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ANTIMICROBIAL, ANTIVIRAL AND CYTOTOXIC ACTIVITY OF NEW ZEALAND LICHENS

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Abstract: A total of 69 species of lichens have been collected from various locations around New Zealand. Screening of extracts of these species for antimicrobial, antiviral and cytotoxic activity showed a high proportion with biological activity. Active extracts were generally from species known to contain phenolic compounds. Bioactivity-directed isolation work on *Cladia retipora*, *Pseudocyphellaria glabra* and *P. homoeophylla* led to the identification of usnic acid as the main antimicrobial, cytotoxic and antiviral component in these three species.

Introduction

Lichens have been used in folk medicine in many countries over a considerable period of time. Their efficacy is due to the synthesis of unique secondary compounds, a number of which have important biological roles as antibiotic, antiherbivore and antimicrobial compounds (Vartia 1974; Richardson 1975, 1988, 1991; Rundel 1978; Lawrey 1984, 1986, 1989).

New Zealand has a richly diverse and well-developed lichen flora, arguably one of the most interesting and best preserved in the world today. Malcolm & Galloway (1997) list 1378 species of lichenized and lichenicolous fungi from New Zealand, an advance on the 966 taxa discussed in the current New Zealand lichen flora (Galloway 1985). Many species are of great size and beauty and, in wetter forested areas, are often dominant components of the epiphytic and ground vegetation (Galloway 1985). New Zealand Maori have a traditional use of long, pendulous species of *Usnea* for nappies and sanitary pads (Galloway 1985; Riley 1994), which took advantage of the antibiotic properties of usnic acid (**1**, see Fig. 1). *Usnea barbata*, a Northern hemisphere species, was erroneously named in Riley (1994: 119–120), but does not occur in New Zealand. The traditional use probably involved the species of *Usnea* common in North Island forests, especially *U. angulata* Ach. [cited as *U. torquescens* Stirt. in Galloway (1985: 602–603)].

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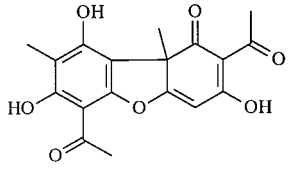
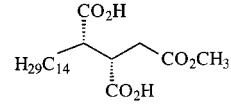
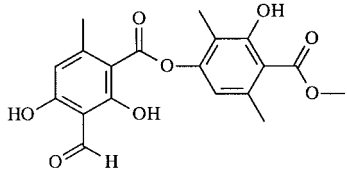


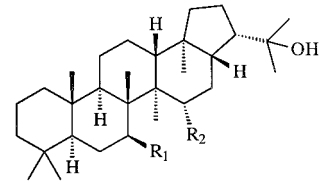
1 Usnic acid



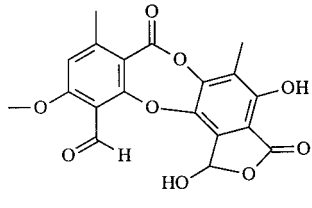
2 Rangiformic acid



3 Atranorin



4 R_1=OAc, R_2=H; 7-Acetoxyhopan-22-ol
5 R_1=H, R_2=OH; Hopan-15,22-diol



6 Stictic acid

FIG. 1. Structures of compounds tested.

A list of the compounds reported from New Zealand lichens is provided by Walker & Lintott (1997) and a few have been included in screening for biologically active (bioactive) compounds. An early discovery was the *in-vivo* antileukaemic activity of an extract of *Pseudocyphellaria coronata* (Müll. Arg.) Malme (then known as *Sticta coronata*) due to a 2,5-dihydroxyquinone, polyporic acid (Burton & Cain 1959). More recently, Calder *et al.* (1986) included 13 lichens in their search for antibiotic activity. All had some activity against the bacterium *Bacillus subtilis*, and all three of the *Stereocaulon* species tested were active against pathogenic fungi. Four lichens from New Zealand's subantarctic islands were screened for cytotoxic and antimicrobial activity by Lorimer *et al.* (1996). Three of these were active, and a revised structure and weak biological activity for rangiformic acid (**2**) from *Cladia retipora* (Labill.) Nyl. were reported by Benn *et al.* (1998).

