Aust. J. Biol. Sci., 1976, **29**, 99–103

Insect-control Chemicals from Plants
III.* Toxic Lignans from *Libocedrus bidwillii*

G. B. Russell,[A] *Pritam Singh*[B] *and P. G. Fenemore*[C]

[A] Applied Biochemistry Division, DSIR, Palmerston North, New Zealand.
[B] Entomology Division, DSIR, Mt Albert, Auckland, New Zealand.
[C] Massey University, Palmerston North, New Zealand.

Abstract

Feeding tests showed that the powdered dried leaves and leaf extracts of *L. bidwillii* are toxic to the larvae of the housefly (*Musca domestica*), and the codling moth (*Laspeyresia pomonella*). The powdered material was not toxic to the light-brown apple moth (*Epiphyas postvittana*). The most active toxin is the lignan β-peltatin-A methyl ether (II) and at a concentration of 100 ppm in a chemically defined diet it gave 98% mortality of housefly larvae.

Introduction

In recent years much interest has centred on the role of natural products influencing the association of plants and insects (Jermy 1966; Fraenkel 1969; Hsiao 1969; Munakata 1970; Chapman 1974). In previous work (Russell *et al.* 1972; Singh *et al.* 1973), we have investigated two New Zealand Podocarpaceae on which very few insects have been reported feeding, in order to define in chemical terms the causes of host specificity. *Podocarpus hallii* Kirk and *P. nivalis* Hook. yielded a number of insect-toxic terpene lactones (Russell *et al.* 1973) which we believe are responsible for there being so few phytophagous insects associated with these plants.

Libocedrus bidwillii Hook. f., the New Zealand mountain cedar, is another conifer with few phytophagous lepidopterous species recorded from it (J. S. Dugdale, personal communication). Foliage from this plant was initially tested for the occurrence of insect active compounds by adding powdered whole leaf to the artificial diets of the codling moth, *Laspeyresia pomonella* (Linnaeus), the light-brown apple moth, *Epiphyas postvittana* (Walker), and the housefly, *Musca domestica*. Using the housefly feeding test as a bioassay the active principles could be isolated from the leaf material and their effects on the development of the housefly evaluated by their addition to a chemically defined diet.

Materials and Methods

Codling Moth and Light-brown Apple Moth Feeding Tests

The composition and preparation of the artificial diet used for rearing the codling moth and the light-brown apple moth are described by Singh (1974*a*). Finely ground powdered leaf material was incorporated into the diet at the rate of 20% of the dry components. The material was thoroughly mixed after the diet had been autoclaved and cooled to 60°C. Two tablets of diet (*c.* 4 g) were required to rear one larva. The larvae were reared individually in glass test tubes at 25 ± 1°C, 50–60% R.H.

* Part II, *Aust. J. Biol. Sci.*, 1973, **26**, 911–15.

and 18-h photoperiod. Fifty larvae were used per treatment. In control treatments, dry leaf powder was replaced by cellulose powder. Details of rearing the light-brown apple moth are described by Singh (1974*b*), and a similar procedure was followed for the codling month.

Housefly Feeding Tests on Milk Diet

The housefly rearing and feeding tests were carried out as previously described (Russell *et al.* 1972). 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 leaf extracts and chromatography fractions were bioassayed for toxicity by coating them on to the paper constituent of the larval diet from a volatile solvent or by dissolving them in the water added to the mix at rates equivalent to 20% of the leaf material. Each test consisted of four replicates, each seeded with 100 eggs and held for 10 days at 25°C. Evaluation was based on numbers and weight of pupae, adult emergence and mortality.

Extraction of Plant Material

Dried milled foliage (1·5 kg) of *L. bidwillii*, collected in the Ruahine Ranges, 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 chloroform. The chloroform fractions were combined and evaporated to dryness under reduced pressure to a gum (29 g).

