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Tetrahedron Letters
Tetrahedron Letters 45 (2004) 6847–6850

Biosynthesis of aglajnes, polypropionate allomones of the opisthobranch mollusc *Bulla striata*

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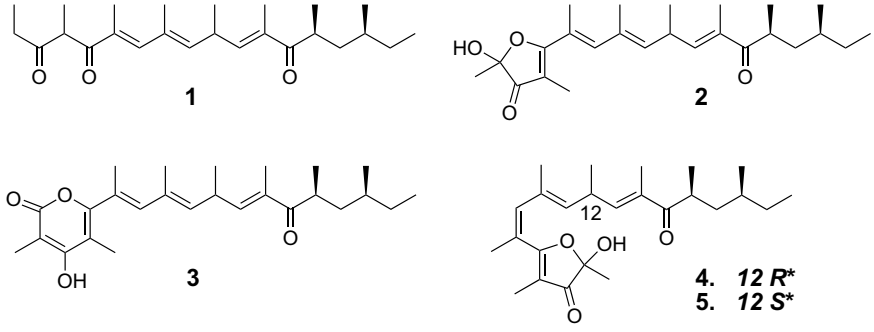
Received 20 May 2004; accepted 24 July 2004

Abstract—The biogenesis of aglajnes, polypropionate allomones of the cephalaspidean mollusc *Bulla striata*, has been investigated in vivo by feeding experiments. Incorporation of the committed precursor, [1-^{14}C]-propionate, into aglajne-1 (**1**) and -3 (**3**) established the de novo origin of these compounds in *B. striata*. In the letter we also discuss briefly the ecological meaning of the origin of polypropionates in *B. striata* and in other cephalaspidean molluscs.

The molluscs of the subclass Opisthobranchia have developed a complex ecological network that includes the use of chemical substances for both defence and communication.[1,2] Most of these products are derived from the diet but, even if less frequently, de novo biosynthesis has also been proved in the most evolved genera.[3] Within the opisthobranchs, cephalaspideans encompass shelled species living on sandy bottoms.[1] These molluscs show the most ancestral characters of the sub-class and are typically considered a transitional group between typical testacean prosobranchs and shellless opisthobranchs. In particular, the Mediterranean *Bulla striata* (Bullomorpha, Bullacea) is provided with a large shell which accommodates the whole animal when it is disturbed.[4] Chemical studies on this mollusc, however, have also shown the presence in its extract of a number of deterrent polypropionates, named aglajnes (**1**–**3**),[4,5] the origin of which has not been clarified to date. In this letter we report the first evidence of the de novo biosynthesis of these allomones in *B. striata,* showing specific incorporation of [1-^{14}C]-propionate into aglajne-1 (**1**) and -3 (**3**) when the animals are supplemented with the sodium salt of the precursor.

The cephalaspidean *B. striata* (8 specimens) was collected by hand using SCUBA at depths of 3–15 m at Capo Miseno in the Gulf of Naples (Italy) during September 2003. Part of the molluscs (5 specimens) were



Keywords: Marine molluscs; Biosynthesis; Polypropionate.
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0040-4039/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2004.07.115

