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Bullatenone, 1,3-dione and sesquiterpene chemotypes of *Lophomyrtus* species

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Abstract

The only known natural source of the volatile bioactive compounds bullatenone **1** and 4-methyl-1-phenylpentane-1,3-dione **2** is the New Zealand endemic shrub *Lophomyrtus bullata* (Myrtaceae). GC and NMR analyses of essential oils and solvent extracts of *L. bullata*, *L. obcordata* and the hybrid *L.* "*ralphii*" showed several chemotypes, which did not correlate with species. Levels of **1** and **2** varied from dominant to low/undetectable and the most common chemotype was rich in *allo*-aromadendrene and other sesquiterpenes. The rare natural product *E*-4-methyl-1-phenyl-1-penten-3-one **4** was detected for the first time in this genus. The non-volatile cytotoxic compound bullataketal **5** co-occurred with bullatenone **1**. An essential oil from the relatively rare bullatenone **1** chemotype showed antifungal activity against *Candida albicans* and *Cladosporium resinae*, and an oil from the 4-methyl-1-phenylpentane-1,3-dione **2** chemotype showed antibacterial activity against *Bacillus subtilis*.

Keywords: *Lophomyrtus*; Myrtaceae; Antifungal; Antibacterial, infraspecific variation; Phenyl diketone; Bullatenone; Sesquiterpene

1. Introduction

Lophomyrtus (family Myrtaceae) is an endemic New Zealand genus with just the two species *L. bullata* (Soland. ex A. Cunn.) Burret and *L. obcordata* (Raoul) Burret (Parsons et al., 1998). Both grow as shrubs or small trees: *L. bullata* on the North Island and the northern South Island; and *L. obcordata* on both North and South Islands (Allan, 1961). *L. bullata* and *L. obcordata* hybrids have been developed as ornamental varieties, e.g. *L.* "*ralphii*" (Hook.f.) Burret var. *purpurea* (Salmon, 1986).

L. bullata (Maori name ramarama) leaves have been used traditionally to treat bruises and cuts, and *L. obcordata* (rohutu) bark and berries have been used to treat dysmenorrhoea (Brooker et al., 1987; Riley, 1994). The first chemical investigation of *L. bullata* essential oil, steam-distilled from leaves, led to the isolation and identification of the unique natural product bullatenone **1** (structures in Fig. 1) (Brandt et al., 1954; Parker et al., 1958). Briggs and White identified six major components in an oil from *L. bullata*: the common monoterpenes α- and β-pinene (32 and 7.5%), the widely distributed sesquiterpene *allo*-aromadendrene (7%), bullatenone **1** (14%), 4-methyl-1-phenylpentane-1,3-dione **2** (19.5%), and 2-isopropylchromone **3** (6.5%) (Briggs and White, 1971). A volatile oil from *L. obcordata* contained a variety of sesquiterpenes and the diterpene hibaene (Briggs et al., 1975). Most recently, our group has discovered the cytotoxic triketone–phloroglucinol–bullatenone hybrid bullataketal **5** in ethanol extracts of *L. bullata*, plus 5-hydroxyflavone, benzyl salicylate and the diterpene phyllocladene (Larsen et al., 2005). Various bioactive applications have been reported for bullatenone **1**: insect repellency (Muta and Amaike, 1991), antiulcer activity (Felman et al., 1992), in sunscreens

