Acetylated Nucleoside Derivatives from a Shallow-Water Marine Bivalve Codakia orbicularis

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BRIEF COMMUNICATIONS

Acetylated nucleoside derivatives isolated for the first time from a shallow-water marine bivalve *Codakia orbicularis* (Lucinidae)

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Codakia orbicularis (Linné, 1758) individuals (40-60 mm shell length) were collected from August to December 2015 by hand from *Thalassia testudinum* sea-grass sediments in Guadeloupe (lat 16°09'01.7"N, long 61°33'42.9"W). These bivalves were identified by Pr. Olivier Gros (bivalve specialist). Fresh gills from 2000 individuals were dissected, pooled and frozen at -20°C before chemical extraction. The first study on the marine Mollusk *Codakia orbicularis* used all soft tissues of this bivalve and led mostly to phospholipids, triglycerides, fatty acids and sterols [1]. Some 20 years later, a second chemical study focusing on gill tissues of this bivalve led to the discovery of a new lectine, called codakine [2]. From the gills of this lucinid, we have recently isolated the new spiro-indolothiazine, named orbicularisine [3]. Three kilograms of gills were extracted by agitation in 200 mL of ethyl acetate (EtOAc) during 12 hours at room temperature. The heterogeneous mixture was filtrated, and the filtrate stored at 4°C. The operation was repeated four times. The filtrates were pooled and evaporated under reduced pressure then under N_2 flow. The residue (24 g) was fractionated on a silica gel column chromatography**,** to yield seventeen fractions, using a solvent mixture of increasing polarity (EtOAc/hexane **:** 20/80, EtOAc/hexane : 40/60, EtOAc/hexane : 60/40, EtOAc/hexane : 80/20, EtOAc, EtOAc/MeOH : 90/10, EtOAc/MeOH : 80/20, EtOAc/MeOH : 50/50 and MeOH). Only polar fractions that have been eluted with EtOAc/MeOH : 80/20 (F_{15}, F_{16}) and EtOAc/MeOH : 50/50 (F_{16}, F_{17}) were further investigated in this report. The major compounds were isolated from each fraction by repeated HPLC using an analytical Sunfire C-18 column (150 x 4.6 mm, 5 µm) and a semi-preparative Sunfire C-18 column (150 x 10 mm, 5 µm). The stepwise gradient milliQ water and 2-100 % MeCN (both acidified by 0.1 % HCOOH, flow rate: 1 mL/min and 4.5 mL/min respectively) was used. The following

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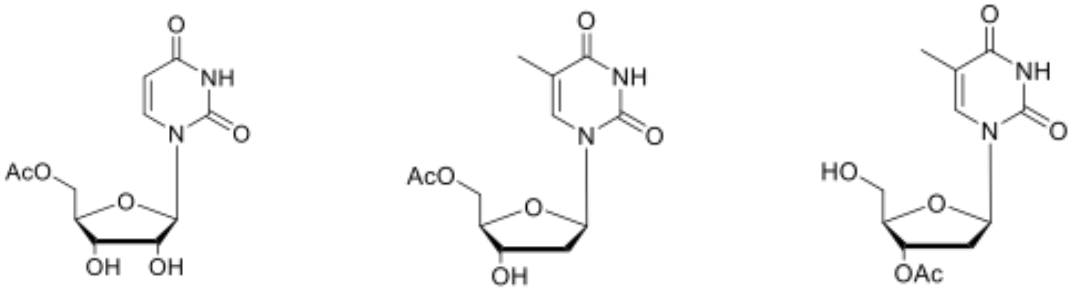
compounds were isolated: compound **1** (1.9 mg, F_{16} and F_{17}), compound **2** (2.0 mg, F_{16}, F_{17}) and compound **3** (0.5 mg, F_{15}). The structures of compounds 1-3 were elucidated by spectroscopic analyses namely HRMS, 1D and 2D NMR but also by comparison of spectroscopic data with those reported in the literature [4, 5].



