isolation of two new and five known polyhalogenated monoterpenes from this species collected along the Portuguese coast [2]. The evaluation of this species for the industrial extraction of phycoerythrin has also been reported [3].

Herein, we wish to report the isolation and characterization of the biopolymer poly(β-hydroxybutyrate) (PHB) **1** and the glycoside 2-*O*-α-D-galactopyranosylglycerol (floridoside) **2**.

RESULTS AND DISCUSSION

Compound **1** was isolated as a colourless crystalline solid from a $CHCl_3$ extract of a fresh sample of *P. cartilagineum*. Its ^1H and ^1H–^1H NMR spectra showed one methyl group at δ 1.23 (*d*, J = 6.3 Hz) coupled to a methine proton at δ 5.22, which forms an ABX-system with a methylene group resonating at δ 2.43 (*dd*, J = 15.6 and 5.7 Hz) and δ 2.57 (*dd*, J = 15.6 and 7.2 Hz). The ^{13}C NMR spectrum displays four resonances at δ 19.7, 40.7, 67.6 and 169.1, whereas the IR spectrum shows an ester function at 1724 cm^{-1}. These spectral data are consistent with a linear head-to-tail polyester and is identical to those of poly(β-hydroxybutyrate) previously isolated from bacteria [4, 5]. The negative optical rotation of **1** corresponds with the D-(−) form of PHB, with the chiral centre of the monomer unit always in the *R* absolute configuration. The observed vicinal coupling constants (J_{AX} = 5.7 Hz, J_{BX} = 7.2 Hz) suggest that the gauche-conformer of the CH_2-CH bonds, in which the carbonyl and the methyl group are antiperiplanar, is predominant in $CDCl_3$ solution, with calculated gauche and *trans* populations of 0.58 and 0.42, respectively [4–6]. Five fractions of PHB with weight-average M_r in the range 4×10^5 to 8×10^5 and different degrees of polydispersity (M_r/M_n, M_n being the number-average M_r), were obtained from the $CHCl_3$ extract (see Experimental). To our knowledge, this is the first described occurrence of PHB in a macroalga.

PHB are linear biodegradable and biocompatible polyesters accumulated as intracellular granules by many bacteria, as carbon and energy storage material, under conditions of restricted growth [7, 8]. Due to these characteristics they have potential applications in medicine, pharmacy and packaging [9]. Among the industrial microbiological sources of PHB, the bacterium *Alcaligenes eutrophus* synthesizes PHB with M_rs in the range 6.0×10^5 to 33×10^5, whereas lower M_rs (0.5×10^5 to 7.0×10^5) are generally recorded for PHB produced by *Pseudomonas* species. The polydispersity usually varies between 1 and 6, depending on the microorganism, carbon sources and environmental conditions. In biotechnological processes, the PHB content of dry cells, accumulated by *A. eutrophus* goes up to 80%, while the *Pseudomonas* sp. accumulate up to 50% [7].

The assumption that the origin of this biopolymer in *P. cartilagineum* could result from an association of bacterial symbionts, cannot be excluded, because there are some examples of algal metabolites assumed to be of microbial origin, including *Pseudomonas* sp. [10]. In order to evaluate PHB from *P. cartilagineum*

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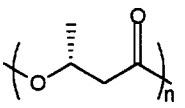

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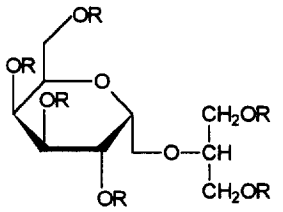
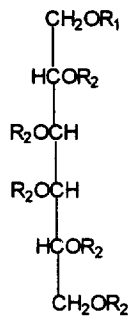


	R
2	H
3	Ac
4	Me



	R_1	R_2
5	Ac	Ac
6	Ac	Me

as a possible candidate for commercial purposes, its physical properties are under investigation.

Reverse-phase chromatography of an ethanol extract of *P. cartilagineum* yielded a water-soluble colourless solid, whose ^1H and ^{13}C NMR spectra (see Experimental) were characteristic of a carbohydrate and in accordance with the reported data for 2-*O*-α-D-galactopyranosylglycerol (floridoside) **2** [11, 12]. The ^1H–^1H, ^1H–^{13}C and DEPT spectra in $CDCl_3$, $CDCl_3$–C_6D_6 and C_6D_6, of the corresponding acetylated derivative, analysed for $C_{21}H_{30}O_{14}$, supported the structure of the hexaacetate **3**, previously isolated from *Ruellia brittoniana* [13]. Acid hydrolysis of **2** afforded α-D-galactose and glycerol, which were separated upon acetylation and identified by comparison with authentic samples. The structure of floridoside was confirmed by GC-mass spectral analysis of the permethylated derivative **4**, the hexaacetate **5** resulting from acid hydrolysis of **2** followed by $NaBH_4$ reduction and peracetylation, and compound **6** obtained by acid hydrolysis of **4** followed by $NaBH_4$ reduction and acetylation.