We now present the results of screening 69 species of New Zealand lichens for antimicrobial, antiviral and cytotoxic activity. We also report the

bioactivity-directed isolation of usnic acid (**1**) from *C. retipora*, *Pseudocyphellaria glabra* (Hook. f. & Taylor) C. W. Dodge, and *P. homoeophylla* (Nyl.) C. W. Dodge.

Materials and Methods

Collections

Lichen samples were collected from various sites in New Zealand (Table 1): site A, Dunedin, 45°50′S 170°30′E; site B, Naseby, 45°00′S 170°10′E; site C, the Maungatua range, 45°50′S 170°10′E; site D, the Rees valley, 44°40′S 168°30′E; site E, the Catlins, 46°30′S 169°20′E; site F, South Island West Coast, mainly from the Cascade area, 44°10′S 168°30′W; site G, Chatham Islands, 44°00′S 175°50′E; and site H, New Zealand's subantarctic islands, see Lorimer *et al*. (1996) for details. The collection date is given by the collection code, for example, sample 960213–24 was collected on 13 February 1996. Voucher samples are held in the Otago University herbarium.

Preparation of extracts

Samples were air dried (30°C) then ground material (5 g) was extracted with alcohol (50 ml of 95% ethanol, 5% H_2O) by shaking overnight. Extracts were filtered through cotton wool into Schott bottles and stored at −20°C.

Assay methods

For antimicrobial assays, extracts (30 µl) were dried onto 6-mm-diam. filter-paper discs, which were then placed onto seeded agar Petri dishes and incubated (24 h for bacteria; 48 h for fungi). The organisms used were the Gram-positive bacterium *Bacillus subtilis* (ATCC 19659), the yeast *Candida albicans* (ATCC 14053) and the dermatophyte *Trichophyton mentagrophytes* (ATCC 28185). Pure compounds were also tested against the Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and the fungus *Cladosporium resinae* (non-ATCC strain). Activity showed as a zone of inhibition around the disc, with its width recorded from the edge of the disc in millimetres. Positive controls were chloramphenicol (30 µg per disc): 12 mm zone against *B. subtilis*; gentamycin (10 µg): 10 mm against *E. coli* and 8 mm against *P. aeruginosa*; and nystatin (100 units): 12 mm against *Candida albicans*, 6 mm against *T. mentagrophytes* and 10 mm against *Cladosporium resinae*.

For the P388 cytotoxicity assays, twofold dilution series of extracts were incubated for 72 h with fast-growing murine leukaemia cells (ATCC CCL 46 P388D1). The concentration of the sample required to inhibit cell growth to 50% of the growth of a cell control (IC_{50}) was determined by interpolation from the absorbances obtained upon staining with MTT tetrazolium. The positive control was mitomycin C (0·06 µg ml^{-1}), which inhibited cell growth by 43–75%. The results for extracts (Table 1) are expressed as follows: 0 = no activity; + = low activity; + + = high activity. For pure compounds the IC_{50}s are expressed in µg ml^{-1} (Table 2).

Cytotoxicity against slow growing BS-C-1 cells (African green monkey kidney, ATCC CCL 26) and activity against the DNA virus HSV (Herpes simplex type 1 virus, ATCC VR 733) were both determined in the same assay. Pure compounds were also tested against BS-C-1 cells infected with PV1 (Polio virus type 1, Pfiser vaccine strain Sabin). Extracts (30 µl) were dried onto 6-mm-diam. filter-paper discs, which were then placed onto the virus-infected cells and incubated (24 h). Activity showed as zones of cytotoxic effects and/or zones of inhibition of viral cytopathic effects around the disc, recorded as: 0 = no zone; + = 1–4 mm zone; + + = > 4 mm.