*5'-O- acetyluridine **(1)***: 1.0 mg, white solid; ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.71 (1H, d, *J* = 8, H-6), 5.83 (1H, d, *J* = 4, H-1'), 5.74 (1H, d, *J* = 8, H-5), 4.35 (1H, dd, *J* = 2.9, 12.9, H-5'$_b$), 4.33 (1H, dd, *J* = 2.9, 12.9 Hz, H-5'$_a$), 4.19 (1H, m, H-2'), 4.15 (1H, m, H-4'), 4.10 (1H, m, H-3'), 2.01 (3H, s, CH_3COOR); ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 172.2 (C, COOR), 166.6 (C, C-4), 152.5 (C, C-2), 142.5 (CH, C-6), 102.9 (CH, C-5), 91.8 (CH, C-1'), 83.0 (CH, C-4'), 75.3 (CH, C-2'), 71.2 (CH, C-3'), 64.9 (CH_2, C-5'), 20.8 (CH_3, CH_3COOR); HRESIMS: *m/z* 287.0837 $[M+H]^+$ (calcd for $\mathbf{C_{11}H_{15}N_2O_7}$, 287.0837). All data were in agreement with the literature [4].
*5'-O- acetylthymidine **(2)***: 1.0 mg, pale yellow gum; ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.50 (1H, s, H-6), 6.26 (1H, dd, *J* = 6.8, 7.2, H-1'), 4.35 (1H, m, H-3'), 4.33 (1H, ddd, *J* = 3.9, 12, 31, H-5'$_b$), 4.27 (1H, ddd, *J* = 4.8, 12, 31, H-5'$_a$), 4.07 (1H, ddd, *J* = 4.8, 6.6, 12, H-4'), 2.27 (2H, m, H-2'), 2.10 (3H, s, CH_3COOR), 1.90 (3H, s, H-7); ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 172.5 (C, COOR), 166.4 (C, C-4), 152.1 (C, C-2), 137.7 (CH, C-6), 111.2 (C, C-5), 86.6 (CH, C-1'), 85.9 (CH, C-4'), 72.4 (CH, C-3'), 65.3 (CH_2, C-5'), 40.5 (CH_2, C-2'), 21.3 (CH_3, CH_3COOR), 12.7 (CH_3, C-7); HRESIMS: *m/z* 285.1086 $[M+H]^+$ (calcd for $\mathbf{C_{12}H_{17}N_2O_6}$, 285.1087). All data were in agreement with the literature [4, 5].
*3'-O- acetylthymidine **(3)***: 0.5 mg, pale yellow gum; ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, *J*/Hz): 7.84 (1H, s, H-6), 6.28 (1H, dd, *J* = 6.8, 7.2, H-1'), 5.31 (1H, m, H-3'), 4.07 (1H, m, H-4'), 3.81 (2H, m, H-5'), 2.35 (2H, m, H-2'), 2.1 (3H, s, CH_3COOR), 1.89 (3H, s, H-7); ^{13}C NMR (150 MHz,CD_3OD, δ, ppm): 173.1(C, COOR), 167.3 (C, C-4), 153.3 (C, C-2), 138.8 (CH, C-6), 112.8 (C, C-5), 87.6 (CH, C-1'), 87.0 (CH, C-4'), 77.3(CH, C-3'), 63.9 (CH_2, C-5'), 39.3 (CH_2, C-2'), 21.8 (CH_3, CH_3COOR), 13.4 (CH_3, C-7); HRESIMS: *m/z* 285.1086 $[M+H]^+$ (calcd for $\mathbf{C_{12}H_{17}N_2O_6}$, 285.1087). All data were in agreement with the literature [5].

Compound **1** was isolated as a white powder and its HR-ESI-MS gave a positive ion peak at *m/z* = 287.0837 corresponding to a molecular formula of $\mathbf{C_{11}H_{15}N_2O_7}$. Because of the presence of some impurities on the ^{1}H NMR spectrum of the compound **1**, 2D NRM have been deemed indispensable to prove the structure of (**1**). ^{1}H NMR spectrum of this product revealed two doublets at δ_H = 7.71 ppm and at δ_H = 5.74 ppm (**Figures S1, S2**) that showed COSY correlations and respectively bound to C-6 and C-5 according to HSQC (**Figure S3**). HMBC data showed equally correlations between theses alkene protons and three quaternary carbons at δ_C = 152.5 ppm (C-2), δ_C = 166.6 ppm (C-4), and δ_C = 172.2 ppm (C-8) (**Figure**

