Host-plant pyrrolizidine alkaloids in *Nyctemera annulata* Boisduval: Their persistence through the life-cycle and transfer to a parasite[1]

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Summary. Larvae of *Nyctemera annulata* Boisduval ingest pyrrolizidine alkaloids from *Senecio spathulatus* A. Rich. which subsequently appear in the adult moths and their eggs; the acquisition of the alkaloids by a parasite of the *N. annulata* larvae provides a further illustration of their ability to persist in insects.

Pyrrolizidine alkaloids commonly occur in plants of *Senecio* and certain other genera[4], and in several cases it has been shown that lepidoptera feeding on these plants acquire the alkaloids[5-9]. Such insects are usually brightly coloured, and not subject to predation by birds. The toxicity of some pyrrolizidine alkaloids to mammalian systems is well-established[5] and it has been plausibly hypothesized that birds learn to identify the lepidoptera as unpalatable, the unpalatability being due, at least in part, to their intake of plant alkaloids[6, 7, 9]: A situation resembling the now famous role of cardenolides in deterring avian predators of Monarch butterflies[10, 11]. We report here a study of the alkaloids present in various stages of *N. annulata* Boisduval (Lepidoptera: Arctiidae) raised on *S. spathulatus* A. Rich., which demonstrates both that this is another example of a moth which retains its host-plant's pyrrolizidine alkaloids and that these alkaloids are exceptionally persistent.

N. annulata flies by day and is brightly patterned. Like their colourful caterpillars, the adult moths are left severely alone by most birds[12]. Known commonly as the Magpie moth, the preference of *N. annulata* for species of *Senecio* as host-plants is so well recognized in New Zealand that when it was included in a recent issue of postage stamps depicting common native lepidoptera the moth was shown (figure) superimposed upon a sprig of *S. minimus* Poiret[13] (= *Erechtites minimus* Poiret, *E. prenanthoides* Hook)[14].

Nyctemera annulata with *Senecio minimus*.

Material and methods. In the Auckland area we have found *N. annulata* larvae feeding on *S. cinerraria* D.C., *S. jacobea* L., *S. mikanioides* Otto ex Walp., *S. vulgaris* L. and *S. spathulatus* A. Rich.[15]. All of these plants contained pyrrolizidine alkaloids but the particularly convenient availability of *S. spathulatus* induced us to concentrate our study on this as host-plant. Mature, flowering plants growing on the DSIR Research Center grounds and environs were used to maintain a laboratory colony of *N. annulata* through several generations.

The presence of host-plant alkaloids in the larvae, and adults therefrom, could be demonstrated by gas liquid chromatography (GLC) and mass-spectroscopy (MS). The alkaloids were isolated conventionally[7] with, and without, a zinc dust-in-sulphuric acid reduction of N-oxides to tertiary bases. Typical GLC analyses were on 2 m × 3 mm of 3% OV-1 or OV-17, on Supelcoport WHP 80-100 mesh, in glass columns at 225 °C, with a nitrogen carrier gas flow of 30 ml/min^{-1}. For GLC-MS analyses (Hewlett Packard Model 5990A GC-MS) we used 0.5 m of 2% OV-101 plus 0.2% carbowax 20M, in glass, at 200 °C with a helium carrier gas flow of 30 ml/min^{-1}.

Results and discussion. The table summarizes our findings. Although a large portion of the larval intake of the plant alkaloids appeared to be excreted unchanged in the frass, the larvae retained some of the bases. Not too surprisingly, these alkaloids appeared in the adults but much more strikingly they were also present in considerable concentration in their eggs. We did not observe the $C_{15}H_{23}NO_5$ metabolite found[7] in *Arctia caja* and *Tyria jacobaeae* L. adults (possibly because the putative precursor seneciphylline is a very minor component of the bases in *S. spathulatus* whereas it is a major one in *S. vulgaris*[7]), and there was no indication of selective metabolism of either of the 2 dominant, geometrically isomeric, alkaloids, i.e. the alkaloid ratio remained constant within experimental error throughout the life cycle, although the amount present fell steadily. This seems to constitute only the second example of the persistence of host-plant pyrrolizidine alkaloids through the entire life-cycle of an insect[16]: the first being reported by Aplin and Rothschild[7], who raised 4 species of

The transfer of pyrrolizidine alkaloids from *S. spathulatus* to *N. annulata*

Species	Alkaloid* content (approximate)
S. spathulatus	0.05%
N. annulata	
Frass	0.2%
Larva	0.2% (ca. 300 μg/larva)
Adult ♂	0.3% (ca. 130 μg/moth)
Adult ♀	0.4% (ca. 160 μg/moth)
Egg	0.4% (ca. 1 μg/egg)

* Largely senecionine and intergerrimine (ca. 1:5), with very minor amounts of seneciphylline. The corresponding N-oxides comprised about 70% of the total bases.

lepidoptera on *S. vulgaris* L. and found that the eggs of one of them, *A. caja* L., contained the plant alkaloids.