Samples of (+)-usnic acid, atranorin and stictic acid from Sigma Chemical Co. were used for the assays reported in Table 2.

Bioactivity-directed isolations

Cladia retipora was collected from site C (code 960330–01), *Pseudocyphellaria glabra* from site A (950116–08) and *P. homoeophylla* from site F (960128–01). Lichens were either extracted with

hot diethyl ether in a Soxhlet apparatus (*C. retipora*), or with cold ethanol followed by extraction of the ethanol extract in a Soxhlet apparatus with hexane, then acetone (*Pseudocyphellaria* species). Antimicrobial and cytotoxicity assays (above) were used to direct the isolation work. Various combinations of crystallization, acid/base extractions and column chromatography over silica gel or C-18 functionalized silica gel solid were used to give pure compounds. These were identified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Results and Discussion

Screening results

The results of screening 111 collections, of 69 species, of New Zealand lichens for antimicrobial, antiviral and cytotoxic activity are summarized in Table 1. These lichens are predominantly macrolichens having substantial biomass and occurring in either lowland to subalpine forest, or alpine grassland habitats of the southern South Island.

Only five species showed no detectable activity in any assay, whereas 38 out of 69 species showed 'high' activity (arbitrarily defined as an antimicrobial zone ≥ 5 mm or '++' cytotoxic or antiviral effects) for at least one extract. These lichen collections yielded a much higher proportion of bioactive extracts than our collections of bryophytes and vascular plants from the subantarctic islands (Lorimer *et al*. 1996). Calder *et al*. (1986) also reported a high level of antimicrobial activity in lichen extracts, compared to extracts of other plants.

Genera for which two or more species were screened can be classified into three groups (Table 1):

(1) all species with high activity: *Cladia*, *Cladina*, *Cladonia*, *Stereocaulon* and *Usnea*;
(2) some species with high activity, some species with low or no activity: *Bunodophoron*, *Nephroma*, *Parmelia*, *Peltigera*, *Pseudocyphellaria* and *Psoroma*;
(3) all species with low or no activity: *Sticta*.

Species of *Sticta* have '. . . a medullary chemistry deficient in acetone-soluble substances such as depsides, depsidones or triterpenoids . . .' (Galloway 1985). In the 20 species of *Pseudocyphellaria*, the genus best represented in our sampling (Table 1), 12 of the 13 species recorded as containing phenolic compounds (Galloway 1988) showed some antibacterial activity, compared to only 3 out of 7 species recorded as containing only triterpenoids. The other group 2 species *Bunodophoron*, *Nephroma*, *Peltigera* and *Psoroma* have either variable contents of phenolics or unexplored chemistry, but *Parmelia* is particularly characterized by the presence of the phenolic depsidone salazinic acid (Galloway 1985). The group 1 species generally contain phenolic compounds. Therefore it seems that the assays used in this screening are sensitive to the phenolic compounds in lichens, and not to the triterpenoids.

However, in some cases assay results varied between different collections of the same species, for example, *Pseudocyphellaria carpoloma* and *P. pickeringii* (Table 1). This could be due to variations in levels of secondary metabolites,

