# INSECT-CONTROL CHEMICALS FROM PLANTS. NAGILACTONE C, A TOXIC SUBSTANCE FROM THE LEAVES OF *PODOCARPUS NIVALIS* AND *P. HALLII*

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*Abstract*

Feeding tests showed that the powdered dried leaves and leaf extract of *P. nivalis* Hook and *P. hallii* Kirk are toxic to housefly larvae when incorporated into a milk diet. An active toxic ingredient has been isolated and identified as the known norditerpene lactone, nagilactone C. Nagilactone C at 50 p.p.m. in a defined medium was toxic to housefly larvae. At lower concentrations it inhibited larval growth, maturation to pupae, and emergence of adults. Nagilactone C was not toxic to 3–4-day-old housefly larvae or to 2–3 day old adults when applied topically at the rate of 5 $\mu$g per insect.

## I. Introduction

During a survey of New Zealand Podocarpaceae for insect moulting hormone activity (Russell and Fenemore 1970), we became interested in *Podocarpus nivalis* Hook, a small prostrate shrub which has only one phytophagous lepidopterous species recorded from it (J. S. Dugdale, personal communication 1969). When leaf material from this plant was incorporated into the milk diet of housefly larvae (*Musca domestica* L.) complete mortality resulted. Other *Podocarpus* species also showed pronounced activity against housefly larvae.

This work reports the isolation of an active principle from leaves of *P. nivalis* and *P. hallii*, and its effects on housefly larvae when incorporated into diets and its effects on larvae and adults when applied topically.

## II. Materials and Methods

### (*a*) *Insect Rearing*

Housefly larvae, strain SP2AB (Spiller 1966), were reared on macerated tissue, whole milk powder, and yeast diet at 25°C and 60–70% R.H., as described by Spiller (1966).

Stock cages of adults were maintained on a dry diet mix of granulated sugar (6·0 g), skim milk powder (6·0 g), autolysed yeast powder (1·3 g), egg yolk powder (1·0 g), and cholesterol (13 mg). Liquid was provided as 2·5% sucrose solution.

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### (*b*) *Feeding Tests on Milk Diet*

Initial tests to determine the activity of whole leaf were conducted by incorporating dry, finely powdered leaf material into the larval diet at the rate of 20% of the dry components. Crude extracts were similarly incorporated, at rates equivalent to 20% of leaf material, by coating them on to the paper constituent of diet from a volatile solvent, or by dissolving them in the water added to the diet mix.

The diet was made up in amounts of 100 g (wet weight) and after mixing, this was subdivided into four 25 g quantities. Each aliquot was placed in a wide-mouthed glass jar (135 ml capacity), seeded with 100 eggs, and held for 10 days at 25°C. Each test consisted of four replicates and samples were tested in groups each containing a control batch.

Assessment of effect was based on numbers and weights of pupae produced 10 days after seeding the larval medium with eggs and the pupae which formed were retained until adult emergence was complete.

### (*c*) *Extraction of Plant Material*

Dried milled foliage (1·9 kg) of *P. nivalis*, collected at Mt. Cheesman, Canterbury, N.Z., was percolated with methanol in a Soxhlet apparatus for several hours. The extract was concentrated to an oily mass, redissolved in methanol–water (4 : 1 v/v), and partitioned between this solvent and light petroleum (three transfers). The aqueous methanolic layers were combined, the methanol removed under vacuum, and the residual aqueous mixture extracted three times with diethyl ether. The aqueous layer was then extracted three times with n-butanol and the butanol layers were each extracted in turn with a second portion of water. The combined butanol layers were concentrated to dryness under reduced pressure to a dark gum (213 g).

Dried milled foliage (1·9 kg) of *P. hallii*, collected in the Kaweka Range, Hawkes Bay, N.Z., was extracted with methanol in a Soxhlet apparatus. The extract was evaporated and treated essentially as described for the *P. nivalis* sample. Evaporation of the butanol solutions gave a brown gum (164 g).

