

Isolation, characterization and antibacterial activity of Aglajne-1: polypropionate isolated from the marine mollusk *Bulla occidentalis*

Abstract

Bulla occidentalis, is a gastropod mollusk belonging to the order Cephalaspidea that is able to produce mucus with defensive products. It is the most common species of the genus *Bulla* in the Caribbean. In this paper we report the presence of Aglajne-1 (1) isolated from *Bulla occidentalis*. This compound is a polypropionate considered a chemical-taxonomic marker for the species of the genus *Bulla*. The compound 1 was evaluated through antibacterial assays, but not significant activity was detected. This is the first report of Aglajne-1 (1) in *Bulla occidentalis*, collected in Venezuela.

Keywords: marine molluscs, polypropionate, cephalaspidea, bulla

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Introduction

Bulla occidentalis A. Adams, 1850 is an heterobranch gastropod mollusk belonging to the order Cephalaspidea Fischer, 1883 (family Bullidae Gray, 1827), and the most common species of the genus *Bulla* Linnaeus, 1758 known in the Caribbean. The species shows a moderately calcified shell that can be very variable in shape, color, and a body that secretes mucus with defensive compounds. *B. occidentalis* typically inhabits shallow muddy bottoms with marine phanerogams and green or red algae, which in Venezuela are usually associated to coastal lagoons.¹ Only a few studies using marine mollusks from Venezuela, and focused on the isolation and characterization of secondary metabolites, have been published to date.¹

Chemical research using species of *Bulla* as a model, revealed the presence of several compounds, generally polyketide-like type. These compounds are suspected to play key ecological and physiological functions, including chemical defense. The polypropionates are the most important source of secondary metabolites in marine mollusk.²⁻⁵ They are polyketides biosynthetically derived by condensation of C₃-Units in regular or mixed acetate/propionate polyketide chain.⁶⁻⁸ Their structural and stereochemical complexity, coupled with their important biological activity, have turned them in an attractive research target for over half a century.⁹ Alternating methyl-hydroxyl-substituted alkyl chains are just one example of the remarkable

structures that have been characterized from Polypropionates. They are found in diverse classes of natural products with biological and medicinal activities. Besides, many applied synthetic approaches to polypropionates have been reviewed recently.¹⁰ The first report for the compound Aglajne-1 (1) was made by Cimino et al from the sea slug *Phillinopsis depicta* (Renier, 1807) (family Aglajidae Pilsbry, 1895).¹¹ In this work the structure of 1 was determined by extensive use of 2D-NMR and confirmed on chemical ground. In the same year Coval et al.¹² isolated two polypropionate compounds: niuhione-A (2) and -B (3) from the mollusk *Phillinopsis speciosa* Pease, 1860 (family Aglajidae), the structure of these compounds compared to 1 revealed an identical carbon skeleton.¹² Polypropionates metabolites Aglajne-2 (4), Aglajne-3 (5) and 1 have been isolated from the sea slug mollusk *Bulla striata Bruguière*, 1972, a prey of the sea slug *P. depicta* confirming their prey-predator relationship (Figure 1).¹³⁻¹⁶ This phenomenon was also observed in the Pacific sea slugs *Bulla gouldiana* Pilsbry, 1985 and *Navanax inermis*, Cooper 1982, which share the same metabolites, including the polypropionates 3 and 5,6-dehydroaglajne-3 (6).¹⁷ The compound 2 and 3 along with a new acyclic polypropionate, named niuhione C (7), have been reported in *B. occidentalis* from the Gulf of Mexico.³ In this communication, the compound 1 isolated from *Bulla occidentalis* is described and presented in Figure 1. The structure of the Aglajne-1 (1) was identified by comparing its spectroscopic and spectrometric data (IR, ESIMS and ¹H NMR) with these reported in the literature.¹¹