TABLE 1. *Bioactivities of New Zealand lichen extracts*

Species	Collection		Antimicrobial*			Cytotoxicity‡		Antiviral HSV§	Usnic acid•
	Code	Site	*B.sub.*	*C.alb.*	*T.ment.*	P388	BSC		
Arthrorhaphis alpina	960213–24	D	0	0	0	0	+	0	
Bunodophoron insigne	951211–40	E	3 (2)	0	0	0	+(2)	0	
B. ramuliferum	960214–09	D	9 (2)	0	3	++(2)	+(2)	++	
Chondropsis semiviridis	950520–01	A	3	0	0	+	0	++	+
Cladia aggregata	930405–03	C	5	0	0	+	++	0	
	920427–01	B	5	0	1	0	+	0	
	940120–13	H	0	0	0	0	0	0	
C. retipora	930405–25	C	9 (2)	0	5 (2)	+(2)	+(2)	++	+
	940120–07	H	3 (2)	0	1 (2)	0	+(2)	0	+
	960330–01	C	8	0	1	+	+	++	+
	970902–11	G	7 (2)	0 (2)	2 (2)	+	+(2)	0 (2)	+
C. sullivanii	930405–04	C	10	0	2	0	+	0	
Cladina confusa	970902–14	G	10	0	3	+	+	++	
C. mitis	960214–05	D	8 (3)	0	5	+	+(3)	++(3)	+
Cladonia fimbriata	920427–04	B	8 (3)	1 (2)	3 (2)	+(2)	++(3)	0	
C. gracilis tenerrima	920427–03	B	9 (2)	2 (2)	3 (2)	+	+(2)	+(2)	
Coccocarpia palmicola	960214–13	D	0	0	0	+	+	0	
Coeleocaulon aculeatum	960213–34	D	1	0	3	+	++	0	
Coenogonium implexum	950525–06	A	0	0	0	0	0	0	
Fuscoderma applanatum	960214–10	D	0	0	0	0	+	0	
Hypogymnia lugubris	940121–07	H	6 (2)	4 (2)	3 (2)	+(2)	++	0	
Lecanora epibryon subsp. broccha	960213–27	D	3	0	4	0	++	0	
Leifidium tenerum	960128–03	F	5	0	1	++	++	0	
Leptogium cyanescens	950116–04	A	0	0	0	+	0	0	
Micarea austroternaria?	960213–23	D	2	0	0	0	+	0	
Nephroma australe	950116–10	A	10 (3)	0 (2)	1	+	++(2)	0	+
	950525–13	A	8	0	2	++	0	++	+
	960214–11	D	4	0	1	+	++	0	+
N. plumbeum var. *isidiatum*	950116–12	A	0	0	0	+	0	0	
	971209–05	D	1	0	0	++	+	0	
Neuropogon acromelanus	960213–36	D	8 (3)	0	2 (2)	+	+(2)	++	+
Pannaria hookeri	960213–32	D	0	0	0	0	+	0	
Parmelia signifera	960214–04	D	4	0	4	0	++(2)	0	
	971208–01	D	2	0	0	0	+	++	
	971208–02	D	0	0	0	0	0	0	
P. tenuirima	960214–03	D	0	0	0	0	+	0	
Peltigera degenii	910821–20	A	0	0	0	+	0	0	
P. dolichorhiza	950116–03	A	0	0	0	+	0	0	
	950525–11	A	5	0	0	++	0	0	
	960214–42	D	0	0	0	+	0	0	
P. membranacea	950116–11	A	1	0	0	++(3)	++	0	
	950525–01	A	0	0	2	+	0	0	
Placopsis trachyderma	960213–29	D	0	0	0	0	+	0	
Pseudocyphellaria ardesiaca	960214–07	D	1	0	0	+	+(2)	0	
P. billardierei	950116–15	A	1	0	0	+	+	0	
P. carpoloma	950525–07	A	0	0	0	+	+	0	
	950525–12	A	0	0	0	+	0	0	
	950628–12	F	4	0	0	+	++	0	

