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Advanced precursors in marine biosynthetic study. Part 2: The biosynthesis of isocyanides and isothiocyanates in the tropical marine sponge *Axinyssa* n.sp.

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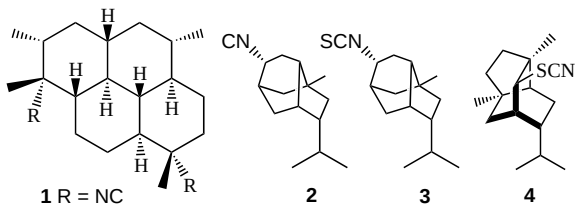
Abstract—The biosynthetic origins of the isocyanide and isothiocyanate groups in 9-isocyanop upukeanane (**2**) and 9-isothiocyanatopupukeanane (**3**) are investigated by incorporation of [^{14}C]-labelled advanced precursors into the sponge *Axinyssa* n.sp.

Research from this group,[1–5] and by others,[6] has clearly established that inorganic cyanide can act as a precursor for the biosynthesis of isocyanide terpenes such as **1** and **2** in marine sponges. Additionally, cyanide is used for the biosynthesis of isothiocyanates (e.g. **3**),[3,4,7] thiocyanates (e.g. **4**)[4] and dichloroimines[7] in marine sponges. Intriguingly, the incorporation of thiocyanate into **1**–**4** has shown that this ambident ion is a precursor for the same suite of functional groups.[3–5,7,8] The interconversion of inorganic cyanide and thiocyanate in marine sponges, first apparent to us when thiocyanate was incorporated into an isocyanide in *Acanthella cavernosa*,[3] has been further confirmed in our laboratory by demonstration of a precursor role for cyanide in the biosynthesis of a thiocyanate in *Axinyssa* n.sp.[4]



1 R = NC
5 R = NCS

2 **3** **4**

The conversion of cyanide to thiocyanate is a well-documented irreversible reaction catalysed by the enzyme rhodanese in microorganisms, plants and higher animals, while peroxidases are known to catalyse the oxidation of cyanide to thiocyanate.[9] Given that cyanide and thiocyanate can both be used by marine sponges to functionalise terpenes,[3–5,7] questions arise as to whether the corresponding secondary metabolites can be interconverted in the sponge, and whether these reactions are enzyme-catalysed. Hagadone et al. suggested that a tricyclic isocyanide was the precursor to an isothiocyanate in *Hymeniacidon* sp., but that the reverse isothiocyanate to isocyanide conversion did not occur. However, their data, obtained using mass spectrometric detection, indicated very low incorporation levels.[10] Recent ^{14}C-labelling experiments carried out in our laboratory and using *Amphimedon terpenensis* have provided evidence for an isothiocyanate to isocyanide conversion; bisisothiocyanate **5** was converted into diisocyanide **1** in this sponge.[8] Unfortunately, the incorporation values were low and variable, possibly because the sponge did not recognise the precursor **5** which has not yet been isolated as a natural product.

Fortunately, the sponge *Axinyssa* n.sp is an ideal choice for further investigation of isocyanide–isothiocyanate conversions, as both 9-isocyanopupukeanane **2** and 9-isothiocyanatopupukeanane **3**, are present in this sponge, as is the thiocyanate **4**.[11] We have previously shown that either cyanide or thiocyanate act as precursor to the isothiocyanate and thiocyanate functional groups in **3** and **4**. However, the incorporation of either cyanide or thiocyanate into the isocyanide functional group of **2** was unexpectedly low.[4] To pursue an advanced precursor study in *Axinyssa* n.sp., we first required the syntheses of [^{14}C]-labelled 9-isocyanopupukeanane **2** and 9-isothiocyanatopupukeanane **3**, which were carried out in a similar manner to the synthesis of [^{14}C]-bisisothiocyanate **5** used in our *A. terpenensis* study.[8]