kept as control and not subjected to any experiment. These animals were frozen and worked up in agreement with Ref. 5b to give aglajne-1 (**1**, 6 mg), aglajne-3 (**3**, 7 mg) and a mixture of aglajne-2 (**2**, 6 mg) together with furanones **4** and **5**. The identity of these compounds was ascertained by comparison of the NMR and MS data with those reported in the literature.[5] The remaining animals were supplemented with sodium [1-^{14}C]-propionate dissolved in sterile marine water (2 μCi of propionate in 60 μL of water per specimen). The radioactive material was directly injected into the digestive gland through the mollusc shell. The opisthobranchs were kept in an aquarium filled with 1 L of refrigerated seawater (18–19 °C) for three days and then frozen. The animals were extracted with acetone and, after removing the volatile solvent, the residue was partitioned between water and diethyl ether to give 20 mg of organic extract (1919 DPM/mg) containing compounds **1**–**5**. Silica gel fractionation of this extract led to 1.7 mg of a radioactive sample (750 DPM/mg) homogeneous to standard aglajne-1 (**1**). Subsequent radio-chromatographic analysis of this fraction carried out on RP-HPLC equipped with a Flo-One (Perkin–Elmer) radiodetector (gradient from 20% H_2O in CH_3CN to 0% in 35 min, flow 1.0 mL/min) supported the labeling of **1**, showing a major radioactive peak (267 cpm) eluted at the same retention time (15.8 min) of aglajne-1 (**1**) (Fig. 1). The peak co-eluting with a standard sample of **1** exhibited significant radioactivity also when chromatographed by radio HPLC in different elution conditions (gradient from 20% H_2O in MeOH to 0% in 25 min, flow 1.0 mL/min, data not shown) thus confirming the specific labeling of aglajne-1 (**1**). Since conversion of propionate to acetate would lead to loss of radioactivity, this is consider as the first direct evidence of propionate incorporation in secondary metabolites of cephalaspidean molluscs.

Although the data were convincing in supporting the de novo origin of polypropionates in *B. striata*, the low yield of incorporation led us to repeat the feeding experiments in order to verify this result. A second group of animals (3 specimens) was collected and injected with ^{14}C-propionate as reported above. After extraction, the ether-soluble material (12 mg, 1709 DPM/mg) was loaded on silica gel column and fractionated. As shown in Figure 2, major levels of radioactivity were associated to the fractions containing aglajnes. It is relevant to note that no other sample showed significant incorporation,

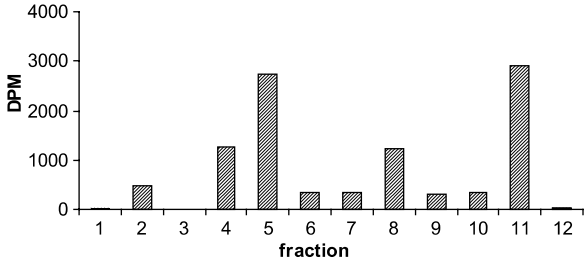

Figure 2. Radioactivity recoveries from the fractionation of the ether extract of *B. striata* fed with [1-^{14}C]-propionate. Fraction 4 and 5 contain aglajne-1 (**1**); fraction 8 contains aglajne-2 (**2**) together with furanones 4 and 5; fraction 11 contains aglajne-3 (**3**). Product identity was ascertained by co-elution with pure standard of 1–5.

thus confirming the predicted selectivity of the propionate. Due to the inherent instability of aglajne-2 (**2**, fraction 8),[5b] this compound was not taken into consideration for further steps of purification, even if the fraction containing it together with **4** and **5** revealed a radioactivity level (260 DPM/mg) significantly higher than the background (about 10 cpm). On the contrary aglajne-1 (**1**, fraction 4 and 5, 439 and 515 DPM/mg) and aglaine-3 (**3**, fraction 11, 562 DPM/mg) were further processed by HPLC. A first analysis was performed on reversed phase columns (MeOH/H_2O gradient as above reported). The chromatographic courses were monitored by UV (234 nm) and each peak was independently collected. The regions of the chromatograms devoid of peaks were also taken into account by collecting fractions every 1 min. Elution of aglajne-1 (**1**, 0.4 mg) and aglajne-3 (**3**, 0.4 mg) (Fig. 3) was ascertained by co-elution with cold material. Aliquots of these fractions were directly measured for radioactivity by a beta-counter that showed a clear labeling of both products (**1**, 744 DPM/mg and **3**, 562 DPM/mg). Significantly, no other region of the chromatogram showed levels of radioactivity above the background. Finally, the fraction containing aglajne-1 (**1**) was further analyzed on normal phase HPLC by eluting with *n*-hexane/ethyl acetate 92:8. Again, the radioactivity (350 DPM/mg) was specifically recovered in the peak co-eluted with a standard sample of the polypropionate (**1**) (Fig. 3).