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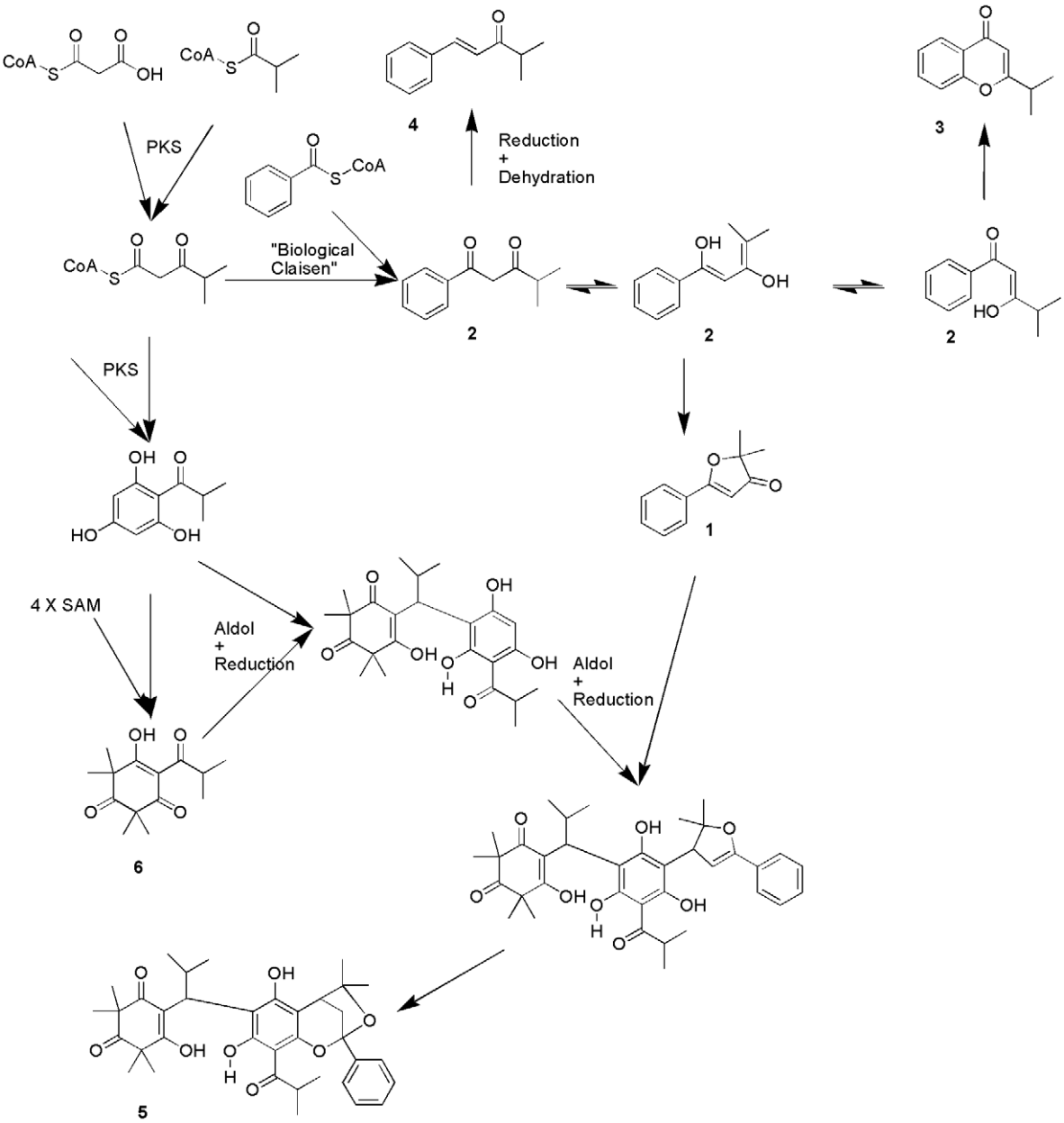

Fig. 1. Structures and proposed phenyl diketone pathway for the *Lophomyrtus* metabolites.

(Amaike, 1990c) and in fragrances (Imaizumi et al., 1988). The other unique *L. bullata* natural products dione **2** and chromone **3** have also been patented for potential use in sunscreens (Amaike, 1990a,1990b).

2. Results and discussion

The starting point for the work described below was the discovery that three *L. bullata* individuals growing in the Dunedin Botanical Gardens (DBG) yielded three very different essential oils. Bullatenone **1** was the predominant component in one of these (voucher code 981125-01, Table 1) and dione **2** in another (981109-01), with both compounds identified by comparison of ^{1}H NMR and mass spectra with the data reported by (Briggs and White, 1971). The third essential oil (981125-02) contained mainly *allo*-aromadendrene and another sesquiterpene bicyclogermacrene, identified by ^{1}H and ^{13}C NMR and mass spectra (Joulain and Konig, 1998). There was almost no overlap in composition between these three plants, but a fourth *L. bullata* from DBG gave a mixture of **1** and **2**. Three *L. bullata* from a natural population on the North Island gave essential oils containing mostly *allo*-aromadendrene plus another common sesquiterpene *trans*-calamenene (Table 1) (Joulain and Konig, 1998).