Continued

TABLE 1. *Continued*

Species	Collection		Antimicrobial*			Cytotoxicity‡		Antiviral HSV§	Usnic acid•
	Code	Site	*B.sub.*	*C.alb.*	*T.ment.*	P388	BSC		
P. cinnamomea	950627–06	F	0	0	0	+	0	0	
P. colensoi	950629–04	F	3	0	0	+ +	+	0	
	960329–03	A	7	0	1	+	+ +	0	
P. coriacea	960329–02	A	1	0	0	+	+	0	
P. coronata	940120–01	H	5 (3)	0 (2)	2 (3)	+(2)	+ +(2)	+(2)	
	950116–16	A	10	0	2	+	+ +(2)	+(2)	
	950525–08	A	10	0	1	+	+ +	0	
	950628–04	F	6	0	0	+	+ +	0	
P. degelii	960214–40	D	0	0	0	0	0	0	
P. dissimilis	950116–01	A	0	0	0	+	0	0	
P. faveolata	950116–14	A	5	0	0	+	0	0	
	950628–15	F	5	0	0	+	+ +	0	
	951211–46	E	1 (2)	0	0	+(2)	+ +(2)	+(2)	
	960421–01	F	5	0	2	+	+ +	0	
P. fimbriatoides	950116–02	A	2	0	0	+	0	0	
P. glabra	950116–08	A	9 (3)	0	2	+	+(3)	+ +(3)	+
	950525–05	A	8	0	2	+ +	0	+ +	+
P. granulata	950116–17	A	6 (2)	0	1	+	+ +(3)	0 (3)	
	960329–01	A	4	0	0	+	+ +	+ +	
	971209–06	D	3	0	0	+	+ +	0	
P. homoeophylla	950628–14	F	9	10	3	+ +	+ +	0	+
	960128–01	F	9	5	2	+	+	+ +	+
P. maculata	960213–37	D	2	0	0	+ +(4)	+	0	
P. multifida	940126–03	H	0	0	0	0	0	0	
P. murrayi	950116–13	A	0	0	0	+	0	0	
P. pickeringii	950116–20	A	7	8	0	0	0	+	
	950525–10	A	10	0	0	+	+ +	0	
	960213–35	D	1	0	1	+	+	+ +	
P. rubella	950525–14	A	5	0	2	+ +	+	+ +	
	960214–12	D	0	0	2	0	+	0	
P. rufovirescens	910821–04	A	2	0	0	+	0	0	
	950116–05	A	0	0	0	+	0	0	
	950525–09	A	0	0	0	+	+	0	
	951211–43	E	0	0	0	+	0	0	
	951211–45	E	0	0	0	+	+	0	
Psoroma buchananii	960213–25	D	0	0	0	0	0	0	
P. hirsutulum	960213–26	D	0	0	0	0	+	0	
P. leprolomum	950116–09	A	3	0	0	+	+ +(2)	0	
P. microphyllizans	950116–07	A	4	0	0	+	+ +	0	
	950525–04	A	0	0	0	+	0	0	
P. pallidum	960214–06	D	3	0	3	+	+ +(2)	0	
	971209–07	D	3	0	0	+	+ +	0	
Siphula dissoluta	960213–31	D	1	0	0	+	+	0	
Sphaerophorus stereocauloides	971209–03	D	4	0	1	0	+ +	0	
Stereocaulon fronduliferum	950116–21	A	4 (2)	0	5	+	+	0	
S. ramulosum	921028–28	F	3	0	0	+	+ +	0	
	950116–06	A	4	0	0	0	+	+ +	
	950525–15	A	5	0	1	0	+ +	0	
	920427–02	B	5	1	1	+	+ +	0	

Continued

TABLE 1. *Continued*

Species	Collection		Antimicrobial*			Cytotoxicity‡		Antiviral	Usnic
	Code	Site	*B.sub.*	*C.alb.*	*T.ment.*	P388	BSC	HSV§	acid•
Sticta filix	950628–13	F	0	0	0	0	0	0	
	951211–17	E	0	0	0	0	0	0	
S. latifrons	950116–18	A	0	0	0	+	0	0	
	951211–44	E	0	0	0	+	0	0	
S. martinii	950116–19	A	0	0	0	+	0	0	
Teloschistes fasciculatus	960213–33	D	1	0	0	0	0	0	
Thamnolia vermicularis	930405–15	C	0	0	0	0	0	0	
	960213–30	D	0	0	0	0	0	0	
Trapeliopsis congregans	960214–14	D	3	0	0	0	+	0	
Umbilicaria cylindrica	960213–28	D	0	0	0	0	+	0	
Usnea capillacea	971209–02	D	7	0	3	+	+	+	+
U. ciliifera	960214–08	D	7 (3)	0	1	0	+ + (2)	+ (2)	+
	971209–01	D	10 (2)	0 (2)	3 (2)	+ (2)	+ (2)	+ + (2)	+
U. cf. *inermis*	920427–05	B	7 (3)	0	1 (2)	0	+ (2)	0	+