It is worth noting that we observed a significant loss of material most likely due to degradation of the com-

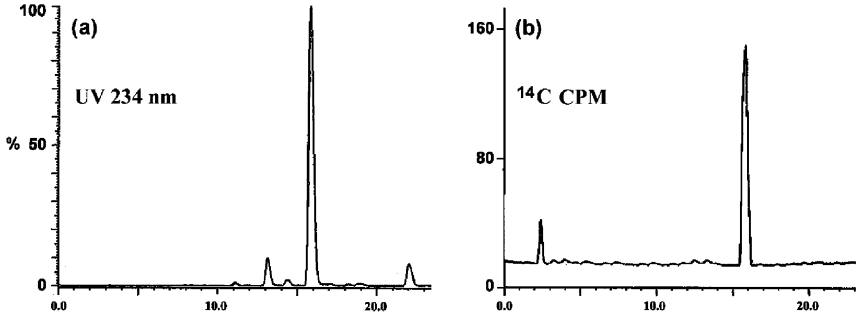

Figure 1. HPLC analysis of aglajne-1 (**1**) isolated from *B. striata* injected with ^{14}C-labeled propionate. (a) UV (234 nm) trace. (b) Radiochromatogram.

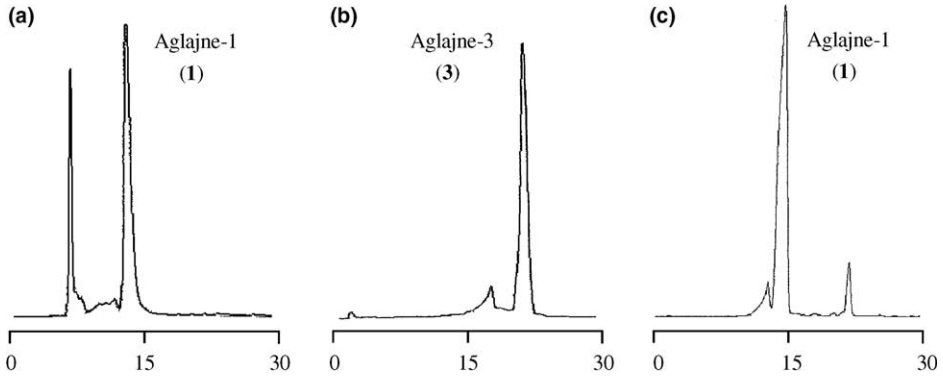

Figure 3. HPLC profiles of purification for aglaine-1 (**1**) on reverse phase (MeOH/H_2O) (a) and normal phase (*n*-hexane/EtOAc) (c) columns, and aglajne-3 (**3**) on reverse phase (MeOH/H_2O) (b) column. Fractions were collected every minute during the elution and 3/10 of each fraction was directly measured for radioactivity by a beta-counter. Background radioactivity was below 14 cpm.

pounds during HPLC purifications. This reduced in an inestimable manner the recovery of the metabolites. Accordingly, the levels of specific radioactivity reported above should be much higher since they were estimated on the basis of a hypothetical yield of purification as high as 100%.

In conclusion, these experiments provided the first evidence for the de novo origin of polypropionates in *B. striata*. The different steps of purification of the mollusc products demonstrate clearly the labeling of the carbon framework of aglajnes (**1–3**) even in the presence of rather a low rate of incorporation (about 0.01%). This last aspect could also be dependent on the inherent instability of **1–3**. However, the data show a coherent presence of radioactivity only in the fractions containing the polypropionates (Fig. 2), with levels that were always much higher than those recorded for the other fractions and the background. These results are also in agreement with the use of propionate as committed precursor of aglajne biosynthesis, since we observed only a reduced effect of random labeling in the other products of the mollusc. Aglajnes **1–3** are also the chemical constituents of the carnivorous bullomorpha *Philinopsis* (=*Aglaja*) *depicta*, a Mediterranean mollusc that preys on other cephalaspideans including *B. striata*.[4,5b] Comparison of the metabolite pattern of different populations of the two molluscs and field observations led the authors to suggest the dietary origin of the polypropionates in *P. depicta*. Our present results are a strong verification of this theory since they confirm at molecular level the implicit assumption that the pattern of polypropionates **1–3** were obtained from the preyed specimens of *B. striata*.