Isolation of the Toxins

The chloroform concentrate was dissolved in benzene–chloroform (3 : 1, 100 ml) applied to a column of silicic acid (Mallinkrodt, 500 g, 5% water) and eluted with this solvent to give a series of active fractions. These were combined (7·3 g) and rechromatographed on neutral alumina (Woelm, 200 g, deactivated with ethyl acetate) with benzene as the eluting solvent. The active fraction contained at least two compounds with very similar R_F values. These were separated with some difficulty after repeated chromatography on silicic acid (Unisil, 100 g, 5% water) with cyclohexane–ethyl acetate (4 : 1) and were crystallized from methanol. The major compound was deoxypodophyllotoxin (I), 1·2 g, m.p. 166–168°C, $[\alpha]_D$ −114° (*c* 0·06, $CHCl_3$), lit. m.p. 167–168°C, $[\alpha]_D$ −116° (Hartwell and Schrecker 1954; Schrecker *et al.* 1956); the second component was β-peltatin-A methyl ether (II), 0·6 g, m.p. 162–163°C, $[\alpha]_D$ −119° (*c* 0·5, $CHCl_3$), lit. m.p. 163–164°C, $[\alpha]_D$ −120° (Hartwell *et al.* 1952; Bianchi *et al.* 1969).

Deoxypodophyllotoxin (100 mg) and β-peltatin-A methyl ether (120 mg) were refluxed in ethanol (5 ml) containing anhydrous sodium acetate (0·3 g) for 18 h to effect epimerization. Deoxypicropodophyllin (V), m.p. 171–172°C, $[\alpha]_D$ +31° (*c* 0·085, $CHCl_3$), lit. m.p. 171–172°C, $[\alpha]_D$ +32°, and β-peltatin-B methyl ether (VI), m.p. 183–184°C, $[\alpha]_D$ +9° (*c* 1·3, $CHCl_3$), lit. m.p. 183–184°C, $[\alpha]_D$ +11°, were obtained in good yield.

Deoxypodophyllotoxin, β-peltatin-A methyl ether and their epimers were fully characterized from their p.m.r., u.v., i.r. and mass spectra (Hartwell and Schrecker 1958; Bianchi *et al.* 1969).

Evaluation of the Toxins in Defined Housefly Diet

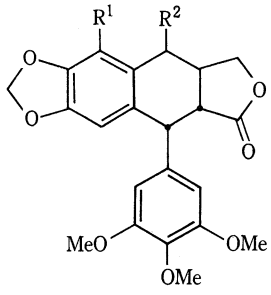
The methods used for rearing housefly larvae under aseptic conditions and evaluating the effects of compounds incorporated into the diet were those of Russell *et al.* (1972). The composition of the chemically defined diet used was similar to that formulated by House and Barlow (1958). The lignans were dissolved at three concentrations (25, 50 and 100 ppm) in ethanol and compared with a control containing 1% solvent only. Three replicates, each of 25 test tubes containing 1 ml of defined diet, were used in each test.

A single neonate sterile housefly larva was added to each tube and reared at 25°C for 8 days in darkness. Daily records were taken of larval survival and stage of development. After the 8th day, surviving larvae were removed from the tubes and placed in Perlite (N.Z. Perlite Ltd. Auckland) for pupation and emergence. Pupae were weighed on the 10th day. Tubes contaminated with microorganisms or those in which larvae died within 24 h were omitted from the results.

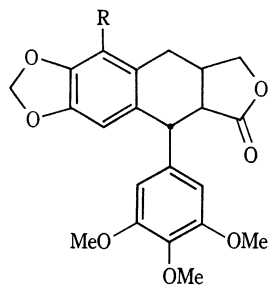
Results and Discussion

When incorporated into the diet at a rate of 20% dry weight, the dry powdered leaf material of *L. bidwillii* gave 100% mortality of the codling moth and housefly larvae. Larvae of the light-brown apple moth were not affected; larval life was not prolonged and 60% adult survival was obtained. This compares favourably with the control.

Using the housefly feeding test as a bioassay the principle toxins from the leaf material could be extracted with methanol, partitioned into chloroform and isolated after repeated chromatography. The known lignans deoxypodophyllotoxin (I) and β-peltatin-A methyl ether (II) were crystallized from the active fraction and appear to account for the toxicity of *L. bidwillii* leaves toward housefly larvae.



