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De Novo Biosynthesis of Polypropionate Metabolites in the Marine Pulmonate *Siphonaria denticulata*

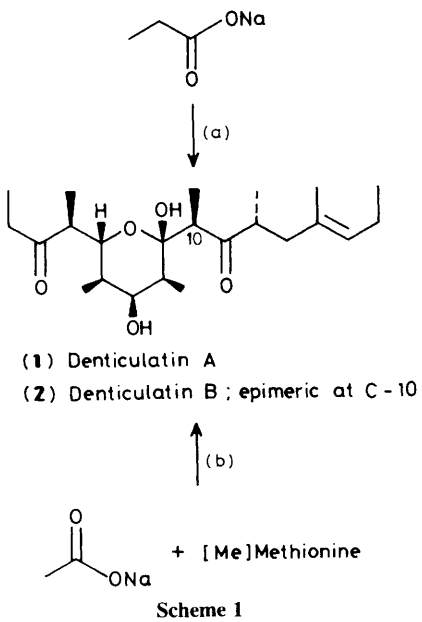
Denise C. Manker,[a] Mary J. Garson,*[b] and D. John Faulkner[a]

[a] *Scripps Institution of Oceanography, La Jolla, CA 92093, U.S.A.*
[b] *Department of Chemistry, University of Wollongong, P.O. Box 1144, Wollongong, N.S.W. 2500, Australia*

The marine pulmonate *Siphonaria denticulata* incorporates label from [1-^{14}C]propionate into denticulatins A (**1**) and B (**2**); the metabolites, which have been suggested to play a role in chemical defence, are located in mucus glands and in the foot of these animals.

Pulmonates of the genus *Siphonaria* are intertidal, air-breathing molluscs which may represent an evolutionary link between marine and terrestrial gastropods. The siphonariids contain unusual secondary metabolites, exemplified by denticulatin A (**1**) and B (**2**) from *Siphonaria denticulata*,[1] which possess a highly methylated skeleton consistent with biosynthesis *via* condensation of propionate units [Scheme 1(a)]. An alternative mode of biosynthesis would be addition of methyl groups derived from C_1-tetrahydrofolate metabolism onto a polyacetate backbone [Scheme 1(b)].

To date, the only known examples of naturally occurring polypropionates are the actinomycete antibiotics, such as erythromycin whose biosynthesis from propionate-derived units has been well documented.[2] However, cases of macrolide biosynthesis in which the chain branches are formed from acetate and methionine have also been reported.[3] We therefore undertook biosynthetic studies to determine which if either of these pathways might operate in *S. denticulata*.



Scheme 1

In biosynthetic studies on marine molluscs,[4] precursor administration has normally been by injection, but this is only successful if the site of storage or of synthesis of the metabolite under study is known. We therefore determined the location of the denticulatin metabolites by dissection of ten animals to separate the foot mantle and digestive organs. T.l.c. and n.m.r. studies confirmed that the metabolites were present in the foot mantle alone; additionally, the mucus excreted by irritated animals was found to contain the denticulatins.

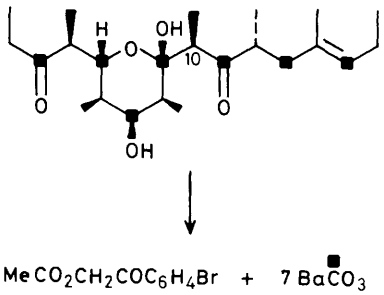
The incorporation of sodium [1-^{14}C]propionate was carried out using two different techniques. A saline solution (50 μCi ml^{-1}; 5 μCi per animal) was injected into the foot of each of 16 animals (Expt. 1) which were maintained in aerated sea water for six days prior to acetone extraction. Alternatively, the precursor was added directly to the aquarium water (Expt. 2) and incorporated by absorption through the skin.[5] In both experiments, the crude organic extract was found to be radioactive (Table 1). The denticulatin fraction, isolated by flash chromatography followed by preparative silica h.p.l.c., was also radioactive, in contrast to the sterol fraction which contained negligible ^{14}C activity. Reverse phase h.p.l.c. of the denticulatin fraction routinely showed at least four peaks, two of which contained ^{14}C activity; one of these two peaks corresponded to the denticulatin mixture. Individual denticulatin isomers† could not be resolved by either form of h.p.l.c., or by fractional crystallisation of denticulatin B (m.p. 137—141 °C) as this isomer was normally the minor component in the mixture. The ^{14}C activity of the purified denticulatin mixture did not however decrease on further h.p.l.c. treatment. When this same sequence was carried out on samples from siphonariids injected with an identical quantity of

† As a masked β-diketone system, facile epimerisation to an equilibrium mixture of the two isomers may be expected.