Eleven *L. obcordata* from a natural population on the South Island also gave essential oils containing mostly

Table 1
Compositions of the essential oils and solvent extracts from individual *Lophomyrtus* plants

Species	Collection code	Location	Oil yield (%)	Levels of main volatiles by GC (peak area as% of total)[a]											Main volatiles by NMR	Bullataketal 5 by NMR[b]
				α-Pin	β-Pin	Lin	Arom	AlloA	Enone	BG	Bull	Cala	Dione	Chrom		
bullata	981109-01	DBG	0.32	15	2	0	2	2	1	2	1	0	59	0	Dione	–
bullata	981125-01	DBG	0.17	10	7	0	1	2	0	2	50	0	1	1	Bull	+
bullata	981125-02	DBG	0.45	2	0	1	1	30	1	17	0	1	1	2	AlloA/bG	–
bullata	981208-01	DBG	0.37	28	3	1	1	1	1	3	45	0	3	0	Dione/bull	+
bullata	990121-01	Foxton	0.19	2	0	0	1	15	1	7	0	19	4	1	AlloA/cala	–
bullata	990121-02	Foxton	0.25	1	0	1	1	10	0	4	0	19	4	2	AlloA/cala	–
bullata	990121-03	Foxton	0.10	1	0	0	1	27	0	4	2	8	2	3	AlloA/cala	–
obcordata	981208-02	DBG	0.44	35	1	2	1	15	1	3	5	1	10	1	Dione/bull/alloA	+
obcordata	981208-03	DBG	0.48	0	0	1	0	24	1	4	0	13	4	1	AlloA/cala	NT
obcordata	981208-04	DBG	0.48	22	28	1	10	1	0	4	0	0	1	0	Arom	–
obcordata	981214-01	Lincoln	0.28	22	0	1	2	6	1	2	9	0	32	2	Dione/bull	+
obcordata	981216-01	Riccarton	0.20	0	0	0	1	24	1	8	1	8	2	1	AlloA/cala	NT
obcordata	981216-02	Riccarton	0.17	5	7	1	1	15	0	5	0	10	3	1	AlloA/cala	NT
obcordata	981216-03	Riccarton	0.25	0	0	1	0	38	0	3	0	9	2	1	AlloA/cala	NT
obcordata	981216-04	Riccarton	0.21	4	5	1	1	26	0	3	0	10	2	0	AlloA/cala	NT
obcordata	981216-05	Riccarton	0.34	0	0	1	1	11	1	7	0	17	4	1	AlloA/cala	NT
obcordata	981216-06	Riccarton	0.25	0	0	1	1	13	1	5	0	17	5	1	AlloA/cala	NT
obcordata	981216-07	Riccarton	0.14	7	9	1	1	17	0	6	1	7	2	1	AlloA/cala	NT
obcordata	981216-08	Riccarton	0.32	5	7	6	0	14	1	6	0	8	2	1	AlloA/cala/lin	NT
obcordata	981216-09	Riccarton	0.12	0	0	1	1	22	1	7	1	8	3	1	AlloA/cala	NT
obcordata	981216-10	Riccarton	0.09	1	0	0	1	39	0	4	1	7	2	0	AlloA/cala	NT
ralphii	981125-03	DBG	0.32	0	0	1	1	22	4	9	0	7	3	1	AlloA/cala	–
ralphii	981125-04	DBG	0.58	30	0	0	1	6	13	2	1	0	9	8	Chrom/enone/alloA	–

[a] In GC retention order: α-pinene; β-pinene; linalol; aromadendrene; *allo*-aromadendrene; enone **4**; bicyclogermacrene; bullatenone **1**; *trans*-calamenene; dione **2**; chromone **3**.
[b] Bullataketal in $CDCl_3$ extract: +, present; –, not detected; NT, not tested.

allo-aromadendrene and *trans*-calamenene (Table 1). Two DBG *L. obcordata* gave mixtures of bullatenone **1**, dione **2** and *allo*-aromadendrene, while another DBG *L. obcordata* had a different major sesquiterpene, aromadendrene (Joulain and Konig, 1998), with a *trans* ring junction instead of the *cis* junction of *allo*-aromadendrene.

