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Polyketide origin of 3-alkylpyridines in the marine mollusc *Haminoea orbignyana*

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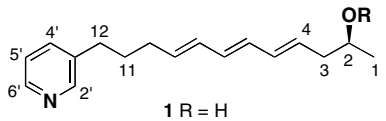
Abstract—The paper reports the biosynthesis of the main alkylpyridine alkaloids, haminol-1 (**1**) and -2 (**2**), in the Mediterranean mollusc *Haminoea orbignyana*. Experiments were carried out by in vivo incorporation of [1,2-$^{13}C_2$ acetate]. Data give full account for a polyketide origin of haminols in the Mediterranean molluscs, showing the biosynthesis of these 3-alkylpyridine alkaloids by elongation with acetate of a starter unit of nicotinic acid.

3-Alkylpyridine alkaloids are a family of marine metabolites showing an outstanding variety of chemical structures formally derived by single or multiple units of a basic 3-alkylpyridine motif.[1,2] Mediterranean of the genus *Haminoea* (*Gastropoda*: *Cephalaspidea*) show the simplest members of this family of compounds and, therefore, represent ideal systems to investigate the biogenesis of this class of natural products.[3] Recently, we have first described the in vivo biosynthesis of the main alkylpyridine compounds, haminol-1 (**1**) and -2 (**2**), in the Mediterranean *Haminoea orbignyana*.[4] Classical feeding experiments with isotopically labelled precursor proved the origin of the 3-alkylpyridine motif from nicotinic acid and acetate units. However, these experiments did not allow us to fully elucidate the biochemical pathway leading to haminols since at least three different routes could be in agreement with experimental results.[4] Herein, we report a further experiment with *H. orbignyana* and give a complete interpretation of the biogenesis of 3-alkylpyridine alkaloids in this mollusc.



1 R = H
2 R = Ac

The animals (65 specimens) were maintained in aquarium and injected twice (day 1 and 4) over a period of 1 week with [1,2-$^{13}C_2$]-acetate (4.6 mg/specimen in 40 μL of distilled water).[5] Subsequent extraction and isolation of the ether-soluble fractions[4] gave 1.0 mg of haminol-2 (**2**, 0.016 mg/specimen). ^{13}C NMR spectrum[6] of this product showed evident differences with that previously reported in the literature (Fig. 1).[4] The changes were particularly severe for the signals of the pyridine ring, thus suggesting the protonation of the heterocyclic moiety as probable origin of the observed spectroscopic variation. In fact, in agreement with the literature of protonated pyridine ring,[7] C-2′ and C-6′ (145.2 and 145.1 ppm) were significantly shifted towards high-field regions (literature[4] values 149.9 and 147.1 ppm), and whereas C-4′ (140.5 ppm) experienced a low-field shift (literature[4] values 135.9 ppm). The bulky effect of these changes was an evident overlapping of the signals in the region of the sp^2 carbons (from 125 to 145 ppm) and a consequent difficulty to discriminate the double bond carbons of the alkyl chain. However, although the signals of the entire spectrum suffered slight variation within respect to the values reported in the literature,[4] the effect was rather marginal on the other carbons and did not preclude the analysis of the ^{13}C–^{13}C coupling constants and the identification of the biogenetically related C_2-units. In fact, besides the evident correlation ($J_{c\text{–}c} = 59.6$ Hz) between the methyl (21.3 ppm) and the carbonyl signals of the acetyl residue (170.4 ppm), very clear was the presence of the coupled doublets flanking the signals of C2 (70.7 ppm, $J_{c\text{–}c} = 38.4$ Hz) and C3 (39.2 ppm, $J_{c\text{–}c} = 38.4$ Hz) (Fig. 2). On the other hand,

