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Biosynthetic Studies on Polypropionates: A Stereochemical Model for Siphonarins A and B from the Pulmonate Limpet *Siphonaria zelandica*.

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Abstract: The pattern of incorporation of sodium [1-^{14}C]propionate and sodium [2,3-$^{14}C_2$]succinate into siphonarins A (1) and B (2), metabolites of the pulmonate limpet *S. zelandica*, is determined by chemical degradation. The absolute configuration of (1) is revised by an x-ray study on the *p*-bromophenylboronate derivative, and together with the chain building pattern, defines a biosynthetic model for these siphonariid polypropionates.

Polypropionate metabolites are frequent products of metabolism in bacteria[1], insects[2] and molluscs.[3] Interest in their biosynthesis, triggered by the potent antibiotic action of polyether and macrolides such as erythromycin, tetronasin, monensin and related compounds, has led to important advances in our understanding of polyketide synthases and provided fascinating insight into the similarity with fatty acyl synthase enzymes.[4] In previous work,[5] we have investigated structural and stereochemical trends among the polypropionate metabolites of siphonariid limpets (phylum Mollusca, class Pulmonata), anticipating that there might be correlations with the biosynthetic trends established for macrolides[6] and polyethers.[7] In this paper, we report the results of recent studies on the absolute stereochemistry and biosynthesis of the *S. zelandica* metabolites siphonarins A (1) and B (2) which lead to a well-defined biosynthetic model (Figure 1).

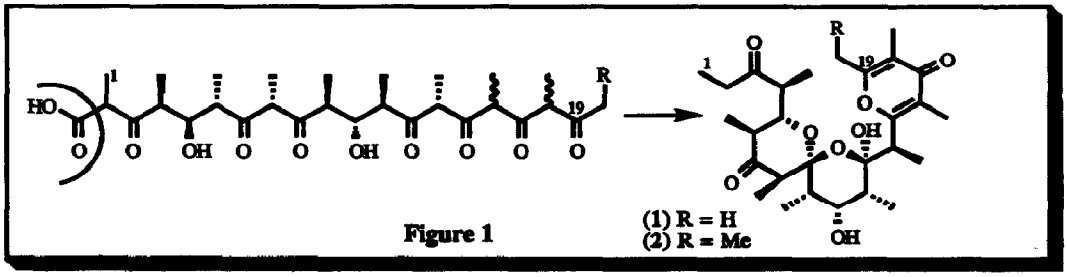
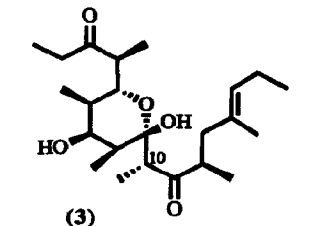


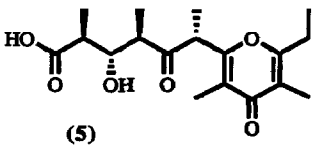
Figure 1

Absolute configuration. A previous x-ray analysis defined the relative stereochemistry of siphonarin A, but did not provide its absolute configuration. [8] The enantiomer arbitrarily chosen had the same configuration in the *tetrahydropyran* ring as the denticulatin metabolites A (3) and B (4) from *S. denticulata* ,[9] whose absolute stereochemistry has been confirmed by total synthesis.[10] The diaxial and 1,3-arrangement of the 11- and 13-hydroxyls in (1) and (2) lent itself to the preparation of crystalline derivatives. A *p*-bromophenylboronate derivative[11] (1.1 eq. *p*-Br phenylboronic acid, DCM, 18 h) was recrystallised as long

colourless needles from ether/hexane and subjected to a low-temperature x-ray study using Cu Kα radiation. The absolute configuration was determined by refinement of the additional parameter η using SHELXTL. The final agreement was R = 0.060 and η = 1.00(9) indicating that the model had the correct absolute stereochemistry.[12] The model (Figure 2) showed clearly the absolute stereochemistry for siphonarin A as shown in (1), i.e. opposite to that shown in the literature.[8] The methyls of the *tetrahydropyrone* ring of (1) thus have the same configuration as those in the *tetrahydropyran* ring of (3) and (4). Our result has also been independently verified by Paterson *et al* in a stereoselective synthesis of the enantiomer of the siphonarin B degradation product (5).[13]