The essential oil of two hybrid *L.* "*ralphii*" individuals from DBG were examined. One oil was mostly *allo*-aromadendrene and *trans*-calamenene, but the other (981125-04, Table 1) contained some different components. Dione **2** was present and 2-isopropylchromone **3** was identified by comparison of ^{1}H NMR and mass spectra with the data of Briggs and White (Briggs and White, 1971). GC–MS showed two peaks (E/Z isomers) with an apparent molecular weight of 174 and a 131 base peak for which a library search suggested the structure 4-methyl-1-phenyl-1-penten-3-one. The larger of these peaks was confirmed as *E*-4-methyl-1-phenyl-1-penten-3-one **4** by matching ^{1}H and ^{13}C NMR peaks in the spectra of the extract with the data reported for synthetic enone **4** (Iwasawa et al., 1997). Compound **4** has only been reported twice before as a natural product, in floral volatiles of two *Elaeagnus* species (family

Elaeagnaceae) (Liu et al., 1988; Yan et al., 1988). The *Z* isomer of **4** has only been reported as a synthetic compound (Iwasawa et al., 1997), and we were not able to isolate any to confirm its identity.

The results for analyses of the essential oils from these 23 individual *Lophomyrtus* plants are combined in Table 1. A principal components analysis on these essential oils showed a separation of the unique high bullatenone **1** and dione **2** chemotypes from the other plants (Fig. 2). The most common chemotype had *allo*-aromadendrene as the main component, together with other sesquiterpenes. Individuals of both *L. bullata* and *L. obcordata* had this chemotype, as did one of the hybrid *L.* "*ralphii*" specimens. The phenyl derivatives **1**, **2**, **3** and **4** were found in individuals of each of *L. bullata*, *L. obcordata* and *L.* "*ralphii*", but the proportions varied widely and *allo*-aromadendrene was also present in some of these. These differences in chemistry are not due to seasonal or environmental factors since the essential oils with the most striking differences were from plants growing at the same location (DBG) and harvested at the same time of year. Therefore the volatile oil chemistry of *Lophomyrtus* is probably genetically controlled, but the chemotypes do not align with taxonomic species boundaries. *L. bullata* is distinguished from *L. obcordata* by its larger leaves, which are bullate (blistered in appearance), but these two species hybridise spontaneously wherever they grow together (Allan, 1961; Salmon, 1986). It seems that the genes controlling biosynthesis of mono- and sesquiterpenes, and the phenyl derivatives **1**, **2**, **3** and **4,** are present in both *L. bullata* and *L. obcordata*. The biosynthesis of **1**, **2**, **3** and **4** has not been studied, but the "phenyl diketone" pathway previously proposed for bullatenone **1** and bullataketal **5** (Larsen et al., 2005), can readily accommodate **2**, **3** and **4** (Fig. 1).