This work may also be useful to redefine the origin of polypropionates in other cephalaspidean molluscs.[6–9] In particular, from both the ecological and chemical point of view, *P. depicta*[5b] shows more than one analogy with *Navanax* (*Aglaja*) *inermis*, a Pacific Aglajdae mollusc containing polypropionates, alkylpyridines and alkylphenols.[6,7] In fact, like *P. depicta*, the Pacific mollusc is an active predator of shelled cephalaspideans of the genera *Bulla* and *Haminoea*.[7] Considering the recent demonstration that *Haminoea orbignyana* is able to synthesize 3-alkylpyridine alkaloids,[10,11] the de novo origin of polypropionates in *B. striata* supports the general assumption that cephalaspideans of these genera possess the ability to construct de novo their defensive molecules. With this line of reasoning, a dietary origin from an unknown *Bulla* species is to suggest for the aglajne-related polypropionates isolated from Aglajide molluscs, including the Pacific species *N. inermis* and *Philinopsis speciosa*.[12]

Acknowledgements

This research was partially supported by 'Programma Nazionale di Ricerca in Antartide (PNRA)'. Dr. Guido Villani is gratefully acknowledged for the biological assistance.

References and notes

1. (a) Karuso, P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer-Verlag: Berlin, 1987, pp 31–60; (b) Cimino, G.; Fontana, A.; Gavagnin, M. *Curr. Org. Chem.* **1999**, *3*, 327–372; (c) Cimino, G.; Fontana, A.; Ciavatta, M. L.; Gavagnin, M. In *Metabolites of Marine Opisthobranchs: Chemistry and Biological Activity*; Tringali, C., Ed.; Bioactive Compounds from Natural Sources. Taylor & Francis: London, 2001; pp 579–588, and references cited therein.
2. Faulkner, D. J. *Nat. Prod. Rep.* ***2002****, 19*, 1–48.
3. Fontana, A.; Cimino, G. *Rec. Res. Develop. Org. Chem.* **2001**, *5*, 89–105.
4. Marin, A.; Alvarez, L. A.; Cimino, G.; Spinella, A. *J. Moll. Stud.* **1999**, *65*, 121–131.
5. (a) Cimino, G.; Sodano, G.; Spinella, A.; Trivellone, E. *Tetrahedron Lett.* **1985**, *26*, 3389–3392; (b) Cimino, G.; Sodano, G.; Spinella, A. *J. Org. Chem.* **1987**, *52*, 5326–5331.
6. Fenical, W.; Sleeper, H. L.; Paul, V. J.; Stallard, M. O.; Sun, H. L. *Pure Appl. Chem.* **1979**, *51*, 1865–1870; Sleeper, H. L.; Paul, V. J.; Fenical, W. *J. Chem. Ecol.* **1980**, *6*, 57–70.
7. Spinella, A.; Alvarez, L. A.; Cimino, G. *J. Chem. Ecol.* **1993**, *49*, 3203–3210.
8. Coval, S. J.; Schulte, G. R.; Matsumoto, G. K.; Roll, D. M.; Scheuer, P. J. *Tetrahedron Lett.* **1985**, *26*, 5359–5362.

9. Szabo, C. M.; Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J. *Tetrahedron* **1996**, *52*, 9681–9686.
10. Cutignano, A.; Tramice, A.; De Caro, S.; Villani, G.; Cimino, G.; Fontana, A. *Angew. Chem. Int. Ed.* **2003**, *42*, 2633–2636.
11. Cutignano, A.; Cimino, G.; Giordano, A.; d'Ippolito, G.; Fontana, A. *Tetrahedron Lett.* **2004**, *45*, 2627–2629.
12. Coval, S. J.; Schulte, G. R.; Matsumoto, G. K.; Roll, D. M.; Scheuer, P. J. *Tetrahedron Lett.* **1985**, *26*, 5359–5362.