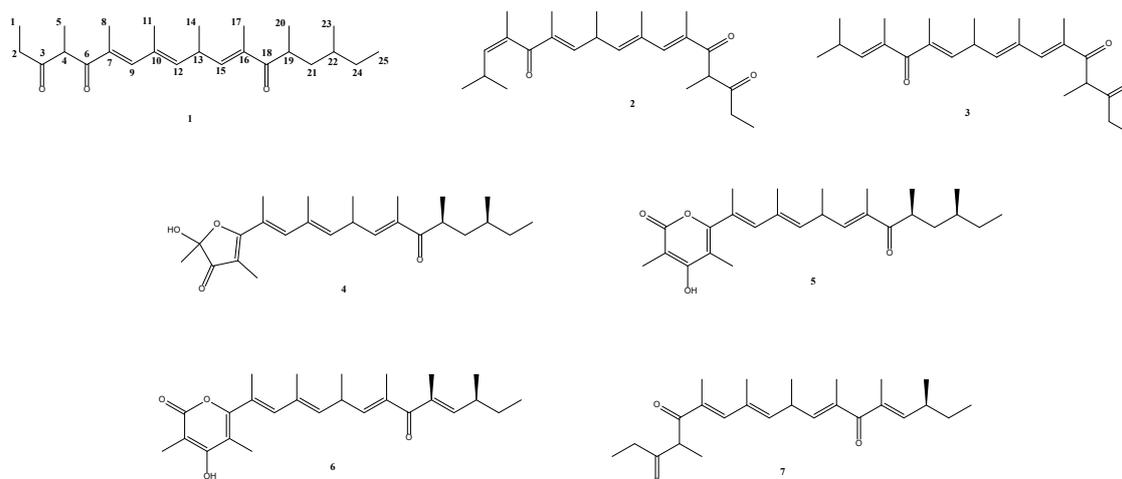


Figure 1 Polypropionates isolated from cephalaspidean mollusks.

Materials and methods

General procedures

All solvents were analytical grade. Compound purity was monitored by TLC on pre-coated silica gel 60 PF254 plates (MERCK). IR spectra were obtained on a Nicolet Magna 560 FTIR spectrometer with KBr disks. Mass Spectra (MS) were performed by Electro Spray Ionisation (ESI) on a TSQ Quantum mass spectrometer. ¹H NMR spectra were measured on a Bruker Advance 500 spectrometer with TMS as the internal standard.

Animal materials

The specimens (25) measured 15-30mm and were collected in July 2009 in the muddy bottom area of the lagoon of “El Ocho”, Higuero, Miranda State, Venezuela (10°32.27’94”N; 66°05’55.3”W). The mucus secreted by the specimens were preserved in 96% ethanol.

Extraction and isolation

The ethanolic extract was filtered and the solvent was concentrated under reduced pressure to give a solid residue (659mg). Part of this extract (121mg, soluble portion in hexane), was fractionated on silica gel column with increasing amount of diethyl ether in petroleum ether (100% hexane, petroleum ether: diethyl ether: 9.5:0.5; 8:2, 7:3, 1:1, 4:6; 3:7) to afford 45 sub fractions, regrouped in 10 sub fractions. The sub fraction FHE (2mg) showed ¹H NMR and MS data identical to those reported in the literature for Aglajne-1 from the sea slug *P. depicta*.¹¹

Aglajne-1 (1)

C₂₅H₄₀O₃, Oil. Rf: 0.4 (petroleum ether: diethyl ether, 7:3). IR (KBr): 2925-2851 (C-H sp³), 1716.94 (C=O), 1640 (C=C), 1014 (=C-H) cm⁻¹. ¹H NMR (500MHz, CDCl₃): 6.94 (1H, s), 6.39 (1H, d, J=9Hz), 5.51 (1H, d, J=9Hz), 4.23 (1H, q, J=7Hz), 3.56 (1H, m), 3.29 (1H, m), 2.46 (1H, dq, J=7.2 y 18Hz), 2.34 (1H, dq, J=7.2 y 18Hz), 1.93 (3H, d, J=1Hz), 1.88 (3H, s), 1.82 (3H, s) 1.72 (1H, m), 1.53 (3H, s), 1.32 (3H, d, J=7Hz), 1.16 (3H, d, J=6Hz), 1.02 (3H, d, J=6.8Hz), 1.008 (3H, t, J=7.25Hz), 0.85 (3H, t, J=7.1Hz), 0.83 (3H, d, J=6.7Hz). MS (EI, 70 eV): m/z=411.29 (M+Na) + (C₂₅H₄₀O₃+Na), m/z=218.30 (M-C₁₂H₁₈O₂)⁺, m/z=175.27 (M-C₁₀H₂₀O)⁺.