**B.sub.* = *Bacillus subtilis*, *C.alb.* = *Candida albicans* and *T.ment.* = *Trichophyton mentagrophytes*. Results are width of inhibition zone in mm (figures in brackets indicate the number of replicates, if any, used to give a mean value).
‡ P388 = murine leukemia cells, BSC = monkey kidney cells. 0 = no activity; + = low activity; + + = high activity.
§ HSV = Herpes simplex type 1 virus. 0 = no activity; + = low activity; + + = high activity.
• + = presence of usnic acid reported by Galloway (1985, 1988).

variability in the assay or trace amounts of potent antibacterial compounds such as usnic acid (**1**) (see Table 2) from contamination of a sample with other lichen species.

Some antibacterial activity against *B. subtilis* was shown by extracts of 47 out of 69 species, but antifungal activity was uncommon (Table 1). Our extracts of *Stereocaulon ramulosum* (Sw.) Rauschel with cold solvent showed almost no antifungal activity, in contrast to those of Calder *et al.* (1986) prepared with boiling solvents. The active compound, methyl haematommate (methyl 2,4-dihydroxy-3-formyl-6-methylbenzoate), may be produced during extraction with boiling methanol (Hickey *et al.* 1990; Hylands & Ingolfsdottir 1985).

A high proportion of the lichen extracts showed cytotoxic activity against one or both of the mammalian cell lines. Differential cytotoxicity is a useful attribute for potential antitumour agents. Several extracts showed antiviral activity (Table 1), which has been less studied than antimicrobial activity. A recent paper reported that some lichen depsides and depsidones are active against a key enzyme of the human immunodeficiency virus, HIV-1 (Neamati *et al.* 1997). The inconsistency in the antiviral activity of some species, for example, *Nephroma australe* (Table 1), may be because cytotoxic effects are difficult to distinguish from viral cytopathic effects in the combined BSC cytotoxicity/antiviral assays.

Bioactivities of pure compounds

Extracts of *Cladia retipora* showed consistent antimicrobial and cytotoxic activity, and variable antiviral activity (Table 1). Bioactivity-directed isolation

TABLE 2. *Bioactivities of pure compounds*

Compound	Antimicrobial*			Cytotoxicity		Antiviral•	
	B.sub.	*C.alb.*	*T.ment.*	P388‡	BSC§	HSV	PV1
Usnic acid (60 µg per disc)	10(3)	1(2)	5(2)	16(2)	+ +(2)	?	?
(30 µg per disc)	6	NT	NT	NT	+ +	+ +	+ +
(7·5 µg per disc)	7	NT	NT	NT	+ +	+ +	+
(1·5 µg per disc)	4(2)	NT	NT	NT	NT	NT	NT
(0·4 µg per disc)	1(2)	NT	NT	NT	NT	NT	NT
Atranorin	0	0	0	>25	0	0	0
Rangiformic acid	1(2)	0	0	>25	+	0	0
Stictic acid (60 µg per disc)	0	0	0	>25	+ +(2)	?	?
(30 µg per disc)	NT	NT	NT	NT	+(2)	?	?
(15 µg per disc)	NT	NT	NT	NT	0	0	0
7β-Acetoxyhopan-22-ol	0	0	0	>25	0	0	0
Hopan-15α,22-diol	0	0	0	9(2)	0	0	0

**B.sub.* = *Bacillus subtilis*, *C.alb.* = *Candida albicans* and *T.ment.* = *Trichophyton mentagrophytes*. Compound tested at 60 µg per disc, unless otherwise noted. Results are width of inhibition zone in mm (figures in brackets indicate the number of replicates to give a mean value). NT = not tested. None of the compounds showed activity against *E. coli*, *P. aeruginosa* or *C. resinae* at 60 µg per disc.