### (*d*) *Isolation of the Toxins*

The toxic butanol fraction (50 g) from *P. nivalis* was warmed with ethanol (100 ml), ethyl acetate (100 ml) added, and the solution filtered and applied to a column of neutral alumina (Woelm, act. 4, 500 g). Ethyl acetate–ethanol (1 : 1 v/v) eluted a series of active fractions which were dissolved in acetone, warmed with carbon, and filtered. The chromatography was repeated with further material and final crystallization from acetone gave pure nagilactone C (Hayashi *et al.* 1968) (1·5 g), m.p. 319–321° (lit. m.p. 290°). $[\alpha]_D + 116°$ (*c*, 1·1 in pyridine), $\lambda_{max}$ (EtOH) 301 nm ($\epsilon$ 5,950), $\nu$ (KBr) 3300, 1770, 1700, 1640, 1550 $cm^{-1}$. The 60 MHz n.m.r. spectrum (pyridine-$d_5$) showed one-proton signals at $\delta$ 6·69 (s), 5·60 (d, *J* 8 Hz), 5·09 (dd, *J* 8, 7 Hz), 4·72 (d, *J* 6 Hz), 3·64 (d, *J* 4 Hz), 3·57 (dd, *J* 7, 3 Hz), 3·40 (m), 2·12 (d, *J* 7 Hz), and showed three-proton signals at $\delta$ 2·08 (s), 1·51 (s), 1·37 (d, *J* 4 Hz), 1·25 (d, *J* 4 Hz). In the mass spectrum the molecular ion at *m/e* 362 ($C_{19}H_{22}O_7$ by mass measurement) was the strongest peak. Nagilactone C gave a monoacetate, m.p. 255°C (lit. m.p. 255°) and a diacetate m.p. 278–279° (lit. m.p. 280°C).

The toxic fraction from *P. hallii* was treated in the same procedure and also gave pure nagilactone C (1·4 g).

### (*e*) *Feeding Tests on Defined Diet*

Housefly larvae were reared aseptically on a defined diet mixture described by Munroe (1962).

The diet mixture (2·25 g) was dissolved in distilled water (22·75 ml), adjusted to pH 6·5 with a few drops of potassium hydroxide solution (4M), and autoclaved for 20 min at 15 lb/in. Vitamins (Munroe 1960) were dissolved in distilled water (50 ml) containing four drops of concentrated ammonia and the solution was sterilized by filtration through a microbiological filter. The vitamin solution (2 ml) and nagilactone C in chloroform–methanol (1 : 1 v/v) were added to the cooled diet medium (60°C) under an aseptic hood. After thorough mixing, the medium (1 ml) was dispensed into sterile vials (75 by 12 mm) and allowed to gel on a slant. Three concentrations

(50, 25, and 12·5 p.p.m.) of nagilactone C in three replications of 25 were set up, together with control batches containing only solvent (1% v/v).

Housefly eggs collected from milk pads were shaken in soapy water (1 g soap/100 ml water) to separate them. After washing several times in distilled water they were transferred to formalin (5% v/v) for 5 min and again washed with distilled water. The eggs were then covered with White's solution and shaken periodically for 1 hr. Finally they were washed five times with sterile distilled water and transferred to sterile Petri dishes containing black filter paper. The newly hatched larvae were transferred with a sterile brush into individual vials containing the diet mixture. The sterilization of eggs and transference of larvae were carried out under an aseptic hood.

The larvae were reared at 26°C in total darkness on the diet containing graded nagilactone C concentrations (see Singh and House 1970 for feeding tests with *Agria affinis* larvae). Growth rate, development, and mortality of larvae were recorded daily. After 10 days the larvae were pooled and allowed to pupate and emerge to determine latent effects.

The $ET_{50}$s of second- and third-instar larvae were calculated, $ET_{50}$ being the time in days required for 50% of the larvae to reach second and third instar respectively.

The mortality of larvae during the first 24 hr was about 10% and since this was assigned to handling it was not considered in the final calculations. Microbial contamination was less than 1% and these larvae were similarly discounted. Percentage mortality in larval instars was corrected according to Abbot's formula (1925).

### (*f*) *Topical Tests*

A solution (1 μl) of nagilactone C in chloroform–methanol (1 : 1 v/v) was applied topically to 3–4-day-old housefly larvae at dosage rates of 5·0, 1·0, and 0·2 μg/larva. Treated larvae (50 at each dosage level) were held for 24 hr at 25°C and 60–70% R.H. before assessment.

Adult houseflies (2–3 days old, 50 of each sex) were treated in a similar manner to larvae and held under the same conditions but with continual illumination and the provision of a dilute sucrose solution.