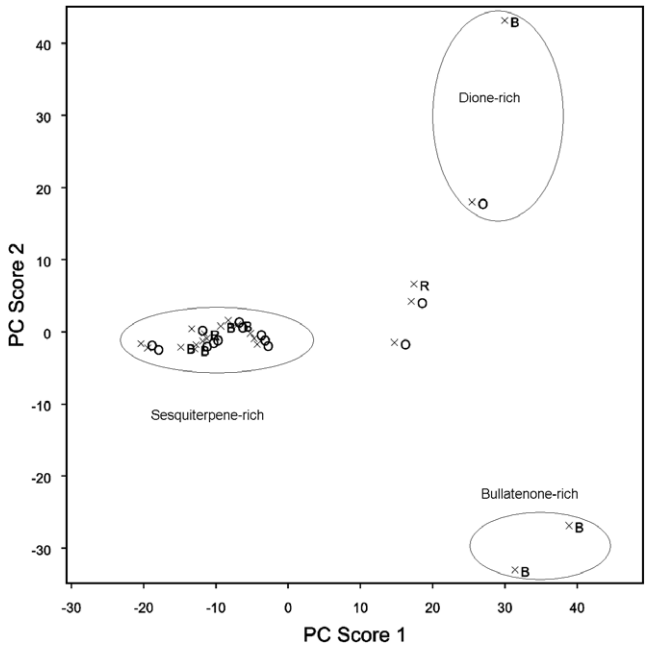

Fig. 2. Principal components analysis (PCA) of *Lophomyrtus* essential oil compositions. O: *L. obcordata*, R: *L.* "*ralphii*", B: *L. bullata*. The main contributors (eigenvectors) to this variation in PC1 (45% of the dataset variation) were α-pinene (+0.51), *allo*-aromadendrene (−0.51), bullatenone (+0.51), calamenene (−0.24) and dione (+0.37), while the main contributors to the variation in PC2 (26% of the dataset variation) were bullatenone (−0.64) and dione (+0.76).

Since the cytotoxic bullataketal **5** contains a bullatenone-derived sub-structure (Larsen et al., 2005), we wanted to know how levels of **5** varied between *Lophomyrtus* individuals. Bullataketal **5** is not volatile enough to be extracted in the essential oil or analysed by GC, but its distinctive low-field ^{1}H NMR signals (16.8, 16.5, 11.5, 11.3, 10.6 and 10.5 ppm (Larsen et al., 2005)) could be detected in small-scale deuterated chloroform extracts of leaves. This method was applied to a range of the *Lophomyrtus* samples that were used to prepare solvent extracts, and showed that bullataketal **5** could only be detected in samples containing bullatenone **1** (Table 1). This same pattern was observed for another 19 individual plants (8 *L. bullata*, 5 *L. obcordata* and 6 *L. ralphii*) growing in the Dunedin region. Only two samples, both *L. bullata* specimens, were high in bullatenone **1** and these also contained bullataketal **5** (data not shown). This co-occurrence of bullatenone **1** and bullataketal **5** suggests that the gene(s) controlling the formation of bullatenone **1** is tightly linked to the gene(s) controlling the further elaboration of **1** to bullataketal (Fig. 1).

Genetic control has been suggested for the essential oil composition of another New Zealand shrub in the Myrtaceae, *Leptospermum scoparium* J. R. et G. Forst. *Le. scoparium* individuals from different regions of New Zealand had distinct chemotypes (Douglas et al., 2004), and those chemotype differences were maintained in plants grown at the same site from seed from the particular regions (Perry et al., 1997). It would be interesting to study the distribution of chemotypes in natural populations of *Lophomyrtus* throughout New Zealand, to see if these also show regional distributions (the DBG plants were from various unrecorded wild populations, and from cultivars).

The essential oil of one chemotype of *Le. scoparium* has antimicrobial activity due to the presence of β-triketones such as flavesone (**6**, Fig. 1) (Perry et al., 1997). Because of the structural similarity of the β-triketones and dione **2** we compared the antimicrobial activity of three of the different *L. bullata* essential oils with the *Le. scoparium* oil that is used commercially as a natural antibiotic (Porter, 2001). We used a simple disc-diffusion assay, measuring the zone of inhibition at three doses to get a measure of potency (low aqueous solubility complicates any antimicrobial testing of essential oils, see Hood et al., 2003). The different chemotypes showed different activity profiles, with the oil rich in bullatenone **1** being most active against the fungi *Candida albicans* and *Cladosporium resinae*, and the oil rich in dione **2** being most active against the Gram-positive bacterium *Bacillus subtilis* (Table 2). Antimicrobial activities have not been previously reported for **1** or **2**, or