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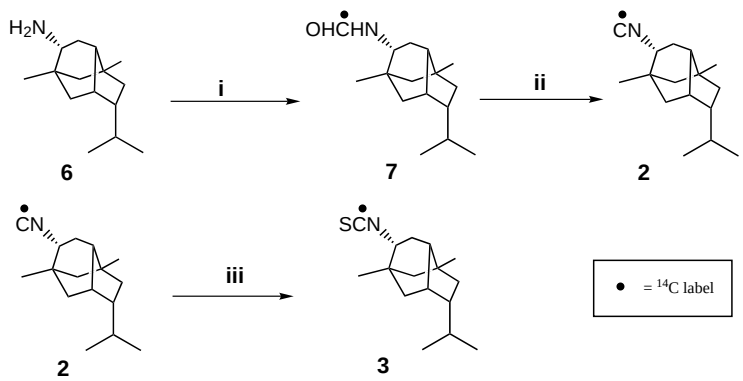
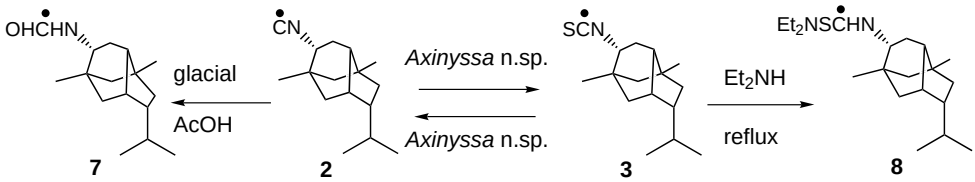


Scheme 1. Synthesis of [^{14}C]-advanced precursors. (i) [^{14}C]-formic-pivalic anhydride, Et_3N, DCM, then silica flash chromatography (hexane to EtOAc gradient); yield 59%; (ii) *p*-TsCl in pyridine, then silica flash chromatography (hexane to EtOAc gradient); yield 84%; (iii) sulfur, 120°C, then silica prep. TLC (hexane); yield 58%.

Amine **6**, available by 6N HCl hydrolysis of the isocyanide **2**, was used as a starting material for the radiochemical synthesis (see Scheme 1). The ^{14}C label was incorporated by formylation of amine **6** with [^{14}C]-formic-pivalic anhydride[12] to give the [^{14}C]-formamide **7**. Dehydration of the formamide with tosyl chloride in dry pyridine gave [^{14}C]-9-isocyanopupukeanane **2**. A portion of this isocyanide was converted to the isothiocyanate by treatment with sulfur at 120°C to give [^{14}C]-9-isothiocyanatopupukeanane **3**. To ensure that the sample of [^{14}C]-9-isothiocyanatopupukeanane **3** used in incorporations contained no unreacted [^{14}C]-9-isocyanopupukeanane **2**, the sample of [^{14}C]-**3** was mixed with unlabelled **2**, the mixture separated and the compounds purified. Two cycles of this purification protocol resulted in unlabelled **2** (with less than 0.0001 μCi recovered), confirming the radiochemical purity of the labelled [^{14}C]-9-isothiocyanatopupukeanane **3** for use in incorporations.

Next, precursor incorporation experiments were performed in the field according to our usual protocol.[1–5,7,8] [^{14}C]-9-Isocyanopupukeanane **2** was dissolved in 1 mL MeOH and added to a glass beaker containing a small piece of *Axinyssa* n.sp. in aerated sea-water kept at ambient temperature and light levels.[13] After overnight incubation, and a 21 day incorporation period, extraction of the sponge, followed by purification to constant specific activity gave a sample of 9-isothiocyanatopupukeanane **3** which was significantly radioactive (see Table 1). Fractions containing 9-isocyanopupukeanane **2** were highly radioactive, but were not purified further. Confirmation of the radiochemical purity of the isolated sample of 9-isothiocyanatopupukeanane **3** was obtained by preparation of the thiourea **8** (Et_2NH reflux),[14] which retained the radioactivity after purification.



In a second experiment, [^{14}C]-9-isothiocyanatopupukeanane **3** was dissolved in 1 mL of acetone and fed to a specimen of *Axinyssa* n.sp in a similar incorporation experiment.[13] The sample of 9-isocyanopupukeanane **2** isolated from this incorporation experiment was significantly radioactive (see Table 1). Fractions containing 9-isothiocyanatopupukeanane **3** were highly radioactive, but were not purified further. Confirmation of the radiochemical purity of the isolated 9-isocyanopupukeanane **2** was achieved by hydration (glacial AcOH) to give formamide **7**, which retained the radioactivity after purification.