Floridoside is the main low M_r carbohydrate present in many red algae [14], although this is the first report of its occurrence in *P. cartilagineum*.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured at 300 and 75.5 MHz, respectively, with TMS as int. standard. M_r determinations of PHB were performed in a Waters GPC apparatus at 30°, using $CHCl_3$ as eluent. A series of three Waters Ultrastyragel columns, 10^3 Å, 10^4 Å and 10^6 Å were used and a calibration curve obtained with monodisperse polystyrene standards was transformed using the Mark-Houwink relationship $[\eta] = K M^a$, where $[\eta]$ is the limiting viscosity number and K and a are the Mark-Houwink constants for PHB in chloroform soln, respectively, $K = 11.8 \times 10^{-3}$ ml g^{-1} and a = 0.78 [15]. GC analysis of **4**–**6** were carried out using a DB-17 column, 30 m × 0.32 mm i.d., under the following conditions: injector and FID detector temps 300°, temp. programmed 150° to 300° at 5° min^{-1}. GC-MS analysis were performed at 70 eV using a PS 255 column, 30 m × 0.32 mm i.d., under the following conditions: injector temp. 250°, interface and ion source temps 250°, temp. programmed 200° to 250° at 5° min^{-1} and 250° to 270° at 10° min^{-1}.

Plant material. Algal material was collected at Figueira da Foz in July, 1990 and air-dried. A voucher specimen is deposited at the herbarium of IPIMAR, Lisbon.

Extraction and isolation of **1** *and* **2**. A specimen of *P. cartilagineum* (L.) Dixon (1 kg) was successively extracted with hexane, $CHCl_3$ and EtOH. Repeated treatment of the $CHCl_3$ extract (8 g) with hexane and Me_2CO afforded five frs of poly(β-hydroxybutyrate) **1** (153 mg), mp 175–176°, $[\alpha]_D^{25}$ −13° ($CHCl_3$, *c* 0.6), FTIR ν_{max}^{KBr} cm^{-1}: 1280, 1724, 2940, 2980. ^1H and ^{13}C NMR: in text. 1st fr. M_r 7.8×10^5, M_r/M_n 2.6; 2nd fr. M_r 4.6×10^5, M_r/M_n 1.6; 3rd fr. M_r 5.4×10^5, M_r/M_n 3.3; 4th fr. M_r 8.5×10^5, M_r/M_n 4.4; 5th fr. M_r 8.1×10^5, M_r/M_n 1.3. Reverse-phase flash CC of the EtOH extract (95 g) using H_2O with increasing proportions of MeOH as eluent, afforded 600 mg of 2-*O*-α-D-galactopyranosylglycerol **2**, mp 120–122° (lit. 128.5° [16]). $[\alpha]_D^{25}$ +128° (H_2O; *c* 0.97) (lit. $[\alpha]_D^{25}$ +165° (H_2O; *c* 3.35) [16]). ^1H NMR (300 MHz, D_2O): δ 3.52–3.56 (6H, *m*, 6-H, H-1′ and H-3′), 3.60 (1H, *m*, H-2′), 3.61 (1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 12 Hz, H-2), 3.70 (1H, *dd*, $J_{2,3}$ = 12 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 3.79 (1H, *dd*, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 0.9 Hz, H-4), 3.89 (1H, *ddd*, $J_{5,6}$ = 6.3 Hz, $J_{4,5}$ = 0.9 Hz, H-5), 4.94 (1H, *d*, *J* = 3.9 Hz, H-1). ^{13}C NMR (75.5 MHz, D_2O): δ 61.0 (C-3′), 61.7 (C-6), 62.0 (C-1′), 69.0 (C-2), 69.8 (C-3), 69.9 (C-4), 71.5 (C-5), 79.3 (C-2′), 98.6 (C-1).

Compounds **3**–**6**. Acetylation of **2** yielded hexaacetate **3** (Found: C, 49.8; H, 6.0; O, 44.2. Calcd for $C_{21}H_{30}O_4$: C, 49.8; H, 5.9%), mp 102–103° (lit. 101° [13]). $[\alpha]_D^{25}$ +111° ($CHCl_3$; *c* 3.0) (lit. $[\alpha]_D^{25}$ +114° (Me_2CO; *c* 3.0) [13]). ^1H NMR (300 MHz, $CDCl_3$–C_6D_6, 1:1): δ 1.86 (1 × OAc), 1.88 (2 × OAc), 1.90 (1 × OAc), 1.91 (2 × OAc), 3.90 (1H, *m*, H-2′), 4.04–4.09 (6H, *m*, 6-H, H-1′ and H-3′), 4.35 (1H, *ddd*, $J_{5,6}$ = 6.9 Hz, $J_{4,5}$ = 0.9 Hz, H-5), 5.16 (1H, *dd*, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 5.34 (1H, *d*, $J_{1,2}$ = 3.9 Hz, H-1), 5.42 (1H, *dd*, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 2.7, H-3), 5.49 (1H, *dd*, $J_{3,4}$ = 2.7 Hz, $J_{4,5}$ = 0.9 Hz, H-4). ^{13}C NMR (75.5 MHz, C_6D_6): δ 20.2 (6 × Me), 61.7 (C-3′), 63.5 (C-6), 63.5 (C-1′), 67.3 (C-2), 67.9 (C-3), 68.5 (C-4), 68.7 (C-5), 74.5 (C-2′), 96.6 (C-1), 169.7 (1 × OAc), 169.9 (1 × OAc), 170.1

(2 × OAc), 170.2 (2 × OAc). ^1H and ^{13}C NMR in $CDCl_3$, see ref. [13]. Ciacanu permethylation [17] of **2** yielded compound **4**; GC-MS data, see ref. [18]. Acid hydrolysis of **2** and **3** followed by $NaBH_4$ reduction and acetylation afforded **5** and **6**, respectively, whose GC-MS data were identical to those of authentic samples obtained from D-galactose.

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