Biological activity

The antibacterial activity was evaluated by the modification of broth microdilution.¹⁸ The five bacterial strains (*Escherichia coli* 35218, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 25923, *Serratia marcescens* 45 and *enterica typhimurium* 323) were seeded in order to obtain isolated colonies and replicated with 16-18hours each strain with a sterile plastic handle. The microplate containing 200μL of Müller-Hinton broth. The bacterial density was adjusted to 0.5 McFarland scale. The solutions were prepared in concentration of 1000 μg/mL using an aqueous solution of 2.5% DMSO (v/v). Because 1 was slightly soluble in DMSO, it was put into the ultrasonic equipment for 30minutes. The optical density was measured at 600nm at 0, 14, 20 and 22hours. With the data, the bacterial growth graphs were obtained.

Results and discussion

The molecular formula of Aglajne-1 (1) is C₂₅H₄₀O₃. The compound was detected as a very small molecular ion in the mass spectrum m/z=411.29 (M+Na)⁺. The ESI mass spectrum contained major fragments at m/z=325.26 (cleavage between C-20 and C-21), m/z=218.26 (cleavage between C-12 and C-14), m/z=204.15 (cleavage between C-14 and C-15) and m/z=75.27 (cleavage between C-15 and C-17). The IR spectrum contained bands for both saturated and unsaturated carbonyl groups (1716 and 1640cm⁻¹). Performing the ¹³C-NMR was not possible there was not sample enough.

The ¹H-NMR (Table 1) showed three olefinic protons at δ 6.94, 6.39 and 5.51, which were assigned to H-9, H-12 and H-15. One quartuplets at δ 4.23, was assigned to H-4; this is a characteristic signal of proton α to carbonyl groups (CO-CH-CO). Two multiplets at δ 3.56 and 3.30, which were assigned to H-13 proton α to olefinic carbon and H-19 proton α to carbonyl groups (CH-CO). Two quartuplets doublets at δ 2.47 and 2.39, assigned to H-2 methylene protons α to carbonyl groups (CH₂-CO). Three singlets at 1.94, 1.88 and 1.82, assigned to methyls H-8, H-11 and H-17. These signals are characteristic of proton α to olefinic carbon. One doublet doublet doublets at δ 1.70, assigned to one proton H-21. Two doublets at δ 1.32 and 1.17, assigned to H-5 and H-14 alifatic methyl β to carbonyl groups and olefinic carbons, respectively. One doublet doublet doublets at δ 1.06, assigned to the

other proton H-21. One multiplet at δ 1.24, assigned to proton H-22. One doublet to doublets at δ 1.02, assigned to aliphatic methylene H-24 and one triplet at δ 1.01, assigned to aliphatic methyl H-20. Finally, one multiplet between δ 0.82–0.87, assigned to aliphatic methyls H-1, H-23 and H-25.

The compound Aglajne-1 (1), a polypropionate considered a chemical-taxonomic marker of marine mollusks belonging to the genus *Bulla*, was isolated in samples of *Bulla occidentalis* from Venezuela for the first time. This paper shows that a lot remains to be done in chemical research using marine fauna from Venezuela, especially identifying secondary metabolites and finding new biological activities for these compounds.

Conclusion

In conclusion, Aglajne-1 (1) was obtained from the ethanolic extract of *Bulla occidentalis*. The structure of the Aglajne-1 (1) was identified by comparing its spectroscopic and spectrometric data (IR, ESIMS and ^1H NMR) with these reported in the literature. In the assay of antibacterial activity, any significative results were observed. This is the first report of Aglajne-1 (1) in *Bulla occidentalis*, collected in Venezuela.