Table 2
Antimicrobial activities[a,b] of *Lophomyrtus bullata* and *Leptospermum scoparium* essential oils

Species/chemotype/collection code	Dose (μg/disk)	Bs	Ca	Cr
L. bullata/bullatenone **1**/981125-01	1200	2	7	2
	600	1	7	1
	300	0.5	5	0
L. bullata/dione **2**/981109-01	1200	5	4	0
	600	3	2	0
	300	2	1	0
L. bullata/alloA/981125-02	1200	1	3	0
	600	1	1	0
	300	1	1	0
Le. scoparium/triketone/[c]	1200	6	2	NT[d]
	600	4	0	NT
	300	1	0	NT

[a] Results are width of inhibition zone in mm.
[b] Test organisms *Bacillus subtilis*, *Candida albicans* and *Cladosporium resinae*. The extracts showed no activity against *Escherichia coli* or *Pseudomonas aeruginosa* at the levels tested.
[c] Results from Perry et al. (1997).
[d] Not Tested.

for the chromone **3**, but a synthetic sample of enone **4** has shown antimicrobial activity (Erciyas et al., 1994).

3. Experimental

3.1. General experimental procedures

NMR spectra (^{1}H at 200 MHz; ^{13}C at 50 MHz) were measured on a Varian Gemini 200 spectrometer using $CDCl_3$ as solvent. GC was performed on a Perkin–Elmer AutoSystem with FID detector, using hydrogen as carrier gas, split ratio 100:1, injector 260 °C, detector 350 °C. The column was a J&W DB-1 (10 m × 0.25 mm, film 0.25 μm), the linear flow rate was 55 cm/s, and the temperature programme was 50–230 °C at 15 °C/min then 5 min at 230 °C. Peak areas were calculated using the Omega software supplied with the instrument. GC–MS was performed on a Finnigan GC8000 with 70 eV EI +ve ion detection, using helium as carrier gas at 0.8 ml/min, and an injector split ratio of 50:1. The column was a J&W DB-1 (30 m × 0.25 mm, film 1 μm), with a 5 m retention gap, and the temperature programme was 50–180 °C at 3 °C/min then 180–240 °C at 10 °C/min, then a 20 min hold.

3.2. Collection and extraction

Leaves and branchlets were gathered from individual plants at the following locations: Dunedin Botanical Gardens (latitude 45° 52′ S, longitude 170° 30′ E); Foxton (40° 25′ S, 175° 10′ E); Lincoln (43° 38′ S, 172° 30′ E); and Riccarton Bush, Christchurch (43° 32′ S, 172° 35′ E). Voucher specimens have been maintained in the Plant Extracts Research Unit collection, with the codes in Table 1 giving the collection date (YYMMDD-XX).

3.3. Extractions and analyses

Samples were dried at 30 °C for 72 h, and then stored in the dark until used. Ground material (ca. 20 g) was added to distilled water (250 ml) and the essential oil was extracted into cyclohexane (ca. 25 ml) using a simultaneous distillation–extraction Likens–Nickerson apparatus for 3 h (Chaintreau, 2001). Subsamples (1 ml) were reserved for GC analysis, and the solvent was removed from the remainder of the organic phase by rotary evaporation at 35 °C, prior to weighing and NMR analysis. Small-scale solvent extracts were prepared by taking dried leaves (100–500 mg), grinding to a powder in liquid N_2 and extracting with $CDCl_3$ (1 ml) overnight. Extracts were filtered through cotton wool plugs directly into NMR tubes for analysis.

Principal components analyses were performed using GenStat (version 9) software on the composition of the 23 essential oils listed in Table 1.

3.4. Antimicrobial assays

Antimicrobial assays against *B. subtilis*, *C. albicans* and *C. resinae* were performed at the Chemistry Department, University of Canterbury, Christchurch, as described previously (Lorimer et al., 1996). Briefly, solutions of extracts for assay were dried on to 6 mm filter paper disks, which were then placed on to seeded agar Petri dishes and incubated. Activity showed as a zone of inhibition around the disk, with its width recorded from the edge of the disk in mm.

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