‡ Murine leukemia cells, IC_{50} in µg ml^{-1}.

§ Monkey kidney cells. Compounds tested at 60 µg per disc, unless otherwise noted. Results are zones of cytotoxic effects: 0 = no zone; + = 1–4 mm zone; + + = >4 mm.

• HSV = Herpes simplex type 1 virus, PV1 = Polio virus type 1. Compounds tested at 60 µg per disc, unless otherwise noted. Results are zones of inhibition of viral cytopathic effects around the disc, recorded as: 0 = no zone; + = 1–4 mm zone; + + = >4 mm. ? = presence/absence of cytopathic effect obscured by cytotoxic effect.

work led to usnic acid [yellow needles, identified by their ^{1}H NMR spectrum (Huneck & Yoshimura 1996)] as the main active component. Usnic acid (**1**) (Table 2) was active against *B. subtilis* and cytotoxic and/or antiviral down to low concentrations [see also Cardarelli *et al.* (1997) and references therein]. Rangiformic acid (**2**) from this lichen (Benn *et al.* 1998) was found to be mostly inactive (Table 2). We also isolated atranorin [identified by its ^{1}H NMR spectrum (Huneck & Yoshimura 1996)] from *C. retipora*. Atranorin (**3**) was inactive at the levels tested in our assays (Table 2), despite previous reports of antimicrobial activity (Cavalcanti *et al.* 1983). These compounds had all previously been reported in *C. retipora* by Galloway (1985).

Extracts of *Pseudocyphellaria glabra* and *P. homoeophylla* also had high antimicrobial, cytotoxic and antiviral activities (Table 1). Usnic acid (**1**) was the main antimicrobial component. The triterpenoids 7β-acetoxyhopan-22-ol (**4**) and hopan-15α, 22-diol (**5**) were also obtained pure, and identified by their ^{13}C-NMR spectra (Wilkins *et al.* 1987). These compounds were generally inactive at the levels tested, apart from P388 cytotoxicity for **5** (Table 2). Galloway (1988) has noted the presence of compounds **1**, **4** and **5** in both these species, as well as stictic acid. Stictic acid (**6**) showed some cytotoxic activity against BSC cells, but not against P388 cells (Table 2).

Conclusions

A high proportion of New Zealand lichen extracts tested showed antimicrobial, cytotoxic and/or antiviral activity. Active extracts were generally from species known to contain phenolic compounds. Usnic acid (**1**) is responsible for these activities in at least three species. The biological activity of usnic acid has been studied previously (Asahina & Shibata 1954; Cardarelli *et al*. 1997; Proksa *et al*. 1996; Yamamoto *et al*. 1995) and it has been used therapeutically (Shaw 1967). Therefore, usnic acid has no potential as a new medicinal agent. Usnic acid could be masking the bioactivities of other compounds, such as the cytotoxic diketopiperazines recently reported from a Sri Lankan *Usnea* species (Williams *et al*. 1998). Our bioactivity-directed isolation work showed that this was not the case for *C. retipora*, *P. glabra* or *P. homoeophylla*. Given the known occurrence of usnic acid (Galloway 1985, 1988) in the lichens screened (see Table 1), it may be responsible for at least part of the bioactivities of *Chondropsis semiviridis* (F. v. Muell. ex Nyl.) Nyl. ex Crombie, *Cladina mitis* (Sandst.) Hustich, *Nephroma australe* A. Rich., *Neuropogon acromelanus* (Stirton) Lamb, and the species of *Usnea*.

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