	R^1	R^2
(I)	H	H
(II)	OMe	H
(III)	OH	H
(IV)	H	OH



(V) R = H
(VI) R = OMe

Deoxypodophyllotoxin and β-peltatin-A (III) have been isolated from several species of *Podophyllum* and *Juniperus*, and β-peltatin-A methyl ether has been isolated from a *Bursera* species (Hartwell 1950; Hartwell *et al.* 1952, 1953; Fitzgerald *et al.* 1957; Bianchi *et al.* 1969). Podophyllotoxin (IV) and its derivatives have received much chemical (Hartwell and Schrecker 1958), biological and clinical attention (Lettre and Wilte 1967) and the above three compounds have been found to be potent cytotoxins (Kelly and Hartwell 1954). In view of their properties it is not surprising that the occurrence of such lignans in the leaves of *L. bidwillii* should make this plant toxic to insects. In the presence of mild base catalysts, all of the podophyllotoxin compounds epimerize smoothly at the 2-position to give lignans in the 'picro' series (Hartwell *et al.* 1952; Schrecker *et al.* 1956; Hartwell and Schrecker 1958) which show little or no cytotoxic activity (Kelly and Hartwell 1954; Hartwell and Schrecker 1958).

Deoxypodophyllotoxin, β-peltatin-A methyl ether and their epimers (V, VI) were incorporated into a defined diet for houseflies and the results of these tests are shown in Table 1. Of all four compounds only β-peltatin-A methyl ether gave complete larval mortality at a concentration of 100 ppm; at 50 ppm there was significant toxicity but at 25 ppm no toxicity was demonstrated. At 50 ppm this material affected pupation, resulting in the formation of smaller pupae, and it also decreased adult emergence. Surprisingly, in view of the reported cytotoxicity, deoxypodophyllotoxin at 100 ppm did not cause significant mortality of larvae, although pupation was

affected. The two picro-epimers were not expected to show significant activity and the results largely support this view. Larval mortality and pupation were only affected to a slight extent with both compounds at 50 and 100 ppm. Deoxypicropodophyllin, however, did have a significant effect on pupal weight and adult emergence and although this material may not be toxic to larvae at these concentrations, it affects growth and maturation in some way.

Table 1. Effect of lignans in defined diet of housefly on larval mortality, pupation and adult emergence

Compound	Concentration (ppm)	Larval mortality after 10 days (%)	Pupation after 10 days (%)	Mean weight of pupae (mg)	Adult emergence[A] (%)
Deoxypodophyllotoxin (I)	100	33·3	47·8	10·1	75·8
	50	31·5	63·0	10·5	71·7
	25	8·1	90·5	11·2	88·1
	0	12·3	82·2	12·3	91·7
β-Peltatin-A methyl ether (II)	100	98·7	0	—	—
	50	45·1	42·3	6·6	43·3
	25	13·5	82·4	9·7	83·6
	0	9·3	89·3	11·3	82·1
Deoxypicropodophyllin (V)	100	23·9	63·4	7·0	11·1
	50	17·6	68·9	9·1	60·8
	25	2·9	87·1	9·9	85·3
	0	2·8	94·4	11·4	85·3
β-Peltatin-B methyl ether (VI)	100	14·7	64·0	9·7	89·6
	50	25·3	66·7	11·1	84·0
	25	14·7	85·3	11·6	78·1
	0	11·1	88·9	12·9	100·0
Least significant differences at 5%		18·3	20·8	1·2	15·8
1%		24·6	27·9	1·6	21·2

[A] Based on number of pupae.

We consider that β-peltatin-A methyl ether and, to a lesser extent, deoxypodophyllotoxin account for the low number of phytophagous lepidoptera associated with *L. bidwillii* and that they probably fulfil a role within the plant's defence mechanisms. It is unlikely that they can be considered as insect control agents, however, because of their toxicity to higher animals.

The mechanism of toxicity and the effects of plants material from the family Podocarpaceae on the oligophagous codling moth and on the polyphagous light-brown apple moth will be discussed in future publications.

Acknowledgments

We are grateful to Misses M. O'Donnell and L. Emms and Mr C. M. Smith for skilled technical assistance.