(3)
(4) epimer at C10



(5)

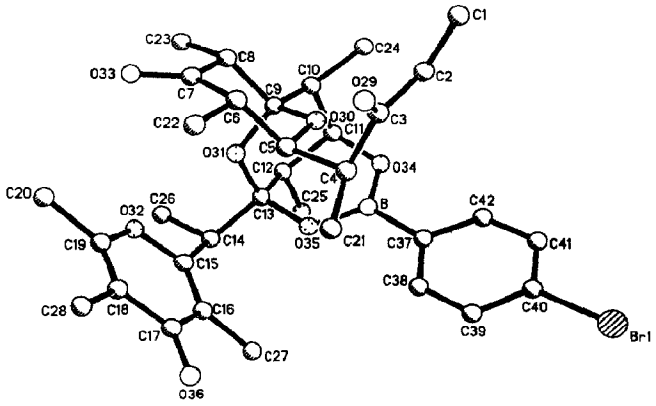


Figure 2

Biosynthesis. We have previously demonstrated the propionate origin of the denticulatins.[14] Injection of [1-^{14}C]propionate (100 μCi) into the foot tissue of *S. zelandica* followed by 4 day aquarium incubation and workup (EtOH extraction, reverse phase HPLC, MeOH/H_2O, 60:40) gave siphonarin A (1) (9447 dpm/mg, 2.17 μCi/mMole, 0.16 % incorporation) and siphonarin B (2) (2498 dpm/mg, 0.59 μCi/mMole, 0.02% incorporation), thus confirming their propionate origin.

Siphonarin A is likely assembled from one acetate[15] and nine propionate units. Two directions of chain assembly, shown in Figure 3, are possible; these differ in that acetate functions either as a chain starter unit (in (a)) or as a chain terminating unit (in (b)). The two assembly modes can be distinguished by determination of the biosynthetic origin of C19. Kuhn-Roth oxidation (CrO_3/H_2SO_4) of [1-^{14}C]propionate-labelled (1) followed by derivatisation (*p*-Br(C_6H_4)$COCH_2Br$, EtOH/H_2O, 9:1, 70°C, 2h) gave *p*-bromophenacyl acetate which was devoid of radioactivity, consistent with use of an acetate chain starter unit as in (a). However, any potentially-labelled acetate isolated from C19 plus its attached methyl is diluted by non-radioactive acetate from the other methyl-substituted centres. A more selective degradation was therefore undertaken. Cleavage of the pyrone ring (O_3, DCM, -78°C, then H_2O) and derivatisation as before gave *p*-bromophenacyl acetate uniquely from C19 plus its attached methyl. [1-^{14}C]Propionate-labelled (1) (9447 dpm/mg, 2.17 μCi/mMole) gave essentially unlabelled *p*-bromophenacyl acetate (11 dpm/mg), consistent with (a). If path (b) had represented the correct direction of polypropionate chain assembly, the *p*-bromo phenacyl acetate would have had a molar specific activity of 0.217 μCi/mMole (1863 dpm/mg). For comparison, ozonolytic degradation of [1-^{14}C]propionate-labelled (2) (2498 dpm/mg, 0.59 μCi/mMole) gave *p*-

bromophenacyl propionate, from C19 plus attached ethyl group, of molar specific activity 0.038 μCi/mMole (theoretical value = 0.065 μCi/mMole = 1/8 that of the labelled (2)).We ascribe the lower than expected labelling of the siphonarin B degradation product to the low recovery (0.5 mg) of propionate derivative. Degradation of [1-^{14}C]acetate-labelled (1) (0.47μCi/mMole) or (2) (0.23μCi/mMole) followed by derivatisation yielded acetate or propionate esters containing 26% or 11% of the radioactivity associated with the natural product, confirming direct use of acetate as a chain starter unit for (1) in addition to indirect incorporation into both (1) and (2) following conversion of acetate to propionate.