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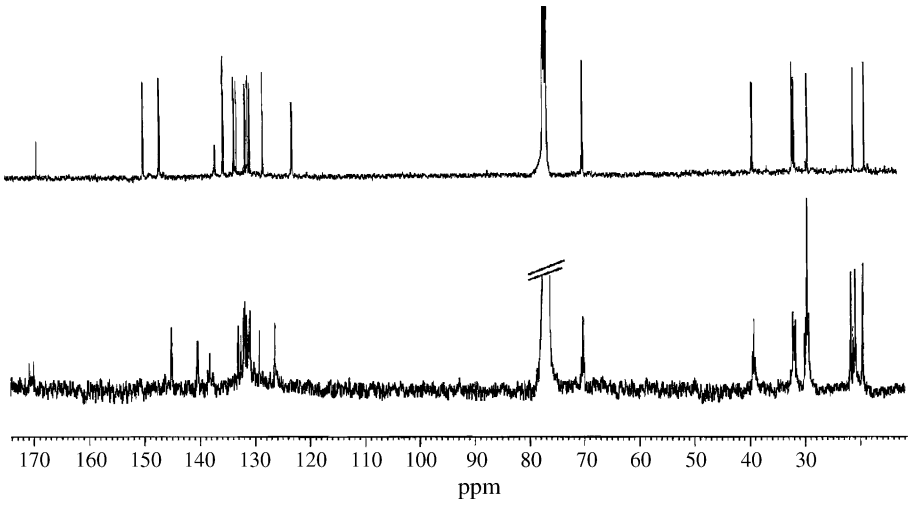


Figure 1. ^{13}C NMR spectra of haminol-2 (**2**). Natural product (upper chart) and product purified after feeding experiment with [1,2-$^{13}C_2$ acetate]. Chemical shift differences between the two samples are due to permanent protonation of the pyridine ring of the labelled product.

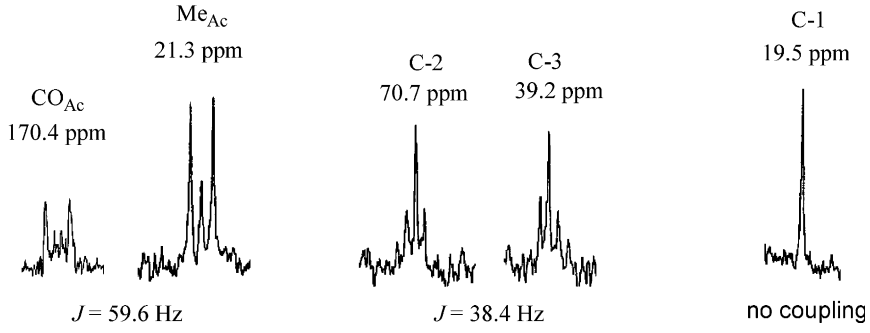
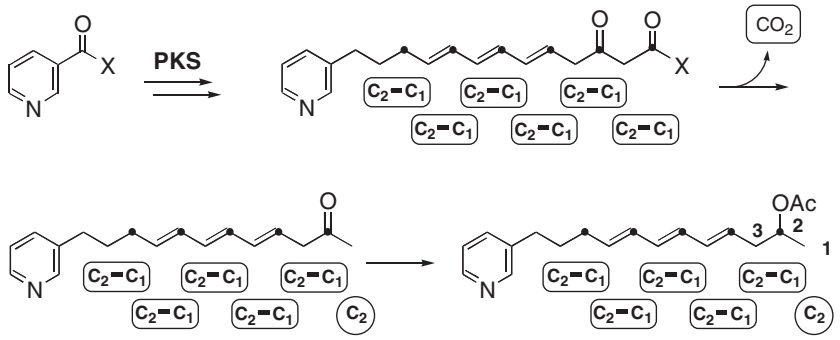


Figure 2. Selected ^{13}C NMR signals of haminol-2 (**2**) isolated from *H. orbignyana* after feeding experiment with [1,2-$^{13}C_2$]-acetate. Spectra were processed with a sensitivity enhancement function (exponential multiplication) by using LB = 3 Hz.

looking at the ^{13}C signals in the high-field region of the spectrum, it was also very evident that C1 (19.5 ppm) did not show any coupling and appeared as a distinct singlet (Fig. 2). Overlapping of the other signals did not allow us to determine unambiguously other correlations but these data were sufficient to establish the incorporation of intact C_2-units at C2–C3 of haminol-2 (**2**) (Scheme 1). Furthermore, considering the labelling of C2, C4, C6, C8 and C10 in experiments with 1-^{13}C acetate,[4] the presence of an intact acetate-derived unit at C2–C3 proved also the origin of haminol-2 (**2**) via a polyketide synthase (PKS) using nicotinic acid as starter unit and six molecules of acetate (or the metabolically equivalent malonate) as extender intermediates (Scheme 1). Loss of