References

Bianchi, E., Sheth, K., and Cole, J. R. (1969). Antitumor agents from *Bursera faragoides* (Burseraceae) (β-peltatin-A methyl ether and 5′-desmethoxy-β-peltatin-A methyl ether). *Tetrahedron Lett.* 2759–62.

Chapman, R. F. (1974). The chemical inhibition of feeding by phytophagous insects: a review. *Bull. Entomol. Res.* **64**, 339–63.

Fraenkel, G. (1969). Evaluation of our thoughts on secondary plant substances. *Entomol. Exp. Appl.* **12**, 473–86.

Fitzgerald, D. B., Hartwell, J. L., and Leiter, J. (1957). Distribution of tumor damaging lignans among conifers. *J. Nat. Cancer Inst.* **18**, 83.

Hartwell, J. L. (1950). Components of Podophyllum. III. Isolation of α- and β-peltatin, structure studies. *J. Am. Chem. Soc.* **72**, 246–53.

Hartwell, J. L., and Schrecker, A. W. (1954). Relationship of anthricin, hernandion and cicutin to deoxypodophyllotoxin. *J. Am. Chem. Soc.* **76**, 4034–5.

Hartwell, J. L., and Schrecker A. W. (1958). The chemistry of Podophyllum. *Fortschr. Chem. Org. Naturst.* **15**, 83–166.

Hartwell, J. L., Schrecker, A. W., and Greenberg, G. Y. (1952). Components of Podophyllum. X. Relation of α-peltatin to β-peltatin. *J. Am. Chem. Soc.* **74**, 6285–7.

Hartwell, J. L., Schrecker, A. W., and Johnson, J. M. (1953). The structure of silicicolin. *J. Am. Chem. Soc.* **75**, 2138–40.

House, H. L., and Barlow, J. S. (1958). Vitamin requirements of the housefly, *Musca domestica* L. (Diptera: Muscidae). *Ann. Entomol. Soc. Am.* **51**, 299–302.

Hsiao, T. H. (1969). Chemical basis of host selection and plant resistance in oligophagous insects. *Entomol. Exp. Appl.* **12**, 777–88.

Jermy, T. (1966). Feeding inhibitars and food preference in chewing phytophagous insects. *Entomol. Exp. Appl.* **9**, 1–12.

Kelly, M. G., and Hartwell, J. L. (1954). The biological effects and the chemical composition of Podophyllum. A review *J. Nat. Cancer Inst.* **14**, 967.

Lettre, H., and Wilte, S. (1967). Experimentelle und Klinische Erfahrungen mit Podophyllin Derivaten in der Tumor Therapie. (F. K. Schattauer-Verlag: Stuttgart.)

Munakata, K. (1970). Insect antifeedants in plants. In 'Control of Insect Behaviour by Natural Products'. (Eds D. L. Wood, R. M. Siverstein and M. Nakajima.) pp. 179–187. (Academic Press: New York.)

Russell, G. B., Fenemore, P. G., and Singh, P. (1972). Insect control chemicals from plants. I. Nagilactone C, a toxic substance from the leaves of *Podocarpus nivalis* and *P. hallii*. *Aust. J. Biol. Sci.* **25**, 1025–9.

Russell, G. B., Fenemore, P. G., and Singh, P. (1973). Structures of hallactones A and B, insect toxins from *Podocarpus hallii*. *Chem. Commun.* 166–7.

Schrecker, A. W., Trail, M. M., and Hartwell, J. L. (1956). Components of Podophyllum. XIV. Isomorphism in the podophyllotoxin series. *J. Org. Chem.* **21**, 292–4.

Singh, P. (1974*a*). Improvements in our relating to artificial dietary mixtures for insects. N.Z. Patent Applied for No. 174449. Date filed 4 June 1974.

Singh, P. (1974*b*). Aseptic rearing of *Epiphyas postvittana* (Lepidoptera: Tortricidae) on a meredic diet. *N.Z. J. Zool.* **1**, 111–17.

Singh, P., Fenemore, P. G., and Russell, G. B. (1973). Insect control chemicals from plants. II. Effects of five natural norditerpene dilactones on the development of the housefly. *Aust. J. Biol. Sci.* **26**, 911–15.

Manuscript received 23 June 1975