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Conflicts of interest

The authors declare that there is no conflicts of interest.

References

1. Gutiérrez MC, Ortea J, Rivero N, et al. The opisthobranch gastropods (Mollusca: Heterobranchia) from Venezuela: an annotated and illustrated inventory of species. *Zootaxa*. 2015;4034(2):201–256.
2. Cimino G, Ciavatta ML, Fontana A, et al. Metabolites of Marine Opisthobranchs: Chemistry and Biological activity. *Cap. 15, Bioactive compounds from Natural Sources*. Corrado Trigali, editor. London and New York: Taylos & Francis; 2001. p.579–637.
3. Cutignano A, Calado G, Gaspar H, et al. Polypropionates from *Bulla occidentalis*: Chemical markers and trophic relationships in cephalaspidean molluscs. *Tetrahedron Letters*. 2011;52(36):4595–4597.
4. Davies–Coleman MT, Garson MJ. Marine polypropionates. *Natural Product Reports*. 1998;15(5):477–493.
5. Marin A, Alvarez LA, Cimino G, et al. Chemical Defence in Cephalaspidean Gastropods: Origin, Anatomical Location and Ecological Roles. *Journal of Molluscan Studies*. 1999;65(1):121–131.
6. Nuzzo G, Cutignano A, Moles J, et al. Exiguapyrone and exiguaone, new polypropionates from the Mediterranean cephalaspidean mollusk *Haminoea exigua*. *Tetrahedron Letters*. 2016;57(1):71–74.
7. Hill RA, Sutherland A. Hot off press. *Natural Product Reports*. 2004;21(5):H17–H21.
8. Moore BS. Biosynthesis of marine natural products: macroorganisms (Part B). *Nat Prod Rep*. 2006;23(4):615–629.
9. Bootwicha T, Feilner JM, Eddie L Myers, et al. Iterative assembly line synthesis of polypropionates with full stereocontrol. *Nature Chemistry*. 2017;9:896–902.
10. Exner CJ, Turks M, Fonquerne F, et al. Concise synthesis of complicated Polypropionates through One-Pot Dissymmetrical two-directional chain elongation. *Chemistry*. 2011;17(15):4246–4253.
11. Cimino G, Sodano G, Spinella A, et al. Aglajne-1, A Polypropionate metabolite from the opisthobranch mollusk *Aglaja depicta*. *Tetrahedron Letters*. 1985;26(28):3389–3392.
12. Coval SJ, Schulte GR, Matsumoto GK. Two polypropionate metabolites from the cephalaspidean mollusk *Phillinopsis speciosa*. *Tetrahedron Letters*. 1985;26(44):5359–5362.
13. Cimino G, Sodano G, Spinella A. New Propionate-Derived Metabolites from *Aglaja depicta* and from Its Prey *Bulla striata* (Opisthobranch Molluscs). *J. Org. Chem*. 1987;52(24):5326–5331.
14. Paul VJ, Ritson-Williams R, Sharp K. Marine Chemical Ecology in benthic environments. *Natural Product Reports*. 2011;28(2):345–387.
15. Fontana A, Cutignano A, Giordano A, et al. Biosynthesis of aglajnes, polypropionate allomones of the opisthobranch mollusc *Bulla striata*. *Tetrahedron Letters*. 2004;45(37):6847–6850.
16. Faulkner DJ. Marine Natural Products. *Natural Product Reports*. 1987;4:539–576.
17. Spinella A, Alvarez LA, Cimino G. Predator-Prey Relationship between *Navanax inermis* and *Bulla gouldiana*: A Chemical Approach. *Tetrahedron*. 1993;49 (15):3203–3210.
18. Cavalieri SJ. Manual de Pruebas de susceptibilidad antimicrobiana. American Society for Microbiology. Washington DC, 2005.