The persistence of the *S. spathulatus* alkaloids certainly makes them candidates as predator-deterrents. However they are largely present in *N. annulata*, as in *S. spathulatus*, as N-oxides (of intergerrimine and senecionine): compounds which are much more water-soluble than the tertiary bases but correspondingly less toxic to vertebrates[4]. For substances to function as efficient feeding-deterrents a likely requirement is for them to be immediately distasteful to predators. It will be interesting to see which, if any, of the pyrrolizidine alkaloids are effective in this regard. Edgar et al.[9] cite unpublished work by T. Eisner, K. Hicks and D. Aneshasley as demonstrating that pyrrolizidine alkaloids are distasteful to some insect predators.

Impressed by the persistence of the *S. spathulatus* alkaloids in *N. annulata* we were curious to see if they would survive transfer to a natural parasite, a species of *Microplitis* (Hymenoptera: Braconidae)[17]. Accordingly we examined the pupae of this braconid, obtained from larvae emerging from late instar *N. annulata* larvae. The plant alkaloids were present in the parasite. So far as we are aware, this is the first report of the transfer of a plant alkaloid through a herbivorous larva to a carnivorous parasite[18], and further attests to the remarkable metabolic stability of the N-oxides of senecionine and intergerrimine in some insects.

1 Acknowledgments. We thank Dr R. Yamdagni of the Chemistry Department, University of Calgary, Canada, and Dr O. Madsen of the Organic Chemistry Department, The Technical University of Denmark, Lyngby, Denmark, for much help with the MS data collection. We are also very grateful to Dr C.C.J. Culvenor, CSIRO Animal Health Lab., Parkville, Victoria, Australia, who most generously provided us with authentic reference specimens of pyrrolizidine alkaloids.
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13 We are grateful to Miss Eileen Mayo of Christchurch, who designed the stamp, for the identification of this plant. We also thank Mr A.R. Anderson of the New Zealand Post Office H.Q., Wellington, for the information that some 419 million copies of this stamp were distributed, plus several million more surcharged to 4c: thus making the figure probably the most widely circulated illustration of an aposematically coloured insect with a host-plant likely responsible for its chemical defence against predators.
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Studies on hydrogen, halides and thiocyanate ions in dioxane-water mixtures at various temperatures

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Summary. The free enrgies of transfer (ΔG_t^0) of H^+, Cl^-, Br^-, I^- and SCN^- ions from water to 10, 20, 30 and 40% dioxane-water mixtures have been studied from potentiometric measurements. For anions the ΔG_t^0 values are in the order $Cl^- > Br^- > SCN^- > I^-$.

The study of electrode potentials of individual electrodes have been a subject of interest to electrochemists. Almost exhaustive work[1-3] has been done to determine the electrode potential of various electrodes in aqueous solutions. Very little work[4-6] has been done in the study of such systems in aquo-organic solvents. These studies should bring out not only the characteristic for aqueous component in influencing the electrolyte but also throw light on the basic character of the solvent. In the present communication, the standard free energies of transfer (ΔG_t^0) have been studies from the standard potentials of Ag-AgBr, Ag-AgI and Ag-AgSCN electrodes in 10, 20, 30 and 40% (w/w) dioxane-water mixture at 15, 25, 35 and 45 °C, and an attempt has been made to deal with the basic character of the solvents.

Materials and methods. The free energy changes (ΔG_t^0) accompanying the transfer of H^+X^- (where X = Br or I or SCN) from water to dioxane-water mixtures have been computed by Feakin's method[7] using E_N^0 values of the 3 electrodes reported in our previous communications[8-10]. The ΔG_t^0 values for H^+Cl^- reported by Das et al.[11,12] have also taken into consideration to calculate the ionic ΔG_t^0 values.

Results and discussion. The ΔG_t^0 values for H^+Cl^-, H^+Br^-, H^+I^- and H^+SCN^- in 10, 20, 30 and 40% (w/w) dioxane-water mixtures are given in table 1. The ΔG_t^0 values are found to be positive for H^+Cl^-, H^+Br^- and negative for H^+I^-, H^+SCN^-. So the transfers of I^- and SCN^- are more favoured than Cl^- and Br^-. The ionic ΔG_t^0 values for H^+ i.e. $\Delta G_t^0(H^+)$ were obtained by plotting ΔG_t^0 values for halogen