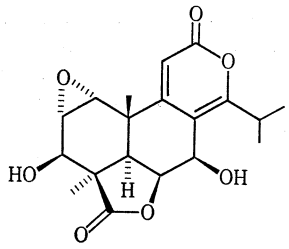
## III. Results and Discussion

When incorporated into the milk diet at a rate of 20% dry weight the dry powdered leaf material of *P. nivalis* gives complete mortality of housefly larvae. The toxic principle can be extracted and the results of feeding leaf extracts and fractions from solvent partitions and chromatography are given in the following tabulation:

| | Sample | | Control | |
|---|---|---|---|---|
| | Pupation (%) | Mean pupal weight (mg) | Pupation (%) | Mean pupal weight (mg) |
| Dried leaf | 0 | — | 64·7 | 20·2 |
| Crude extract | 0·2 | 7·6 | 60·7 | 13·3 |
| Light petroleum fraction | 42·3 | 9·3 | 60·7 | 13·3 |
| Methanol–water fraction | 0·2 | 9·5 | 60·7 | 13·3 |
| Diethyl ether fraction | 71·2 | 18·7 | 65·9 | 19·5 |
| Water fraction (1) | 0 | — | 65·9 | 19·5 |
| Butanol fraction | 0·7 | 6·8 | 74·8 | 18·0 |
| Water fraction (2) | 27·5 | 8·6 | 74·8 | 18·0 |
| Chromatography fractions | 0 | — | 68·5 | 15·1 |

The known norditerpene lactone, nagilactone C (I), previously isolated from *P. nagi*, was crystallized from the active fractions and this compound accounts for most of the toxicity of *P. nivalis* leaves. Nagilactone C, whose spectral and physical properties are consistent with those previously published (Hayashi *et al.* 1968), was

also obtained from *P. hallii* leaves. The leaves of this plant and the leaf extracts gave similar feeding results as those in the above tabulation.



(I)

Nagilactone C was incorporated into a defined diet for houseflies and the results of these tests (Table 1) show that the material is toxic at a level of 50 p.p.m. At 25 p.p.m. the rate of growth and development is inhibited and pupae are almost half the weight of the controls. Although the effects on the larval instars are not severe at 12·5 p.p.m., the formation of pupae and emergence of adults is adversely affected.

TABLE 1

EFFECT OF NAGILACTONE C IN DEFINED DIET ON LARVAL DEVELOPMENT, PUPATION, ADULT EMERGENCE, AND MORTALITY OF THE HOUSEFLY

Results are averages of three replicates each of 25 larvae

| Concn. (p.p.m.) | $ET_{50}$ of larvae | | Increase in time of larval development (%) | Larvae in third instar after 10 days (%) | Pupation (%) | Average weight of pupae (mg) | Adult emergence (%) | Mortality in larval instars after 10 days* (%) |
|---|---|---|---|---|---|---|---|---|
| | Second instar | Third instar | | | | | | |
| Control | 1·5 | 3·5 | 0 | 93·3 | 93·3 | 13·8 | 75·0 | 6·7 |
| 12·5 | 1·7 | 5·0 | 40·0 | 78·3 | 60·0 | 11·0 | 36·7 | 21·4 |
| 25·0 | 2·5 | 7·9 | 126·0 | 50·0 | 15·5 | 7·0 | 1·8 | 46·4 |
| 50·0 | – | – | – | 3·0 | 0 | – | 0 | 91·2 |

* Corrected according to Abbott's (1925) formula.

Topical treatments using pure nagilactone C at the rate of 5 $\mu$g per insect (larvae and adults) were without effect, indicating that nagilactone C is only active by oral ingestion.

A careful examination of the extracts of *P. nivalis* failed to reveal the presence of any other lactones but the mother liquors of the *P. hallii* extraction, on the other hand, gave small quantities of several unknown lactones. Until these materials are finally purified it is not known whether or not they also contribute to the toxicity of the lactone fraction from this species.

A previous study on the termite resistance of *P. macrophyllus* (Isako, Summato, and Kondo 1970) revealed that inumakilactone, another diterpene lactone, is responsible for the anti-termite activity and it is not unexpected that the toxic principle of *P. nivalis* and *P. hallii* is a similar compound. Lactones of this type also show plant growth inhibitory activity (Galbraith *et al.* 1970) and this dual role with plants and insects is of considerable interest.

The toxicity of nagilactone C will be further investigated with other orders of

insects and although the toxicity to plants and mammals is not known, these lactones may offer new avenues in the search for selective insect-control agents. Their presence in plants suggests an interesting insect–host interrelationship.

## IV. Acknowledgments

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