S4). All of these data confirmed an uracil moiety [6]. The remaining proton signals δ_H = 4.35, 4.33, 4.19, 4.15 and 4.10 ppm were assigned to a sugar moiety [7]. The last one was connected to the uracil moiety to form uridine because HMBC correlations have been shown between the proton at δ_H = 5.83 ppm and the carbons C-6 at 142.5 ppm and C-2 at 152.5 ppm (**Figures S4, S5**). Unlike in uridine, metabolite well known [8] compound **1** was an acetylated product with a sharp singlet at δ_H = 2.01 ppm on its ^{1}H NMR spectrum (**Figure S2**). Moreover, on the basis of HMBC data these protons shown a correlation with a sp^2 quaternary carbon at δ_C = 172.2 ppm. Only C-5' location of the acyl group in the structure of (**1**) explained chemical shifts around 4.33 ppm for H-5'$_a$ and H-5'$_b$ instead of 3.50 ppm in uridine [9]. Compound **1** was then identified as 5'-O-acetyluridine. Compounds **2** and **3** were isolated as a pale-yellow gum. Both HR-ESI-MS indicated a positive ion peak at *m/z* = 285.1086 with a molecular formula of $\mathbf{C_{12}H_{17}N_2O_6}$ (calcd for 285.1087). The latter could be attributed to three acetylated thymidine isomers namely the 3'-O- acétylthymidine, the 5'-O-acetylthymidine and the 3-acetyl-5-methyl-2′-deoxyuridine [10, 11]. The comparison of the 1D NMR data of (**2**) and (**3**) with those previously extracted from [10] a soft coral shown that the acetylation was not done on N-3 position because chemical shifts of the thymine part were not modified [12]. However, for the compound **2** the downfield shifts of H-5'$_a$ and H-5'$_b$ (**Figures S6, S7**) were observed with a difference $\Delta\delta$ around + 0.54 ppm compared to those of thymidine at respectively 3.74 ppm and at 3.78 ppm [13]. The above observations proved that the acetylation has been done at the location 5' and established the structure of compound **2** as 5'-O- acétylthymidine. By the same analogy, the acetylation at location 3' deshielded the hydrogen H-3' at δ_H = 4.40 ppm in the thymidine [13] to δ_H = 5.31 ppm in the compound **3** (**Figures S8, S9**). This proved that (**3**) was the 3'-O-acetylthymidine. Bacteriostatic activity of compounds **1 - 3** was evaluated on several target strains of Gram positive and Gram-negative bacteria. No inhibition zone of bacterial development could be detected (**Figures S10, S11 and S12**). 5'-O-acetyluridine (**1**) was first isolated as natural product from a fungal endophyte of *Huperzia serrata* [13] and later from a soft coral-derived fungus *Pestalotiopsis* sp. [14]. The 5'-O-acetylthymidine (**2**) and the 3'-O-acetylthymidine (**3**) were found in the soft coral *Cladiella australis* (associated with bacteria) [10]. According to the literature, the class of the Bivalves is the richest in lipids in the branching of the Mollusks [15]. There are essentially sterols, triacylglycols, fatty acids (particularly PUFA) [16]. These molecules often have a structural role or are energetic sources for these mollusk species [17]. These data have confirmed the previous study performed by Berg *et al.* in *C. orbicularis* [1]. Otherwise, the large quantities of free aminoacids found in Bivalves as glycine, glutamic

acid, alanine or taurine could be a stress indicator [18]. The filtering organisms like Bivalves produce bioactive peptide or bioactive non-peptidic metabolites to protect themselves against microbes from the water [19]. Exogenous molecules like toxins are often isolated from Bivalves but come from the consumption of phytoplanctons, cyanobacteria or dinoflagellates [20, 21].

Up to date, during the investigations on *C. orbicularis*, no toxin was found probably because the bacterial gill-endosymbionts provide up to 90 % of energy needs by this lucinid host [22]. *Codakia orbicularis* is a bivalve mollusk belonging to the order Veneroida in which numerous carotenoids have been described [23]. Derivative nucleosides containing arsenic were also isolated from a giant clam *Tridacna maxima* (Verenoida) [24]. In conclusion, to our knowledge, there is no acetylated nucleosides isolated from Bivalve Mollusks until now. This is the first report on the isolation of 5'-O-acetyluridine (**1**), 5'-O-acetylthymidine (**2**) and 3'-O-acetylthymidine (**3**) from *C. orbicularis* and more generally from Bivalve Mollusks.

Acknowledgments

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Supporting Information

Supporting Information accompanies this paper on:

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