Table 1. Incorporation of [1-^{14}C]propionate into denticulatins A/B.

	Experiment 1[c]		Experiment 2[d]		Experiment 3[e]	
	Weight/ mg	dpm mg^{-1}	Weight/ mg	dpm mg^{-1}	Weight/ mg	dpm mg^{-1}
Crude extract	58.0	14 340	19.0	12 880	20.0	8 900
Crude denticulatins[a]	26.0	19 230	8.0	17 090	9.0	13 290
Pure denticulatins[b]	4.7	24 150	2.0	13 410	2.5	15 900
Molar specific activity denticulatins/μCi mmol^{-1}	4.35		2.41		2.86	
% Incorporation	0.1		0.02		0.04	
Molar specific activity barium carbonate[f]/μCi mmol^{-1}	0.066[g]		—		—	

[a] Flash chromatography (30% ethyl acetate–hexane). [b] Silica h.p.l.c. (15% ethyl acetate–hexane), then C_{18} h.p.l.c. (10% H_2O in MeOH) followed by silica h.p.l.c. (15% ethyl acetate–hexane). [c] By injection; 50 μCi; 16 animals; 6 days. [d] By absorption; 50 μCi; 10 animals; 7 days. [e] By absorption; 50 μCi + 14 mg unlabelled sodium propionate; 10 animals; 7 days. [f] Denticulatins A/B (4.35 μCi mmol^{-1}) were diluted with unlabelled material to give denticulatins A/B of specific activity 1.2 μCi mmol^{-1} prior to Kuhn–Roth degradation. [g] Theoretical result 0.16 μCi mmol^{-1}.



Scheme 2. Kuhn-Roth oxidation of denticulatins.

[1-^{14}C]acetate, the purified denticulatins were not significantly labelled (radioactive content <600 dpm mg^{-1}). The results of these experiments thus suggest that the denticulatins arise from condensation of propionate-derived units and not by methylation of a standard polyacetate chain. The low incorporation of acetate is most likely *via* succinate.[6]

The denticulatin A/B mixture from the propionate experiment was subjected to Kuhn–Roth degradation (Scheme 2) under N_2, yielding acetic acid (isolated as the *p*-bromo phenacyl ester) with negligible radioactivity and barium carbonate which was found to be radioactive. Theoretically, the molar specific activity of the barium carbonate sample should be one-seventh that of the parent denticulatin mixture. Experimentally, the sample contained only 41% of the anticipated radioactivity.‡ It was not therefore possible to establish from this degradation whether the denticulatins were uniformly labelled by propionate. Experiments with ^{13}C- and ^{2}H-labelled precursors are required to clarify this point. In a trial experiment, the incorporation of [1-^{14}C]propionate admixed with 14 mg non-labelled propionate gave a denticulatin mixture whose ^{14}C content corresponded to a dilution factor of 830. Stable isotope study with mutiply-labelled precursors may therefore be feasible.

Other fractions from both the flash chromatography separation and the reverse phase h.p.l.c. were found to contain ^{14}C activity. Proton n.m.r. studies suggest that this activity is associated with minor quantities of other polypropionate metabolites. These compounds are currently being investigated further since their structures may shed light on the biosynthetic steps leading to the denticulatin metabolites.

The identification of a common biosynthetic precursor for the denticulatins and for erythromycin raises intriguing questions as to the origin of the siphonariid metabolites. However, the lack of detection of denticulatin metabolites in the gut of the animals argues against the involvement of microfloral symbionts in their formation. Despite our demonstration that these metabolites are located in the part of the animal most susceptible to predation, we have found that siphonariids are commonly eaten by starfish and by other predators. The suggestion[1] that these metabolites are involved in chemical defence requires reassessment.

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‡ After subtraction of barium carbonate derived from atmospheric carbon dioxide (determined in a control experiment).[7]

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