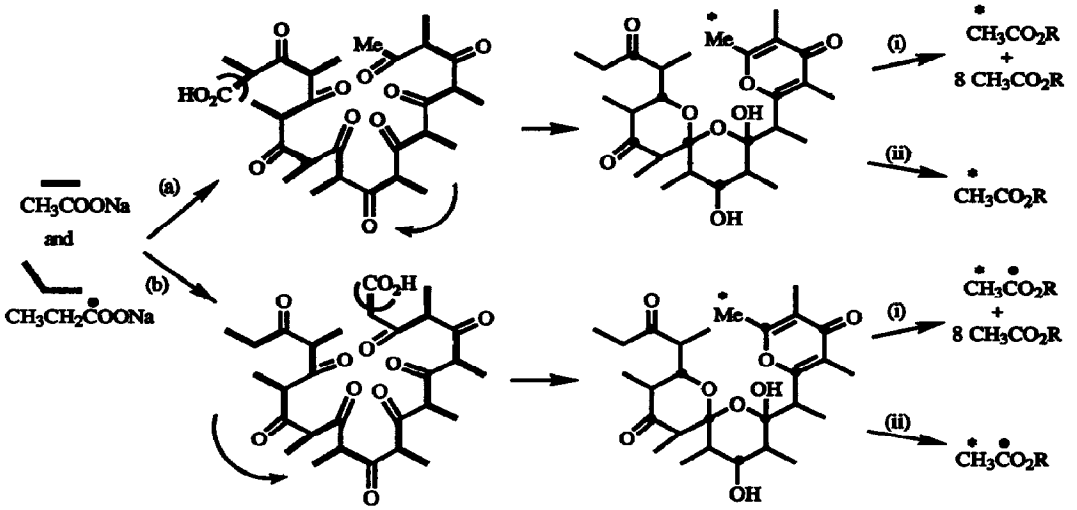


Figure 3 (i) CrO_3/H_2SO_4; then *p*-Br-$C_6H_4COCH_2Br$, EtOH/H_2O (9:1), 70° (ii) O_3, DCM, -78°; then H_2O, then *p*-Br-$C_6H_4COCH_2Br$, EtOH/H_2O (9:1), 70°.

The incorporation of [2,3-$^{14}C_2$]succinate into siphonariid metabolites gives comparable molar specific activities to those obtained in experiments with propionate. Thus [2,3-$^{14}C_2$]succinate-labelled (1) (5159 dpm/mg, 1.19 μCi/mMole, 0.14% incorporation) generated, after ozonolysis, *p*-bromophenacyl acetate 176 dpm/mg, 0.02 μCi/mMole; 15% of that of (1) showing the availability of succinate-derived acetate (chain starter unit) and propionate (chain building units). [2,3-$^{14}C_2$]Succinate-labelled (2) (2096 dpm/mg, 0.50 μCi/mMole) gave *p*-bromophenacyl propionate (371 dpm/mg, 0.046 μCi/mMole, 92% of theoretical value calculated on the basis that only 1 out of 10 evenly-labelled units is isolated in this degradation).

These results thus indicate a preference for an acetate chain starter unit in the biosynthesis of (1), define the direction of chain assembly as from C19 to C1, and demonstrate the presence of a functioning methylmalonyl-CoA mutase in *S. zelandica*.

Biosynthetic Model. The acyclic precursor which generates the cyclised siphonarin metabolites is shown in Figure 1. We have previously proposed configuration models to interrelate the various ketal metabolites produced by siphonariids,[5,16] which need to be refined in the light of the above results,[17] and correlated with existing polyether/macrolide models.[6,7]

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11. Satisfactory analytical and spectroscopic data were obtained.
12. All measurements were done on a Siemens R3m diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.54180 Å). The crystal was orthorhombic, with cell parameters a = 11.473(2) Å, b = 16.622(6) Å and c = 21.348(8) Å determined by a least-squares fit of 25 diffractometer-measured 2θ values in the range of 35-45^{0} and of space group $P2_12_12_1$ with one molecule per assymetric unit. A total of 4962 reflections (including Friedel pairs) with $2\theta \leq 105^{0}$ were collected using a variable speed θ:2θ scan technique. No absorption or decomposition errors were made. After correction for Lorentz, polarisation and background effects, 3816 of the 4425 independent reflections were (86%) judged observed ($|Fo| \geq 4\sigma Fo$). The structure was solved by heavy atom Patterson methods and refined by full-matrix least-squares methods using the SHELXTL program package. Anisotropic thermal parameters were employed for the non-hydrogen atoms. Additional crystallographic details are available and the data have been deposited in the Cambridge Crystallographic Data Centre.
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