Scheme 1. Biogenetic proposal for the formation of 3-alkylpyridine in *Haminoea* molluscs via a polyketide synthase (PKS) using nicotinic acid as starter unit and acetate (or the metabolically equivalent malonate) as extender intermediates. The alkyl chain framework derives from a final decarboxylation, presumably due to a post-PKS process. (•) = labelling pattern from experiment with [1-^{13}C]-acetate;[4] (C_1–C_2) = intact acetate units incorporated from feeding experiment with [1,2-$^{13}C_2$]-acetate; X = OH or a metabolically equivalent group.

the terminal carbon of the PKS-emerging chain, presumably due to a post-PKS decarboxylation, provides the carbon framework of **2**. In fact, this last step produces the break of the final C_2-unit with resulting loss of the correlation between the carbon atoms of this acetate-derived fragment. Since C1 does not have any other observable coupling, decarboxylation of the alkyl chain is in agreement with the absence of doublets flanking the signal of this carbon, thus explaining why this signal appears as a singlet in the ^{13}C NMR spectrum (Fig. 2).

In conclusion, the experiments reported here allow us to conclude that haminols are produced in *H. orbignyana* through a polyketide pathway. The results rule out other hypotheses about the formation of the alkyl pyridine framework. In particular, the alternative routes that we had discussed in a former publication[4] are excluded since both of them require the presence of intact acetate unit between C1–C2 and C3–C4 of the haminol framework. To the best of our knowledge, this is the first report of a polyketide pathway enable to process nicotinic acid as starter unit. As stated above, the 3-alkylpyridine motif of haminols is common to a large family of alkaloids isolated from marine sponges (generally of the order Haplosclerida), such as saraines and manzamines.[1] The origin of these last compounds has been discussed by several authors[1,8] and our studies do not exclude that the biosynthesis of more complex structures can follow other biochemical mechanisms. Further investigations are necessary to clarify these aspects of the lipid metabolism in marine organism. First attempts have begun to explore the biogenesis of alkylpyridine in *Haminoea* mollusc at genetic and enzymatic level. Unfortunately, no significant progress has been achieved to date.

Acknowledgements

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References and notes

1. Andersen, R. J.; van Soest, R. W. M; Kong, F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: London, 1996; Vol. 10, pp 301–355, references cited therein.
2. (a) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059–2062; (b) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C.; Boughtflower, R. J.; Mutton, I. M.; Upton, R. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2661–2663.
3. (a) Tsuda, M.; Kobayashi, J. *Heterocycles* **1997**, *46*, 765–794; (b) Kitigawa, I.; Kobayashi, M. *Gazz. Chim. Ital.* **1993**, *123*, 321–325; (c) Baldwin, J. E.; Whitehead, C. R. *Tetrahedron Lett.* **1992**, *33*, 2059–2062; (d) Cimino, G.; Scognamiglio, G.; Spinella, A.; Trivellone, E. *J. Nat. Prod.* **1990**, *53*, 1519–1525.
4. Cutignano, A.; Tramice, A.; De Caro, S.; Villani, G.; Cimino, G.; Fontana, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 2633–2636.
5. *H. orbignyana* (180 specimens) was collected by hand using SCUBA at depths of 3–6 m at Miseno Lake (Gulf of Naples) in June 2003. Part of the molluscs (115 specimens) was kept as control group and not subjected to any experiment. The remaining animals (65 specimens) were maintained alive in aquaria (1000 mL of sterilized marine water) for 7 days. On days 1 and 4 the animals were injected into the hepatopancreas with [1,2-$^{13}C_2$]-acetate (40 μL of 1.3 M solution in distilled water). On day 7 all the animals were frozen at −80 °C and each group of molluscs were processed separately as reported in Ref. 4, to give 1.0 mg of ^{13}C-labelled haminol-2.
6. ^{13}C NMR spectra (32 K) of natural and isotopically labelled samples were recorded at 75 MHz in $CDCl_3$ with decoupling during acquisition and an interval delay of 2 s (ns = 35,000).
7. Kalinowski, H. O.; Berger, S.; Braun, S. *Carbon13 NMR Spectroscopy*; Wiley–VHC: New York, 1988.
8. Sanchez-Salvatori, M. d. R.; Marazano, C. *JOC* **2003**, *68*, 8883–8889.