These results clearly show that 9-isocyanopupukeanane **2** and 9-isothiocyanatopupukeanane **3** can be interconverted by the sponge *Axinyssa* n.sp., and that the rate of conversion is similar in either direction. The radio-

Table 1. Molar specific activities of **2** and **3** and reaction products

Precursor	Compound	Molar specific activity (μCi/mmol)	Incorporation (%)	Radioactivity (%)
[^{14}C]-**2**	**3**	4.6	0.12	100.0
	8	4.1	–	89.7[a]
[^{14}C]-**3**	**2**	0.14	0.16	100.0
	7	0.14	–	99.8

[a] The small amount and difficulty in purification meant the specific activity was lower than desired; it is quite unlikely that this level of labelling would be retained after purification if the compound was unlabelled.

label is unlikely to have been incorporated into the sesquiterpene portion of the natural products as the precursors had been specifically labelled by an unambiguous synthetic procedure, and the possibility of metabolic degradation and reincorporation of the label via general metabolism is remote given the incorporation yields obtained. The conversion of 9-isothiocyanatopupukeanane **3** into 9-isocyanopupukeanane **2** confirms that the samples of **2** obtained from the earlier incorporations of cyanide and thiocyanate were indeed genuinely labelled.[4]

Our ^{14}C labelling results therefore give contrasting results to those obtained by the Scheuer group using ^{13}C precursors in *Hymeniacidon* sp.[10] A clearer picture of isocyanide–isothiocyanate transformations can be obtained by additional study in other sponge systems; these experiments, and others to determine the role of enzymes, are in progress in our laboratory.

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References

1. Garson, M. J. *J. Chem. Soc., Chem. Commun.* **1986**, 35–36.
2. Fookes, C. J. R.; Garson, M. J.; Macleod, J. K.; Skelton, B. W.; White, A. H. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1003–1011.
3. Dumdei, E. J.; Flowers, A. E.; Garson, M. J.; Moore, C. J. *Comp. Biochem. Physiol. A* **1997**, *118*, 1385.
4. Simpson, J. S.; Garson, M. J. *Tetrahedron Lett.* **1998**, *39*, 5819.
5. Garson, M. J.; Simpson, J. S.; Flowers, A. E.; Dumdei, E. J. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, A., Ed.; 2000; Vol. 21, p. 329.
6. Karuso, P.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 2092–2095.
7. Simpson, J. S.; Raniga, P.; Garson, M. J. *Tetrahedron Lett.* **1997**, *38*, 7947.
8. Simpson, J. S.; Garson, M. J. *Tetrahedron Lett.* **1999**, *40*, 3909.
9. Westley, J. In *Cyanide in Biology*; Vennesland, B.; Conn, E. E.; Knowles, C. J.; Westley, J.; Wissing, J., Eds.; Academic Press: London, 1981; Vol. 1, Chapter 5, pp. 61–76.
10. Hagadone, M. R.; Scheuer, P. J.; Holm, A. *J. Am. Chem. Soc.* **1984**, *106*, 2447–2448.
11. Simpson, J. S.; Garson, M. J.; Hooper, J. N. A.; Cline, E. I.; Angerhofer, C. *Aust. J. Chem.* **1997**, *50*, 1123–1127.
12. [^{14}C]-Formic-pivalic anhydride was prepared by dissolving sodium-^{14}C-formate (750 μCi) in formic acid (22 μL), then adding pivaloyl chloride (70 μL). The resulting solution was then added to amine **6** (116 mg) in DCM (1 mL) and triethylamine (360 μL).
13. Sponge samples were collected on SCUBA at Coral Spawning, Heron Island (23°27′S, 151°55′E), Great Barrier Reef (14–16 m) in April 1999. A voucher sample (registry number QM G312575) is lodged at The Queensland Museum, Brisbane. A specimen of *Axinyssa* n.sp. (w. wt. 19.2 g) was placed in an aquarium containing 400 mL aerated seawater at ambient temperature. [^{14}C]-9-Isocyanopupukeanane **2** (20.3 μCi, 578 μCi/mmol) in methanol (1 mL) was added and the sponge allowed to assimilate radioactivity for 12 h overnight. The sponge was kept in running seawater in a 10 L aquarium at ambient temperature for 21 days, then frozen for subsequent radiochemical analysis. A DCM extract was processed by our previously reported method,[4] followed by repeated silica HPLC (μ-partisil, 0.25% EtOAc/hexane) to give 9-isothiocyanatopupukeanane **3** (2.4 mg, 0.012% chemical yield; 29440 dpm/mg, 0.16% incorporation yield). In an otherwise identical experiment, *Axinyssa* n.sp. (w. wt. 18.1 g) was treated with [^{14}C]-isothiocyanatopupukeanane **3** (14.6 μCi, 619 μCi/mmol) in acetone (1 mL), and after extraction and purification gave 9-isocyanopupukeanane **2** (29.8 mg, 0.165%; 1320 dpm/mg, 0.12%).
14. All new compounds gave satisfactory spectroscopic and analytical data.